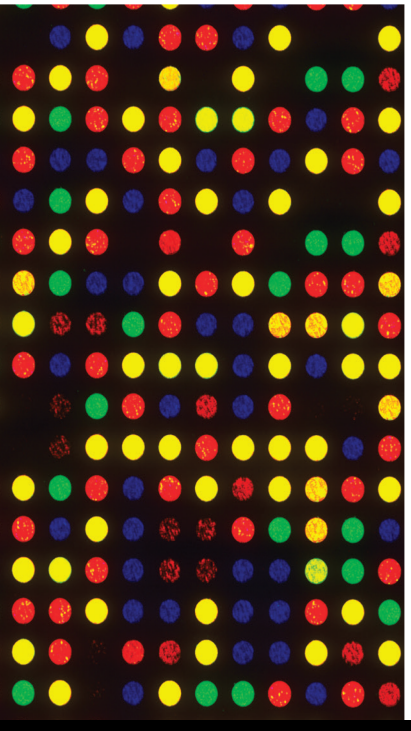
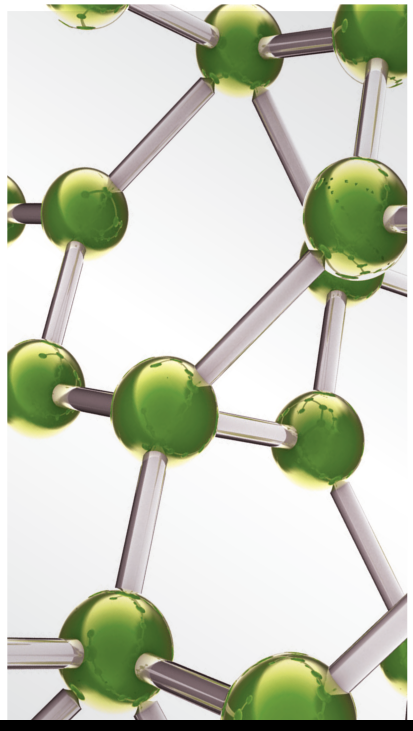
Evidence-Based Complementary and Alternative Medicine

Heavy Metal-Induced Toxicity, Complications, and the Therapeutic Role of Natural Products

Lead Guest Editor: Rajadurai Murugan
Guest Editors: Ramachandran Saravanan and Murugavel Ponnusamy






Heavy Metal-Induced Toxicity, Complications, and the Therapeutic Role of Natural Products

Evidence-Based Complementary and Alternative Medicine

Heavy Metal-Induced Toxicity, Complications, and the Therapeutic Role of Natural Products

Lead Guest Editor: Rajadurai Murugan
Guest Editors: Ramachandran Saravanan and Murugavel Ponnusamy

Contents

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 8011953, 11 pages
https://doi.org/10.1155/2022/8011953

Review Article

Mutagenic, Carcinogenic, and Teratogenic Effect of Heavy Metals

Sukeerthi Dasharathy,[1] Selvam Arjunan,[1] Anusha Maliyur Basavaraju,[2] Vidya Murugasen,[3] Saravanan Ramachandran,[4] Rohini Keshav,[1] and Rajadurai Murugan[2]

[1]*Department of Biotechnology, Faculty of Life and Allied Health Sciences, Ramaiah University of Applied Sciences, Bangalore 560054, Karnataka, India*
[2]*Department of Food Technology, Faculty of Life and Allied Health Sciences, Ramaiah University of Applied Sciences, Bangalore 560054, Karnataka, India*
[3]*Proiuvo Private Limited, Bangalore, Karnataka, India*
[4]*Department of Marine Pharmacology, Chettinad University, Kelambakkam, Chennai, Tamil Nadu, India*

Correspondence should be addressed to Rajadurai Murugan; rajadurai.ft.ls@msruas.ac.in

Received 13 May 2022; Revised 10 August 2022; Accepted 16 September 2022; Published 5 October 2022

Academic Editor: Ammar Al-Farga

Heavy metal (HM)-induced toxicity and its associated complications have become a major issue in the medical world. HMs are not biodegradable, enter into the food chain, and gets accumulated in the living systems. Increased concentrations and accumulation of HMs can cause severely damaging effects and severe complications in living organisms and can even lead to the death of the organism. In Ayurvedic medicine, ingredients of natural origin, including whole plants or certain portions of the plant, animal sources, and minerals, are used for therapeutic purposes as medicine, both alone and in combination. HM such as cadmium, copper, zinc, lead, chromium, nickel, and arsenic cause hazardous effects on animals, human health, and the environment. This review focuses on mutagenic, carcinogenic, and teratogenic effects of HM , mechanism, organ toxicity, available remedies in the market, and their side effects. Also, emphasis is given to alternative systems of medicine to treat HM toxicity.

1. Introduction

The naturally occurring metals with relatively high density are called as heavy metals (HMs). These metals with metalloids like arsenic are toxic and can cause health effects. Due to the increase in the application of these metals in different fields, including agriculture and industries, exposure to these harmful chemicals has increased drastically; it has become a global health concern [1].

Though these HMs are present in trace amounts in the environment, the exposure to these metals increases as the mining and smelting activities grow. Further, the use of these metals in many industries, including agriculture, and pharmaceutical also increases, which also leads to human exposure. Figure 1 shows the severity of HM toxicity cases in the US. Environmental contamination of these metals can also occur due to corrosion, leaching of HM, soil erosion, and atmospheric deposition [2]. Figure 2 shows the statistical reports of HM toxicity concerning the route of entry.

These metals including arsenic, lead, cadmium, and mercury are nonessential elements and interact with different cell components like cell membrane, proteins, mitochondria, and DNA, causing reactive oxygen species (ROS)-related damages, transcriptional changes, DNA damage to cells leading to many diseases, and disorders like multiorgan damage and cancer [3].

The mechanism of toxicity differs with different HMs. Hence, understanding the exposure and toxicity of different metals is important for curing diseases and reducing the pollution caused by HMs. It's not possible to discuss and review all the HMs and their hazardous effects. Hence, we decided to concentrate on lead, mercury, and arsenic. This review focuses on mutagenic, carcinogenic, and teratogenic effects and its mechanism action of HM

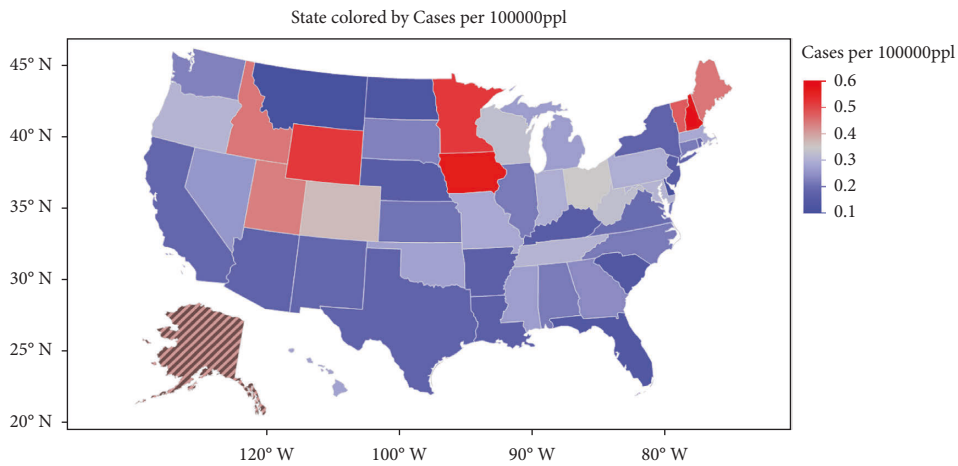


Figure 1: Severity of heavy metal (HM) toxicity cases (lead, arsenic, and mercury) in the USA. Adapted and redrawn from data available from the National Capital Poison Centre.

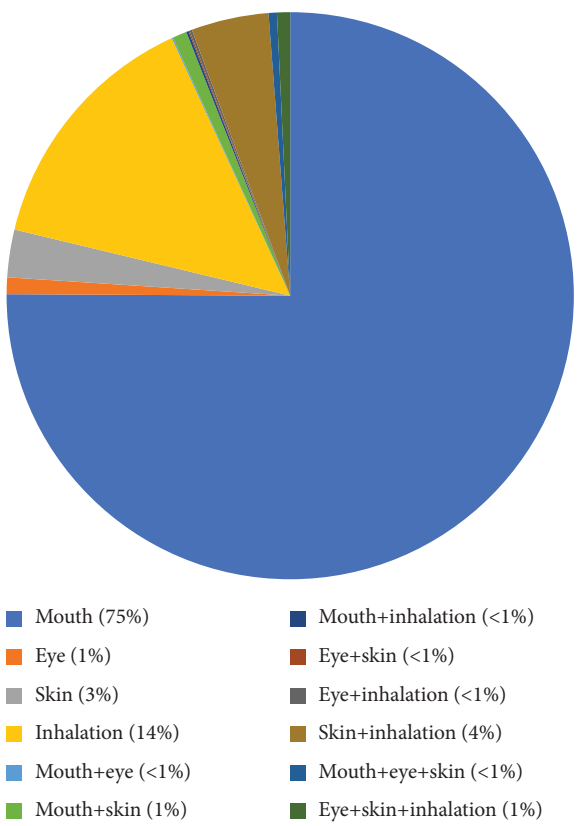


Figure 2: Heavy metal (HM) toxicity concerning the route of entry. Adapted and redrawn from data available from the National Capital Poison Centre.

toxicity and complications associated with specific organs, and it also deals with the available remedies for HM toxicity and alternative system of medicine to treat HM toxicity.

2. Methodology

Required data were searched/collected from the online data bases including Wiley, Google, PubMed, Google Scholar, ScienceDirect, and Scopus. Keywords used in this search are HM-induced toxicity, alternative system of medicine, lead, mercury, cadmium, and mutagenicity, carcinogenicity, and teratogenicity. Latest published data were selected [3].

3. Lead

Lead has a wide range of applications in industries, agriculture, and domestic uses because of its physicochemical properties such as softness, high malleability, ductility, low-melting point, poor conductibility, and corrosion resistance. It is one of the oldest known occupational and environmental toxicants which is not biodegradable. The International Agency for Research on Cancer (IARC) has distinguished inorganic lead compounds under group 2*A* (probable human carcinogens), whereas lead metal under group 2*B* (possible human carcinogen) [4]. On exposure to lead, it accumulates in bones and teeth over a decade. Although the usage of lead has been discontinued in many parts of the world, it is still being used for smelting and manufacturing batteries. It is one of the highly toxic metals which affects major organs in the body [5]. The present review focuses on the sources, occupational exposure, mechanism of toxicity, available remedies, and alternate system of medicine.

3.1. Occupational Exposure and Sources. Elemental pollution is due to many man-made activities like industrialization, mining, and manufacturing. Lead has been used for the production of lead-acid batteries, ammunitions, oxides for paint, glass, pigments, chemicals, and devices to shield X-

rays. The exposure to lead is being controlled by the leading industries (battery manufacturers), but there are some industries (demolition industry) where the exposure still occurs occasionally. Lead is found naturally in mineral deposits and they combine with the environment through industrial activity [6]. Lead poisoning can occur majorly from two routes, that is, inhalation and ingestion. Inhalation of lead can be through fumes or lead dust produced by industrial activities such as automobiles, smelting, storage batteries production, and lead-glazed ceramics. Cigarette smoking also releases lead. Ingestion through consumption of drinking water or food contaminated with lead enters into the food chain. The water pipes and solder made of lead may leach into drinking water. Children are more vulnerable to lead toxicity than adults. They are exposed through toys, deteriorated paint, dust, or bare contaminated soil. Automobiles emit lead components that are absorbed by the plants. Also, increased urbanization has led to an increase in the lead content in the sewage sludge and soil, thereby entering into the food chain [7]. Exposure to lead during pregnancy may be detrimental as the lead absorbed can be transferred to the fetus [1]. Dogs and cats are the most affected animals in urban areas [8]. The intake of polluted food sources, such as aquatic animals consuming lead-affected sediments in mining sites, can expose avian species to high levels of lead. Increased oxidative stress and reduced antioxidant enzymes in hepatic and renal tissue are two toxicities seen in these avian species [9].

3.2. Mechanism of Toxicity. Various studies have been reported the different mechanisms by which lead exerts its toxicity, one of them being through oxidative stress. Exposure to lead causes oxidative stress, that is, an imbalance in the generation of ROS and antioxidant defense to nullify it [10]. The onset of oxidative stress is depicted by two pathways that occur simultaneously: (a) depletion of antioxidant reserves and its removal and (b) generation of ROS. Glutathione is a major intracellular antioxidant that can occur in both reduced (GSH, 90%) and oxidized (GSSG, glutathione disulfide, 10%) forms under normal conditions. Lead forms covalent attachment by electron sharing with sulfhydryl groups of antioxidant enzymes such as glutathione, glutathione reductase (GR), and glutathione peroxidase (GPx) which are the targets for inactivation [7]. Figure 3 shows the mechanism of toxicity of lead. Lead causes oxidative stress in the hepatocytes by raising free radical levels, causing lipid peroxidation, and lowering a number of antioxidants (MDA, SOD, etc.). NOX2 and CYP2E1 are two examples of genes whose transcription is disrupted by lead. Lead alters DNA, DNA methylation, mitochondrial stress, the electron transport chain, the IRE1-JNK pathway, RER damage in the ER, nuclear pyknosis, inflammation, and overexpression of NF-κB, alterations in CYP7A1 gene expression, and HMGR, which alters cholesterol metabolism and causes hepatocytic necrosis [11].

The second possible mechanism of lead exerting its toxicity on the hemopoietic system is through δ-aminolevulinic acid (ALA). ALA is a molecule that is part

Figure 3: Mechanisms of toxicity of lead.

of porphyrin synthesis leading to heme synthesis. In the mitochondria, it is formed by the condensation of glycine and succinyl-CoA by the enzyme ALA synthase. δ-Aminolevulinic acid dehydratase (ALAD), a cytosolic enzyme catalyzing the formation of porphobilinogen [12]. Lead can inhibit ALAD causing accumulation of ALA, generating free radicals such as superoxide ($O_2^{-\cdot}$) and hydrogen peroxide (H_2O_2) radicals. The generated free radicals interact with oxyhemoglobin, generating hydroxyl ($^{\bullet}OH$) radicals. Increased lipid peroxidation causes oxidation of hemoglobin followed by hemolysis. Lipid peroxidation is a biomarker for oxidative stress. Lead can bind to phosphatidylcholine in the cellular membrane altering its properties [13].

3.3. Organ Toxicity and Associated Complications. The adverse effect of this compound is based on the level of exposure. Acute exposure although uncommon or through occupational exposure can affect the gastrointestinal tract, kidney, and brain, are more common, and meanwhile, chronic exposure has adverse effects on the nervous system, hematopoietic, renal, cardiovascular, and reproductive systems [14]. The nervous system plays a crucial role in the normal functioning of the body, and it is prone to be affected by lead toxicity due to its sensitivity. Lead affects both the central nervous system (CNS) in children and the peripheral nervous system (PNS) in adults predominantly. Acute encephalopathy is a condition associated with lead exposure to CNS, and some of the major symptoms are dullness, irritability, poor attention, muscular tremor, loss of memory, and hallucination [15]. At a lower level, fetuses and young children are vulnerable to lead toxicity as the lead can enter the blood brain barrier (BBB) resulting in hyperactivity and

irritable behavior in them, while at a higher level it can cause permanent brain damage and death [16].

The hematopoietic system is affected by lead as low as 10 μg/dl in the blood. Lead restrains the synthesis of hemoglobin, affects the heme biosynthetic pathway, and reduces the lifespan of erythrocytes as they become fragile leading to anemia. Hemolytic anemia (acute high-level lead exposure) and frank anemia (blood lead level elevated for a long duration) are caused by lead toxicity. Lead affects three key enzymes involved in the heme biosynthetic pathway, that is, aminolevulinic acid synthetase (ALAS), δ-aminolevulinic acid dehydratase (ALAD), and ferrochelatase [17].

The renal system is affected by exposure to lead as low as 10 μg/dl and causes renal dysfunction at a concentration above 60 μg/dl [18]. The contribution of lead to the cardiovascular system remains unclear, but a positive correlation has been reported between blood lead levels and hypertension [19]. Studies indicate that lead exposure decreases the bioavailability of nitric oxide, increases ROS production, and changes cytokine production which support atherogenesis and plaque development from elevated cholesterol levels [20]. An adverse effect of lead can affect the reproductive system in women and men. In women, lead exposure can make them susceptible to miscarriage, infertility, premature delivery, pre-eclampsia, and low birth weight of child born. Lead present in the blood can enter the fetus through the placenta as well as through breast milk increasing the toxicity. Exposure to low doses of lead in men reduces their sperm count, while exposure to high doses can cause a reduction in the sperm count and motile sperm number. Studies reported that lead can act as an endocrine disruptor molecule, i.e., it can delay puberty and alter menopause onset [21].

Lead toxicity is known to impair the immune (innate and adaptive) system. Occupational exposure to lead causes the generation of ROS in neutrophils and affects eicosanoid metabolism in the mature macrophages indicating its negative aspect on the innate immune system [22]. Lead affects the adaptive immune system through *T*-lymphocytes by interferring with the critical *T*-helper 1 (Th1)/*T*-helper 2 (Th2) lymphocyte balance required for resistance against infectious diseases. It reduces the $CD^{4+}T$ helper cells and increases the expression of 2,3-dioxygenase required for N-formyl-kynurenine formation. N-formyl-kynurenine is a metabolite of tryptophan metabolism causing apoptosis of *T*-lymphocytes and cell-cycle arrest explaining the depletion of $CD^{4+}T$ cells [23].

3.4. Treatment and Alternate System of Medicine. Chelation therapy is the treatment of choice to remove HM-induced toxicity. It involves chelate formation and excretion from the body upon administrating a chelating agent that binds lead [24]. The chelating agents are calcium disodium ethylenediamine tetraacetate ($CaNa_2EDTA$) or dimercaprol and for oral chelation, it is D-penicillamine or *meso*-2,3-dimercaptosuccinic acid (DMSA). British Anti-Lewisite (BAL) has been used to treat severe lead poisoning where it evades precipitation of encephalopathy. Succimer (DMSA) is an oral chelation agent approved by the United States Food and Drug Administration (USFDA) effective in treating lead poisoning in children with BLL between 45 and 69 μg/dl. The side effects associated with DMSA are an increase in hepatic transaminase, loss of appetite, nausea, and diarrhea. An individual with glucose 6 phosphate dehydrogenase (G6PD) deficiency should not be administered succimer as it can cause hemolytic anemia. Although chelation therapy has been the method of choice for the removal of lead from the body, it causes renal damage along with the loss of essential metals such as zinc, iron, and manganese [25].

Dietary strategies to overcome lead toxicity by excretion of lead involve supplementation of iron, zinc, calcium, and thiamine supplementation. Essential metals reduce lead burden by competing with them for intestinal absorption and binding to enzymes' active sites. Zinc supplementation protects the blood ALAD and alleviates oxidative stress as zinc is a cofactor for copper zinc-superoxide dismutase (Cu/Zn SOD), an antioxidant enzyme. Selenium also has a protective effect against lead toxicity as it contributes to the antioxidant defense by being a cofactor of GPx [26]. The deficiency of thiamine, pyridoxine, and ascorbic acid increases the sensitivity toward lead toxicity. The pyrimidine ring of thiamine interacts with lead, thereby mitigating its toxicity and increasing its excretion from the body. The nitrogen atom of pyridoxine ring chelates lead before its absorption, reducing its accumulation in the tissues and reducing inhibition of ALAD activity. Ascorbic acid and vitamin E have a positive impact on reducing BLL as it is a natural nonenzymatic antioxidant capable of attenuating oxidative damage [27]. Organosulphur compounds (diallyl sulfide and diallyl tetrasulfide) found in alliums such as garlic, ginger, and onion prevent intestinal absorption of lead and promote its excretion [28]. *Lactobacillus, Bifidobacterium, Saccharomyces,* and *Bacillus* are some of the commercial probiotic strains. *Lactobacillus rhamnosus, Lactobacillus plantarum*, and *Bifidobacterium longum* are some of the lactic acid bacteria (LAB) which bind HMs *in vitro*. The protective effect of *Lactobacillus plantarum* CCFM8661 against lead toxicity has been reported where they were found to reduce the BLL and prevent the oxidative stress caused by the metal [29].

4. Arsenic

4.1. Occupational Exposure/Sources. Arsenic has been employed for a variety of purposes throughout history, from medicines to poisons. Arsphenamine (marketed as Salvarsan) was used to cure syphilis until antibiotics were developed in the early 1900s; it was also used to treat trypanosomiasis and amoebic dysentery. Rheumatism, arthritis, asthma, malaria, trypanosome infection, TB, and diabetes were all treated using medicinal solutions such as potassium arsenite, arsenic iodide, and arsphenamine [29]. Arsenic contamination in humans can occur via arsenic-containing industrial effluent, agricultural pesticides, or natural mineral deposition in soil, air, food, and drinking

water. Arsenic is observed to accumulate greater in paddy, green vegetables, and subterranean vegetables. Arsenic can also be found in fish, shellfish, pork, chicken, dairy products, and cereals; however, exposure from these foods is often considerably lower than exposure from contaminated groundwater [30]. The greatest risk to public health starts from contaminated groundwater. Arsenic in the form of inorganic ions is naturally found at higher levels in nearly 108 countries in the groundwater of many countries like Bangladesh, China, Mexico, Argentina, the United States of America, India, and Chile [31].

4.2. Mechanism of Toxicity. When arsenic enters the body through consumption, it is quickly absorbed into the bloodstream by the digestive tract. Arsenic in the blood binds to oxyhemoglobin and causes severe red blood cell death, limiting the blood's ability to carry oxygen to important organs and interfering with the kidney and liver's ability to function effectively [32]. Arsenic is a protoplastic toxin that specifically targets the sulphydryl group of cells, impairing cell enzyme function and cellular respiration [33]. Figure 4 shows the mechanism of toxicity of arsenic. Humans and organisms (fungi, bacteria, and algae) enzymatically methylate toxic inorganic arsenic compounds after chronic arsenic exposure to form less dangerous monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which are expelled through urine. Monomethylarsonic acid (MMA III) is more toxic due to its high affinity for sulfhydryl groups. It cannot be excreted in the urine, and its accumulation is linked to arsenic-induced carcinogenesis [34].

4.3. Organ Toxicity and Associated Complications. Arsenic toxicity is known to cause anemia, gastrointestinal symptoms, skin lesions, neuropathy, hyperpigmentation, vascular lesions, liver damage, and renal damage [35]. Chronic arsenic poisoning has been shown to harm the cardiovascular system, neurological system, pulmonary system, endocrine system, and reproductive system. *T* cell and *B* cell functions are harmed by maternal arsenic exposure [36]. Vomiting, abdominal pain, and diarrhea are the first signs of acute arsenic poisoning. In severe cases, they are followed by numbness and tingling in the extremities, muscle cramps, and death [37].

Long-term exposure to arsenic can lead to malignancies of the bladder and lungs, in addition to skin cancer. Developmental impacts, diabetes, lung disease, and cardiovascular disease are some of the other negative health outcomes linked to long-term ingestion of inorganic arsenic. Myocardial infarction caused by arsenic, in particular, can be a major cause of death. Exposure to Arsenic has been related to "Blackfoot disease," a severe illness affecting blood vessels and leading to gangrene [38]. Arsenic and phosphorus belong to the same periodic table group, and phosphate and arsenate chemicals have chemical similarities, allowing arsenic to substitute in key molecules or reactions such as DNA methylation disruption, DNA repair inhibition, oxidative stress disruption in ATP generation, and so on [39].

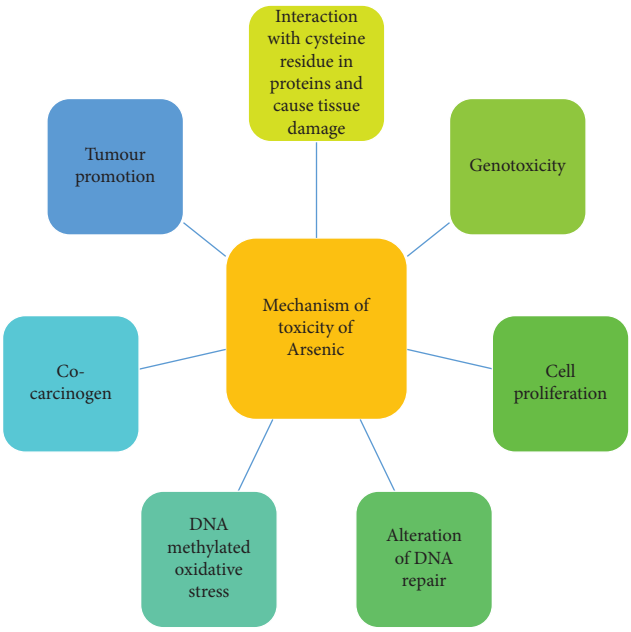


Figure 4: Mechanisms of toxicity of arsenic.

4.4. Treatment and Alternate System of Medicine. The first and foremost treatment of arsenic poisoning is dependent on the symptoms that have manifested; the patient must be removed from the source of exposure. In patients with significant burdens, chelating agents such as succimer and dimercaprol are essential. Hemodialysis should be considered for patients with kidney failure [40]. Plants and plant products have been used to treat ailments since the dawn of time. Plant-based medicine's main advantages appear to be perceived efficacy, low frequencies of serious side effects, and inexpensive cost. The current literature review found that medicinal plants like *Withania somnifera, Ipomea aquatica, Phyllanthus emblica, Triticum aestivum, Tephrosia purpurea, Camellia sinensis, Vitis vinifera, and Moringa oleifera* [41–43] and natural products like rutin, β-carotene, α-tocopherol, curcumin, resveratrol, and quercetin with arsenic toxicity alleviative effects simultaneously demonstrated a good intrinsic antioxidant effect by suppressing arsenic-induced oxidative stress through multimodal augmentation of endogenous defense mechanisms, resulting in arsenic toxicity amelioration [44, 45].

The natural compounds (phytochemicals) are well-known nutraceuticals and natural antioxidants. This demonstrates the need for antioxidant supplementation and backs up the suggestion for antioxidant therapy in humans. However, the biological advantages of these compounds must be confirmed in human subjects with chronic arsenic exposure. A potentized homeopathic medicine, Arsenicum album-30, was given to a group of arsenic patients, and the amount of arsenic in their urine and blood was measured regularly after that. The findings indicate that the medicine may be able to treat Arsenic poisoning in humans [46].

5. Mercury

5.1. Occupational Exposure and Sources. Mercury is a d-block element on the periodic table. It is the only common metal that is liquid at room temperature. It can exist in two oxidation states: I (mercurous) and II (mercuric). Inorganic, organic, and elemental forms of mercury show a slightly varied toxic profile, for instance, organomercuric compounds are considered most toxic owing to their ability to penetrate the BBB [47]. Gaseous mercury (used in electron tubes, argon lamps, etc.) is most difficult to control, owing to its high volatile nature. Dissolved elemental mercury, Hg (0), is widely observed in sediments and water which can get metabolized by microorganisms into organic mercury compounds such as methyl mercury which can then enter the food chain and accumulate [48].

Mercury emission can be from natural sources such as volcanic activity, weathering of rocks, oceans, soil, and biosphere accumulates, but the majority of the emission is due to anthropogenic activities such as combustion of coal, mercury-containing fuels [49], incineration of waste, mining, iron, and steel production. Global mercury emissions in 2000 were majorly from fossil fuel combustion which accounted for about 65%, gold production of around 11%, and nonferrous metal production and cement production with a mere 7% and 6% emission rate, respectively [50]. According to the WHO, a major cause of mercury exposure is due to dental amalgams and ingestion of contaminated seafood [51]. It is reported that carnivorous fish and animals have a higher chance of mercury toxicity than those of fishes and animals with herbivorous food habits [52]. Similar to what occurs in humans, higher animals (like mammals) can become poisoned by HM. The interaction of HM with invertebrates often results in genetic variation for tolerance and creates modifications in life-history traits. Invertebrates that have evolved to HM have shorter lifespans and make more effort during reproduction [53].

5.2. Mechanism of Toxicity. The two aspects that determine the toxicity of mercury are its solubility and its ability to form bonds with sulfhydryl groups. Primarily, the absorption rate of inorganic mercury into the body is around 2-38%, whereas organic mercury is almost completely absorbed into the bloodstream [54]. As aforementioned, it can enter the BBB and the CNS [55]. Also, the fat-friendly nature enables the toxin to enter the cell membrane conveniently. Being electrophilic, mercury tends to interact with nucleophile components such as sulfhydryl (–SH) or selenohydryl (–SeH) groups and forms stable complexes [56].

The chemical interactions lead to the formation of ROS, thus inducing oxidative stress. ROS are known to cause cell death by damaging enzymes, lipids, and nucleic acids [57]. MeHg is also known to target and disrupt the mitochondrial electron transport chain and causes a reduction in the glutathione levels in the body by bonding with the thiol group in GSH and forming a GS-$HgCH_3$ complex [56]. The free thiol group is particularly important for normal membrane functioning, and hence mercury blocks the physiological and metabolic functioning of the cell leading to oxidative stress and also damaging calcium homeostasis [58]. The blocking of the free thiol groups by mercury is one of the major causes of neurotoxicity and neuroinflammation. Apart from this, the tubulin protein of microtubules is also attacked by mercury compounds [59]. Figure 5 shows the mechanism of toxicity of mercury. It has been determined that mercury has deteriorated the effects of the antioxidant system and lipid peroxidation in addition to increasing the levels of superoxides. It has been reported that the signaling of the Nrf2-Keap1 molecules undergoes modifications. The DNA damage as a result of DNA breaks is one of the genetic alterations. Mitochondrial swelling, excessive H_2O_2 production, RER dilation, and alterations in the phosphorylation of JNK and GRP78 protein activation are a few noticeable ultrastructural changes [11].

Figure 5: Mechanisms of toxicity of mercury.

5.3. Organ Toxicity and Associated Complications. The bonding of mercury with -SH groups is known to bring about secondary changes in the structure of DNA and RNA and structural changes in the ribosomal proteins [60, 61]. Mercury affects the activities of many neurotransmitters such as acetylcholine, serotonin, and norepinephrine [51]. It is shown to affect the immune system by inducing cytokine expression as well as IL-1 release [52] and also reducing the uptake of vitamin B12 and plasma ascorbic acid [52, 62]. Mercury is shown to induce immunosuppression, type-4 hypersensitivity, and autoimmunity by affecting cytokine production [63]. Prenatal exposure to organic mercury have been linked to mental retardation, deafness, blindness, and dysarthria in children [64].

Exposure to inorganic mercury on the other hand is shown to be associated with insomnia, weight loss,

Table 1: Toxicity of lead, arsenic, and mercury.

Element	Route	Toxicity	Reference
Mercury	Inhalation	Deposited in the brain, thyroid, adrenals, skin, and pancreas and can impair the organs	[82, 83]
	Ingestion	Weakness, fatigue, anorexia, weight loss, and gastrointestinal disturbance	[83]
		Kidney targeting—abdominal pain, vomiting, and bloody diarrhea with potential necrosis of the gut mucosa	[84]
Arsenic	Ingestion of water or accidental ingestion of pesticides and insecticides	Vomiting, nausea, cyanosis, confusion, diarrhea, cardiac arrhythmia, and hallucinations	[85]
	Inhalation of arsenic gas	Shortness of breath, cough, bronchitis, lung cancer, chronic obstructive pulmonary disease, and bronchiectasis	
	Ingestion of water	Pigmentation of feet, hands, fingers, and keratosis	
		Central and peripheral vascular and cardiovascular disease, malignant diseases such as bladder, kidney, and liver cancer, diabetic millets, low blood count, and numbness	
Lead	Ingestion	Loss of neuron myelin sheath, reduction in the number of neurons, and it interferes with neurotransmission and decreases neuronal growth	[86]
		Adverse effects on certain organ systems like the central nervous system, the cardiovascular system, kidneys, and the immune system	[87]
		Affects osteoclasts and osteoblasts. Accelerates processes of bone formation and mineralization, which results in the formation of poor quality bones	[88]

erythema, pruritus, excessive perspiration and hypersalivation, renal tubular dysfunction, and neuropsychiatry disorders [58]. There are reports of deposition of mercury in the kidney, liver, brain, and muscles. Mercury also has an affinity for *T* cell surface binding sites and sulfhydryl groups, which influences *T* cell function. Health effects of mercury include neurotoxicity, immunotoxicity, cardiovascular issues, carcinogenicity, and reproductive issues [65–67].

5.4. Treatment and Alternate System of Medicine. A few of the most used chelating agents for mercury toxicity are BAL, DMPS, DMSA, Penicillamine, Ca EDTA, etc. DMPS and DMSA are chelating agents that form complexes with various HMs including mercury. Much research suggests that administration with DMPS and DMSA resulted in increased urinary mercury output as compared to chelators such as BAL [68–70]. Iranmanesh et al. [71] have proved that the combined use of deferasirox and deferiprone is effective for treating mercury intoxication *in vivo*. DMSA and DMPS are administered orally and are known to effectively remove mercury through the kidney [71]. Additionally, DMSA is reported to be more efficient at the removal of methylmercury, including from the brain region [68, 72, 73]. Selenium plays a role in the metabolism of mercury in the body. Although the exact mechanisms remain unclear, selenium is shown to reduce the toxicity of mercury [74]. DMSA is considerably less toxic as compared to DMPS and Ca EDTA, which makes it the preferred chelating agent [75]. Despite being less toxic, these chelating agents possess numerous drawbacks. Adverse symptoms such as fever, muscle pain, and in severe cases difficulty in breathing, heart failure, and kidney damage are known to occur during the course of treatment and as a result, arises a need to find alternative natural sources of treatments [70, 75].

Owing to the side effects of chelation treatment, numerous other treatments have gained popularity in combination with chelation. In case of high toxicity, plasma exchange treatment can be used as an efficient alternative. It is most efficient for inorganic mercury poisoning. Induced sweating is found to be an effective treatment, while surprisingly hemodialysis was found to be ineffective [69]. The use of Ayurvedic medicine for the same is also gaining popularity [76]. The extracts of *Plathymenia reticulata, Connarus favosus* are shown to reduce acetylcholinesterase activity and methylmercury-induced lipid peroxidation, thus reducing mercury poisoning in zebrafish [77]. A recent study evaluated the protective effect of zinc (Zn) and N-acetylcysteine (NAC) on mercury toxicity in lactating rats [78]. Numerous natural antioxidants such as vitamin E, vitamin C, uric acid, and carotenoids are known to reduce the ROS released due to mercury toxicity [79–81]. Table 1 shows the health complications of HM toxicity.

6. Conclusion

The research efforts have increased the awareness related to HM-induced toxicity. Also, this review insists the important and effectiveness of alternative system of medicine. Nowadays, HM exposure has become a part of our day-to-day life, which cannot be avoided completely, but can be reduced significantly. This communication has thrown light on the adverse effects of these HMs. Even though modern medicines are effective in controlling HM-induced toxicities, which also cause severe side effects, they alter the normal metabolic process and sometimes extremely worsen the condition. However, alternative systems of medicine with little or no effects to treat the HM-induced toxicity will be the major focused area of research in the coming days. Plant products are the best sources to control metabolic disorders, including HM toxicity.

6.1. Recommendations. According to this communication, the recommendation is to go for alternative therapy for HM-induced toxicity than allopathic therapy because alternative system of medicine has few or no side effects.

Data Availability

No data were used to support the study.

Ethical Approval

Ethical approval was not required for the study.

Conflicts of Interest

All the authors declare that there are no conflicts of interest.

Authors' Contributions

Conceptualization: R. M. and S. D; validation: S. A. and A. M. B.; formal analysis: V. M: S. R; and R. K; writing-original draft preparation; R. M. and S. D; writing-review and editing: R. M. and S. D; supervision: R. M. The final manuscript was read and approved by all authors.

References

[1] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, "Heavy metal toxicity and the environment," *Molecular, Clinical and Environmental Toxicology*, vol. 101, pp. 133–164, 2012.

[2] Z. L. He, X. E. Yang, and P. J. Stoffella, "Trace elements in agroecosystems and impacts on the environment," *Journal of Trace Elements in Medicine & Biology*, vol. 19, no. 2–3, pp. 125–140, 2005.

[3] S. Sambu, U. Hemaram, R. Murugan, and A. A. Alsofi, "Toxicological and teratogenic effect of various food additives: an updated review," *BioMed Research International*, vol. 2022, Article ID 6829409, 11 pages, 2022.

[4] M. Abdulla, "Essential and Toxic Trace Elements and Vitamins in Human Health," *Chapter 13-Lead*, Elsevier, Amsterdam, Netherlands, 2020.

[5] S. Acharya, "Lead between the lines," *Nature Chemistry*, vol. 5, no. 10, p. 894, 2013.

[6] N. C. Papanikolaou, E. G. Hatzidaki, S. Belivanis, G. N. Tzanakakis, and A. M. Tsatsakis, "Lead toxicity update. a brief review," *Medical Science Monitor: International*

Medical Journal of Experimental and Clinical Research, vol. 11, no. 10, pp. RA329–RA336, 2005.

[7] P. Sharma and R. S. Dubey, "Lead toxicity in plants," *Brazilian Journal of Plant Physiology*, vol. 17, no. 1, pp. 35–52, 2005.

[8] R. Levin, C. L. Z. Vieira, M. H. Rosenbaum, K. Bischoff, D. C. Mordarski, and M. J. Brown, "The urban lead (Pb) burden in humans, animals and the natural environment," *Environmental Research*, vol. 193, Article ID 110377, 2021.

[9] R. J. Williams, S. D. Holladay, S. M. Williams, and R. M. Gogal, "Environmental lead and wild birds: a review," *Reviews of Environmental Contamination & Toxicology*, vol. 245, pp. 157–180, 2018.

[10] J. A. Timbrell, "Biochemical mechanisms of toxicity: specific examples," *Principles of Biochemical Toxicology*, Informa Health Care, London, UK, 2008.

[11] K. Renu, R. Chakraborty, H. Myakala et al., "Molecular mechanism of heavy metals (lead, chromium, arsenic, mercury, nickel and cadmium)-induced hepatotoxicity-a review," *Chemosphere*, vol. 271, Article ID 129735, 2021.

[12] S. Piomelli, "Childhood lead poisoning," *Pediatric Clinics of North America*, vol. 49, no. 6, pp. 1285–1304, 2002.

[13] G. Flora, D. Gupta, and A. Tiwari, "Toxicity of lead: a review with recent updates," *Interdisciplinary Toxicology*, vol. 5, no. 2, 2012.

[14] K. Kalia and S. J. Flora, "Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning," *Journal of Occupational Health*, vol. 47, no. 1, pp. 1–21, 2005.

[15] J. Hsiang and E. D. Diaz, "Lead and developmental neurotoxicity of the central nervous system," *Current Neurobiology*, vol. 2, no. 1, pp. 35–42, 2011.

[16] A. L. Wani, A. Ara, and J. A. Usmani, "Lead toxicity: a review," *Interdisciplinary Toxicology*, vol. 8, no. 2, 2015.

[17] P. Mitra, S. Sharma, P. Purohit, and P. Sharma, "Clinical and molecular aspects of lead toxicity: an update," *Critical Reviews in Clinical Laboratory Sciences*, vol. 54, no. 7-8, pp. 506–528, 2017.

[18] E. B. Ekong, B. G. Jaar, and V. M. Weaver, "Lead-related nephrotoxicity: a review of the epidemiologic evidence," *Kidney International*, vol. 70, no. 12, pp. 2074–2084, 2006.

[19] N. D. Vaziri and H. C. Gonick, "Cardiovascular effects of lead exposure," *Indian Journal of Medical Research*, vol. 128, no. 4, pp. 426–435, 2008.

[20] C. Sevim, E. Dogan, S. Comakli, and S. Comakli, "Cardiovascular disease and toxic metals," *Current Opinion in Toxicology*, vol. 19, pp. 88–92, 2020.

[21] I. Mazumdar, K. Goswami, and M. S. Ali, "Status of serum calcium, vitamin D and parathyroid hormone and hematological indices among lead-exposed jewelry workers in Dhaka, Bangladesh," *Indian Journal of Clinical Biochemistry*, vol. 32, no. 1, pp. 110–116, 2017.

[22] A. J. Theron, G. R. Tintinger, and R. Anderson, "Harmful interactions of non-essential heavy metals with cells of the innate immune system," *Journal of Clinical Toxicology*, 2012.

[23] K. Mishra, R. Rani, V. S. Yadav, and S. Naik, "Effect of lead exposure on lymphocyte subsets and activation markers," *Immunopharmacology and Immunotoxicology*, vol. 32, no. 3, pp. 446–449, 2010.

[24] K. S. Gunturu, P. Nagarajan, P. McPhedran, T. R. Goodman, M. E. Hodsdon, and M. P. Strout, "Ayurvedic herbal medicine and lead poisoning," *Journal of Hematology & Oncology*, vol. 4, no. 1, pp. 51–56, 2011.

[25] R. J. Prasanthi, C. B. Devi, D. C. Basha, N. S. Reddy, and G. R. Reddy, "Calcium and zinc supplementation protects lead (Pb)-induced perturbations in antioxidant enzymes and lipid peroxidation in developing mouse brain," *International Journal of Developmental Neuroscience*, vol. 28, no. 2, pp. 161–167, 2010.

[26] M. Shaban El-Neweshy and Y. Said El-Sayed, "Influence of vitamin C supplementation on lead-induced histopathological alterations in male rats," *Experimental & Toxicologic Pathology*, vol. 63, no. 3, pp. 221–227, 2011.

[27] A. G. Farag, M. E. Elhalwagy, and H. E. Farid, "Effect of ginger supplementation on developmental toxicity induced by fenitrothion insecticide and/or lead in albino rats," *Pesticide Biochemistry and Physiology*, vol. 97, no. 3, pp. 267–274, 2010.

[28] Q. Zhai, A. Narbad, and W. Chen, "Dietary strategies for the treatment of cadmium and lead toxicity," *Nutrients*, vol. 7, no. 1, pp. 552–571, 2015.

[29] E. Merian and T. W. Clarkson, *Metals and Their Compounds in the Environment: Occurrence, Analysis, and Biological Relevance*, VCH, Weinheim, NY, USA, 1991.

[30] A. Shrivastava, "Dietart arsenic exposure: sources and risks," in *Arsenic Toxicity: Challenges and Solutions*, N. Kumar, Ed., Springer, Singapore, 2021.

[31] E. Shaji, M. Santosh, K. V. Sarath, P. Prakash, V. Deepchand, and B. V. Divya, "Arsenic contamination of groundwater. a global synopsis with focus on the Indian Peninsula," *Geoscience Frontiers*, vol. 12, no. 3, Article ID 101079, 2021.

[32] V. R. Mohan Chandrasekaran, I. Muthaiyan, P. C. Huang, and M. Y. Liu, "Using Iron precipitants to remove arsenic from water: is it safe?" *Water Research*, vol. 44, no. 19, pp. 5823–5827, 2010.

[33] A. T. Jan, M. Azam, K. Siddiqui, A. Ali, I. Choi, and Q. Haq, "Heavy metals and Human health: mechanistic insight into toxicity and counter defense system of Antioxidants," *International Journal of Molecular Sciences*, vol. 16, no. 12, pp. 29592–29630, 2015.

[34] M. T. T. Jaishankar, T. Tseten, N. Anbalagan, B. B. Mathew, and K. N. Beeregowda, "Toxicity, mechanism, and health effects of some heavy metals," *Interdisciplinary Toxicology*, vol. 7, no. 2, pp. 60–72, 2014.

[35] Agency for Toxic Substances and Disease Registry (ATSDR), *Toxicological Profile for Arsenic (Update)*, U.S. Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA, USA, 2007.

[36] T. B. Huy, T. T. Tuyet Hanh, R. Johnston, and H. Nguyen-Viet, "Assessing health risk due to exposure to Arsenic in drinking water in Hanam Province, Vietnam," *International Journal of Environmental Research and Public Health*, vol. 11, no. 8, pp. 7575–7591, 2014.

[37] J. C. Saha, A. K. Dikshit, M. Bandyopadhyay, and K. C. Saha, "A review of Arsenic poisoning and its effects on human health," *Critical Reviews in Environmental Science and Technology*, vol. 29, no. 3, pp. 281–313, 1999.

[38] Y. S. Hong, K. H. Song, and J. Y. Chung, "Health effects of chronic arsenic exposure," *Journal of Preventive Medicine and Public Health*, vol. 47, no. 5, pp. 245–252, 2014.

[39] S. Shen, X. F. Li, W. R. Cullen, M. Weinfeld, and X. C. Le, "Arsenic binding to proteins," *Chemical Reviews*, vol. 113, no. 10, pp. 7769–7792, 2013.

[40] S. J. Flora, S. Bhadauria, S. C. Pant, and R. K. Dhaked, "Arsenic induced blood and brain oxidative stress and its response to some thiol chelators in rats," *Life Sciences*, vol. 77, no. 18, pp. 2324–2337, 2005.

[41] A. Kumar, R. Kumar, M. S. Rahman et al., "Antidote effects of plants of Himalayan sub-origin against arsenic-induced toxicity," *Biological Chemical Research*, vol. 2, pp. 99–109, 2015.

[42] T. K. Dua, S. Dewanjee, M. Gangopadhyay, R. Khanra, M. Zia-Ul-Haq, and V. De Feo, "Ameliorative effect of water spinach, Ipomea aquatica (Convolvulaceae), against experimentally induced arsenic toxicity," *Journal of Translational Medicine*, vol. 13, no. 1, p. 81, 2015.

[43] S. Sayed, N. Ahsan, M. Kato, N. Ohgami, A. Rashid, and A. A. Akhand, "Protective effects of *Phyllanthus emblica* leaf extract on sodium arsenite-mediated adverse effects in mice," *Nagoya Journal of Medical Science*, vol. 77, no. 1-2, pp. 145–153, 2015.

[44] K. Sárközi, A. Papp, Z. Máté, E. Horvath, E. Paulik, and A. Szabo, "Rutin, a flavonoid phytochemical, ameliorates certain behavioral and electrophysiological alterations and general toxicity of oral arsenic in rats," *Acta Biologica Hungarica*, vol. 66, no. 1, pp. 14–26, 2015.

[45] R. Das, A. Das, A. Roy, U. Kumari, S. Bhattacharya, and P. K. Haldar, "β-carotene ameliorates arsenic-induced toxicity in albino mice," *Biological Trace Element Research*, vol. 164, no. 2, pp. 226–233, 2015.

[46] A. R. Khuda-Bukhsh, S. Pathak, B. Guha et al., "Can homeopathic arsenic remedy combat arsenic poisoning in humans exposed to groundwater arsenic contamination? a preliminary report on first human trial," *Evidence-Based Complementary and Alternative Medicine*, vol. 2, no. 4, pp. 537–548, 2005.

[47] F. Maqbool, K. Niaz, F. I. Hassan, F. Khan, and M. Abdollahi, "Immunotoxicity of mercury: pathological and toxicological effects," *Journal of Environmental Science and Health, Part C*, vol. 35, no. 1, pp. 29–46, 2017.

[48] L. Yang, Y. Zhang, F. Wang, Z. Luo, S. Guo, and U. Strähle, "Toxicity of mercury: molecular evidence," *Chemosphere*, vol. 245, Article ID 125586, 2020.

[49] A. Thanos Bourtsalas and N. J. Themelis, "Major sources of mercury emissions to the atmosphere: the US case," *Waste Management*, vol. 85, pp. 90–94, 2019.

[50] E. G. Pacyna and J. M. Pacyna, "Global emission of mercury from anthropogenic sources in 1995," *Water, Air, and Soil Pollution*, vol. 137, no. 1/4, pp. 149–165, 2002.

[51] R. Siblerud, J. Mutter, E. Moore, J. Naumann, and H. Walach, "A hypothesis and evidence that mercury may be an etiological factor in Alzheimer's disease," *International Journal of Environmental Research and Public Health*, vol. 16, no. 24, p. 5152, 2019.

[52] D. F. de Oliveira, B. S. de Castro, M. C. N. do Nascimento Recktenvald et al., "Mercury in wild animals and fish and health risk for indigenous Amazonians," *Food Additives and Contaminants: Part B*, vol. 14, no. 3, pp. 161–169, 2021.

[53] Z. Rahman and V. P. Singh, "The relative impact of toxic heavy metals (THMs) (arsenic (As), cadmium (Cd), chromium (Cr) (VI), mercury (Hg), and lead (Pb)) on the total environment: an overview," *Environmental Monitoring and Assessment*, vol. 191, no. 7, pp. 419–421, 2019.

[54] D. R. Abernethy, A. J. DeStefano, T. L. Cecil, K. Zaidi, and R. L. Williams, "Metal impurities in food and drugs," *Pharmaceutical Research*, vol. 27, no. 5, pp. 750–755, 2010.

[55] Y. S. Hong, Y. M. Kim, and K. E. Lee, "Methylmercury exposure and health effects," *Journal of Preventive Medicine & Public Health*, vol. 45, no. 6, pp. 353–363, 2012.

[56] M. Farina, J. B. Rocha, and M. Aschner, "Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies," *Life Sciences*, vol. 89, no. 15-16, pp. 555–563, 2011.

[57] X. Wu, S. J. Cobbina, G. Mao, H. Xu, Z. Zhang, and L. Yang, "A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment," *Environmental Science and Pollution Research*, vol. 23, no. 9, pp. 8244–8259, 2016.

[58] R. Ynalvez, J. Gutierrez, and H. Gonzalez-Cantu, "Mini-review: toxicity of mercury as a consequence of enzyme alteration," *Biometals*, vol. 29, no. 5, pp. 781–788, 2016.

[59] T. Syversen and P. Kaur, "The toxicology of mercury and its compounds," *Journal of Trace Elements in Medicine & Biology*, vol. 26, no. 4, pp. 215–226, 2012.

[60] J. M. Goodrich, N. Basu, A. Franzblau, and D. C. Dolinoy, "Mercury biomarkers and DNA methylation among Michigan dental professionals," *Environmental and Molecular Mutagenesis*, vol. 54, no. 3, pp. 195–203, 2013.

[61] J. Richard Pilsner, A. L. Lazarus, D. H. Nam et al., "Mercury-associated DNA hypomethylation in polar bear brains via the luminometric methylation assay: a sensitive method to study epigenetics in wildlife," *Molecular Ecology*, vol. 19, no. 2, pp. 307–314, 2010.

[62] L. Zeneli, A. Sekovanić, M. Ajvazi, L. Kurti, and N. Daci, "Alterations in antioxidant defense system of workers chronically exposed to arsenic, cadmium and mercury from coal flying ash," *Environmental Geochemistry and Health*, vol. 38, no. 1, pp. 65–72, 2016.

[63] M. Berlin, R. K. Zalups, and B. A. Fowler, G. F. Nordber and B. A. Fowler, "Mercury," in *Handbook on the Toxicology of Metals, Specific Metals II*, M. Nordberg, Ed., Academic Press, Amsterdam, Netherlands, 2015.

[64] O. Boucher, G. Muckle, J. L. Jacobson et al., "Domain-specific effects of prenatal exposure to PCBs, mercury, and lead on infant cognition: results from the environmental contaminants and child development study in nunavik," *Environmental Health Perspectives*, vol. 122, no. 3, pp. 310–316, 2014.

[65] A. P. Sanders, M. J. Mazzella, A. J. Malin et al., "Combined exposure to lead, cadmium, mercury, and arsenic and kidney health in adolescents age 12–19 in NHANES 2009–2014," *Environment International*, vol. 131, Article ID 104993, 2019.

[66] Y. Ma, Y. X. Zheng, X. Y. Dong, and X. T. Zou, "Effect of mercury chloride on oxidative stress and nuclear factor erythroid 2-related factor 2 signalling molecule in liver and kidney of laying hens," *Journal of Animal Physiology and Animal Nutrition*, vol. 102, no. 5, pp. 1199–1209, 2018.

[67] L. Y. Zheng, A. P. Sanders, J. M. Saland, R. O. Wright, and M. Arora, "Environmental exposures and pediatric kidney function and disease: a systematic review," *Environmental Research*, vol. 158, pp. 625–648, 2017.

[68] R. A. Bernhoft, "Mercury toxicity and treatment: a review of the literature," *Journal of Environmental and Public Health*, vol. 2012, Article ID 460508, 10 pages, 2012.

[69] J. J. Kim, Y. S. Kim, and V. Kumar, "Heavy metal toxicity: an update of chelating therapeutic strategies," *Journal of Trace Elements in Medicine & Biology*, vol. 54, pp. 226–231, 2019.

[70] Y. Cao, M. A. Skaug, O. Andersen, and J. Aaseth, "Chelation therapy in intoxications with mercury, lead and copper," *Journal of Trace Elements in Medicine & Biology*, vol. 31, pp. 188–192, 2015.

[71] M. Iranmanesh, S. J. A. Fatemi, M. R. Golbafan, and F. Dahooee Balooch, "Treatment of mercury vapor toxicity by combining deferasirox and deferiprone in rats," *Biometals*, vol. 26, no. 5, pp. 783–788, 2013.

[72] M. J. Kosnett, "The role of chelation in the treatment of arsenic and mercury poisoning," *Journal of Medical Toxicology*, vol. 9, no. 4, pp. 347–354, 2013.

[73] M. Rafati-Rahimzadeh, M. Rafati-Rahimzadeh, S. Kazemi, and A. A. Moghadamnia, "Current approaches of the

management of mercury poisoning: need of the hour," *Daru Journal of Pharmaceutical Sciences*, vol. 22, no. 1, pp. 46–10, 2014.

[74] G. Bjørklund, "Selenium as an antidote in the treatment of mercury intoxication," *Biometals*, vol. 28, no. 4, pp. 605–614, 2015.

[75] G. Bjørklund, J. Mutter, and J. Aaseth, "Metal chelators and neurotoxicity: lead, mercury, and arsenic," *Archives of Toxicology*, vol. 91, no. 12, pp. 3787–3797, 2017.

[76] L. Breeher, M. A. Mikulski, T. Czeczok, K. Leinenkugel, and L. J. Fuortes, "A cluster of lead poisoning among consumers of ayurvedic medicine," *International Journal of Occupational and Environmental Health*, vol. 21, no. 4, pp. 303–307, 2015.

[77] K. Gombeau, R. B. De Oliveira, S. L. F. Sarrazin, R. H. V. Mourão, and J. P. Bourdineaud, "Protective effects of *Plathymenia reticulata* and *Connarus favosus* aqueous extracts against cadmium-andmercury-induced toxicities," *Toxicological Research*, vol. 35, no. 1, pp. 25–35, 2019.

[78] V. A. Oliveira, N. de Souza da Costa, M. Mesquita et al., "Mercury toxicity in pregnant and lactating rats: zinc and N-acetylcysteine as alternative of prevention," *Environmental Science and Pollution Research*, vol. 27, no. 32, pp. 40563–40572, 2020.

[79] V. D. Subramaniam, S. V. Prasad, A. Banerjee et al., "Health hazards of nanoparticles: understanding the toxicity mechanism of nanosized ZnO in cosmetic products," *Drug and Chemical Toxicology*, vol. 42, no. 1, pp. 84–93, 2019.

[80] U. Ghosh, M. Nagalakshmi, R. Sadhukhan, and A. Girigoswami, "Biocapped nanoparticles are more bioactive to HeLa cells than its chemical counterpart," *Advanced Science, Engineering and Medicine*, vol. 5, no. 8, pp. 783–788, 2013.

[81] V. Unsal, "Natural phytotherapeutic antioxidants in the treatment of mercury intoxication-a review," *Advanced Pharmaceutical Bulletin*, vol. 8, no. 3, pp. 365–376, 2018.

[82] G. Guzzi, M. Grandi, C. Cattaneo et al., "Dental amalgam and mercury levels in autopsy tissues: food for thought," *The American Journal of Forensic Medicine and Pathology*, vol. 27, no. 1, pp. 42–45, 2006.

[83] B. A. Fowler, L. T. Friberg, G. F. Nordberg, and M. Nordberg, *Handbook on the Toxicology of Metals*, Elsevier, Amsterdam, Netherlands, 2007.

[84] J. L. Barnes, E. M. McDowell, J. S. McNeil, W. Flamenbaum, and B. F. Trump, "Studies on the pathophysiology of acute renal failure," *Virchows Archiv B Cell Pathology Including Molecular Pathology*, vol. 32, no. 1, pp. 201–232, 1980.

[85] Y. C. Janga, Y. Somanna, and H. Kimb, "Source, distribution, toxicity, and remediation of arsenic in the environment—a review," *International Journal of Applied Environmental Sciences*, vol. 11, no. 2, pp. 559–581, 2016.

[86] C. D. Rudolph, *Rudolph's Pediatrics*, Appleton & Lange, Norwalk, CT, 2003.

[87] L. L. Bergeson, "The proposed lead NAAQS: is consideration of cost in the clean air act's future?" *Environmental Quality Management*, vol. 18, no. 1, pp. 79–84, 2008.

[88] Ż. Ciosek, K. Kot, D. Kosik-Bogacka, N. Łanocha-Arendarczyk, and I. Rotter, "The effects of calcium, magnesium, phosphorus, fluoride, and lead on bone tissue," *Biomolecules*, vol. 11, no. 4, 2021.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 6369150, 15 pages
https://doi.org/10.1155/2022/6369150

Review Article

Pharmacology, Toxicology, and Rational Application of Cinnabar, Realgar, and Their Formulations

Huifang Guan,[1] **Yan Xu**,[2] **Chunyu Ma**,[1] **and Dexi Zhao**[3]

[1]*College of Traditional Chinese Medicine, Changchun University of Chinese Medicine, Changchun 130117, China*
[2]*College of Pediatrics, Henan University of Chinese Medicine, Zhengzhou, Henan 450000, China*
[3]*Department of Encephalopathy, The Affiliated Hospital of Changchun University of Chinese Medicine, No. 1478 Gong-Nong Road, Changchun, Jilin 130021, China*

Correspondence should be addressed to Dexi Zhao; dexizhao1006@163.com

Received 14 April 2022; Accepted 20 August 2022; Published 27 September 2022

Academic Editor: Saravanan Ramachandran

Ethnopharmacological Relevance. Mineral medicines are widely used traditional Chinese medicines with curative effects. These medicines are used for many refractory diseases. *Aim of the Review*. In this review, cinnabar (HgS) and realgar (As_2S_2) serve as examples of mineral medicines, and their pharmacology, therapeutic toxicity, use in traditional medicine mixtures, and research perspectives are discussed. *Materials and Methods*. A search was performed for the literature on cinnabar and realgar in PubMed, the Chinese Pharmacopeia, Google, and other sources. The search included studies using single herbs, traditional formulations, or novel dosage forms. *Results*. Cinnabar and cinnabar formulas exhibit good efficacy for sedation, sleep improvement, anxiety alleviation, and brain protection. However, previous studies on neurotransmitters have reached different conclusions, and detailed pharmacological mechanisms are lacking. Realgar and its formulas exert promising antitumor activity through regulation of cell cycle arrest, intrinsic and extrinsic apoptosis, induction of differentiation, autophagy, metabolic reprogramming, matrix metalloproteinase-9 (MMP-9) signaling, and reactive oxygen species (ROS) generation. In addition, realgar can be used to treat a variety of refractory diseases by regulating immunity and exerting antibacterial, antiviral, and other effects. However, the existing pharmacological research on the use of realgar for epidemic prevention is insufficient, and animal experiments and research at the cellular level are lacking. Inappropriate applications of cinnabar and realgar can cause toxicity, including neurotoxicity, liver toxicity, kidney toxicity, and genotoxicity. The toxicological mechanism is complex, and molecular-level research is limited. For clinical applications, theory and clinical experience must be combined to guide scientific and rational drug use and to achieve reduced toxicity and increased efficacy through the use of modern preparation methods or combined drugs. Notably, when cinnabar and realgar are used to treat targeted diseases, these agents have a bidirectional effect of "treatment" and "toxicity" on the central nervous system in pathological and normal states. The pharmacological and toxicological mechanisms need to be elucidated in greater detail in the future. Overall, systematic research is needed to provide a basis for better promotion of the rational use of cinnabar and realgar in the clinic. *Conclusion*. Mineral medicines are multicomponent, multiactivity, and multitargeted substances. The pharmacology and mechanisms of the toxicity and action of realgar and cinnabar are extremely complex. A number of Chinese medicinal preparations of realgar and cinnabar have demonstrated unique efficacy in the treatment of refractory diseases.

1. Introduction

Mineral medicines include raw minerals (cinnabar, calamine, natural copper, realgar, gypsum, etc.), processed products of raw mineral materials (calomelas, mirabilite, etc.), and animal bones or fossils of animal bones (bone or teeth fossils of large mammals) and are characteristic of traditional medicines used in China.

Throughout history, mineral medicines have played an important role in traditional Chinese medicine (TCM). TCM has a long application history with the accumulation of rich clinical experiences and is still widely used today. The

earliest record of the roles of mineral medicines is documented in The Classic of Mountains and Seas, which states that taking mineral medicines can "make people healthy and live longer." In addition, the modern 2020 edition of the Pharmacopoeia of the People's Republic of China (2020ChP) [1] affirms the wide range of clinical applications of mineral medicines in internal medicine, orthopedics, gynecology, and otorhinolaryngology. Cinnabar and realgar are two mineral medicines commonly used in the clinic with confirmed curative effects. However, their safety is of great public concern. Therefore, these medicines will be the focus of this minireview.

Cinnabar is described by the 2020ChP [1] as follows: "This product is the sulfide mineral cinnabar of the cinnabar family, which mainly contains Mercury sulfide (≥96%), which has the effects of clearing away the heart-fire and sedative, relieving uneasiness of body and mind, improving eyesight, and detoxifying." Cinnabar is a critical sedative component of numerous Chinese prescriptions [2], such as Zhusha Anshen Pill (ASAS) and Baizi Yangxin Pill (BZYX). In addition, modern research has found that cinnabar has antianxiety, tranquillizing, antioxidative stress, and anti-brain-damage effects, and the mineral is used in compound preparations for the treatment of insomnia, anxiety disorders, brain trauma, stroke, and neuroinflammation [3].

Realgar is described by the 2020ChP [1] as a sulfide mineral of the realgar family that mainly contains arsenic disulfide (As_2S_2) and has the functions of "detoxifying and killing insects, drying dampness and removing phlegm, and treating malaria." Modern research has further revealed that realgar can be combined with other drugs to treat a variety of blood system diseases [4], including acute promyelocytic leukemia (APL) [5], myelodysplastic syndrome (MDS) [6–9], and lymphoma. Realgar has also shown significant pharmacological effects against primary or metastatic cancers, such as breast cancer (cells) [4, 10, 11], cervical cancer [12], lung cancer [13], osteosarcoma [14], gastric cancer [15], oral cancer (cells) [16], and liver cancer [17], in nonclinical studies. In addition, realgar exhibits good immunoregulatory, antiviral, and antiepidemic effects, which may provide new ideas for the treatment of viral infectious diseases.

In recent years, the occurrence of phytotoxicity incidents caused by the improper use of mineral drugs has increased the concern regarding their toxicity. Cinnabar and realgar have become popular discussion topics because their main components (Mercury and arsenic) are known toxic metals/metalloids. Some have even questioned the necessity of heavy metal minerals, such as cinnabar and realgar, in medicinal formulas.

Several studies have found that different forms of Mercury can be absorbed through the gastrointestinal tract, respiratory tract, and skin and that excess levels of Mercury and its compounds have acute or chronic toxic effects on the human body [18–23]. Arsenic toxicity is associated with liver tumors, diabetes, and cardiovascular, neurological, and other diseases [24–26]. However, some in vivo experiments in mice have indicated that cinnabar does not significantly affect transporter genes in the liver and kidneys [27, 28]. This contradiction underscores the need for further research on the pharmacology and toxicology of cinnabar and realgar.

When discussing Mercury toxicity and arsenic toxicity, it is necessary to distinguish the chemical forms. In traditional Chinese medicine, Mercury and arsenic are used orally in sulfide forms [29]. The chemical forms of cinnabar and realgar are significant determinants of their activity and toxicity. Both cinnabar and realgar are far less toxic than the well-known Mercury [21, 30–32] and arsenic [33]. In addition, studies have demonstrated that cinnabar (α-HgS) differs from environmental Mercury ($HgCl_2$, MeHg) based on its bioavailability, intestinal transport levels, distribution, metabolism, elimination, and toxicity [29, 32]. Similarly, realgar (As_2S_2, As_4S_4) differs from sodium arsenate (As^{5+}) and sodium arsenite (As^{3+}) in structure, disposition, efficacy, and toxicity [29].

Thousands of years of clinical practice have suggested the effectiveness of cinnabar and realgar. However, the exact pharmacodynamic and toxic mechanisms of these minerals and how to optimize their clinical applications remain unclear; an in-depth research with modern research techniques is needed. A literature search of the use of Chinese materia medica in the treatment of diseases reveals that cinnabar and realgar are seldom used alone. These agents are commonly used in multiherb/metal mixtures. Therefore, we recommend that analyzing the mechanisms of clinically effective formulations at the molecular, cellular, and organismal levels represents a potentially effective method for assessing the value of traditional medicines.

In this article, we briefly summarize the main pharmacological and toxicological characteristics and usage specifications of the mineral medicines cinnabar, realgar, and their commonly used preparations, aiming to provide a theoretical basis for the rational and safe clinical use of cinnabar and realgar.

2. Cinnabar

For thousands of years, cinnabar has been used to treat different diseases [2]. According to the 2020ChP [1], approximately 10%–30% of Chinese compound prescriptions contain cinnabar. Representative prescriptions include ASAS and Baizi Yangxin Pill. According to traditional Chinese medicine, cinnabar taken orally can clear away heart fire, exert sedative effects, relieve uneasiness in the body and mind, improve eyesight, and detoxify. Modern research has shown that cinnabar can improve sleep, combat anxiety and oxidative stress, and protect the brain from damage.

However, the use of cinnabar in clinical practice has been controversial because it contains the heavy metal Mercury. Epidemiological investigations and animal experiments have shown that excessive intake of cinnabar can exert toxic effects on the kidneys, liver, and nervous system. Therefore, the pharmacology and toxicology of cinnabar and traditional medicines containing cinnabar have attracted attention.

2.1. Pharmacological Research on Cinnabar Alone

2.1.1. Sedative and Anxiolytic Mechanisms. Cinnabar has long been used in combination with other Chinese materia medica as a sedative for more than 2000 years [2]. The

sedative effect of cinnabar has been verified in animal experiments. For example, studies have shown that, in mice administered low-dose cinnabar (10 mg/kg/d) for 11 weeks, motor activity is decreased, and pentobarbital-induced sleeping time is prolonged, suggesting that cinnabar has sedative effects [34]. Wang et al. confirmed the anxiolytic effect of cinnabar on anxiety-like behaviors in mice using the elevated plus maze test; this neuropharmacological effect may have been similar to the sedative and soothing effects of cinnabar. The results suggest that cinnabar exerts anxiolytic effects when administered chronically at effective doses and is associated with reduced brain serotonin (5-HT) levels [35].

2.1.2. Antioxidative Stress and Brain Injury Protection Mechanisms. Oxidative stress plays a key role in neuronal death and underlies neurodegenerative diseases. Thus, antioxidants can play important roles in treating several neurodegenerative diseases [36]. Reactive oxygen species (ROS) are markers of oxidative stress that can indicate the state of redox balance in the body.

One study confirmed that cinnabar can reduce the disappearance of antioxidant enzymes and the overproduction of ROS through regulation of the 5-HT metabolism pathway under hypoxic conditions at the cellular level and in zebrafish in vivo, ultimately alleviating hypoxia-induced oxidative stress and significantly improving the survival of neuronal cells under high-ROS conditions [37]. This finding highlights one of the potential mechanisms by which cinnabar exerts neuroprotective effects.

In the endoplasmic reticulum (ER) stress response, protein kinase RNA-like ER kinase (PERK) induces the activation of C/EBP homologous protein (CHOP). Activated CHOP then induces apoptosis via the apoptosis receptor BCL2 family proteins and the death receptor caspase family proteins of the downstream mitochondria-dependent pathway [38]. Low cinnabar concentrations induce antioxidative and antiapoptotic effects on cells and inhibit intracellular stress responses. The possible mechanism involves blockade of ER stress-induced apoptosis via downregulation of PERK expression and indirect downregulation of CHOP activation [39]. In an emerging microbiome study, cinnabar reduced neuronal stress and inflammation through the gut–brain axis by altering the microbiome structure via reductions in Verrucomicrobiaceae and increases in Enterobacteriaceae, suggesting that HgS-containing traditional medicines can mechanistically target gut microbiota to exert their therapeutic effects [40]. The pharmacological mechanism of cinnabar is presented in Figure 1.

2.1.3. Research on Cinnabar Formulas. Given its good curative effect, cinnabar has been widely used as a sedative ingredient in TCM prescriptions. However, the mechanisms of cinnabar and its various formulations have been the subject of few studies. Some pharmacological studies on the use of cinnabar and its formulations for the treatment of insomnia have been reported in the Chinese literature but are not available in PubMed. One study found that the formula ASAS, which includes cinnabar as the main therapeutic ingredient, exhibits good sedative and antianxiety effects. A study on ASAS in rats with conditioned fear showed that this pill can antagonize conditioned fear; the antagonistic effect is mainly reflected in the fading stage of fear memory and may be involved in regulating the content of monoamine neurotransmitters and the expression of c-Fos protein in the basolateral amygdala. This mechanism increases the efficiency of fear extinction and improves sleep in rats [41]. The mechanism is related to hippocampal neuron protection, hippocampal synaptic structure regulation, and functional plasticity [42]. In the sleep phase, moderate and high doses of a decoction of ASAS obviously decrease the duration of wakefulness and increase the total sleep time. A moderate dose of the decoction obviously increases the duration of slow-wavestage-1 sleep, whereas high doses of the decoction obviously increase the duration of slow-wavestage-2 sleep. Although low doses do not decrease the duration of wake, they can increase the duration of slow-wavestage-2 sleep [43]. The mechanism may be related to inhibition of the production of 5-HT and norepinephrine (NE) monoamine neurotransmitters in the ventrolateral preoptic area [44] that increase the content of γ-aminobutyric acid in the brain [45].

2.2. Toxicological Research on Cinnabar. Cinnabar is a natural medicinal substance containing metallic Mercury [46]. Regarding toxicology, cinnabar has known toxic effects on the kidneys, liver, and nervous system. Wei et al. [47] applied a nuclear magnetic resonance metabolomics method and found that cinnabar altered biochemical indexes with time-anddose-dependent effects. Proteomics has shown that therapeutic and toxic doses of cinnabar affect different pathways and potential targets in the mouse cerebral cortex [48]. In addition, a study found that the cerebellum is more sensitive to cinnabar treatment than the cerebrum [19]. Some studies have revealed that exposure to a high dose (1.0 g/kg/day for 7 or 14 consecutive days) of cinnabar or HgS causes ototoxicity [20] and neurotoxicity [21, 49], whereas 0.01 g/(kg day) causes no toxic effects [21, 49].

Cinnabar toxicity may occur because high dose or long-term use of Chinese compound preparations containing cinnabar leads to the accumulation of Mercury in the kidneys, resulting in kidney damage, such as kidney inflammation and mild fibrosis [22]. Cinnabar may also cause proximal tubular damage, which may be related to the activation of the renal tubular apoptosis pathway and the expression of organic anion transporters (OATs) and the expression of tubular basal transporters organic anion transporter OAT1 and OAT3 [23, 50]. One study found that the gut microbiota is a potential target for the dual effects of cinnabar and that oligopeptide transporter 1 may represent an important transporter of cinnabar into the intestinal epithelium [32]. Under the influence of human intestinal bacteria, cinnabar is transformed into nontoxic products (mercuric polysulfides) rather than methylmercury [51].

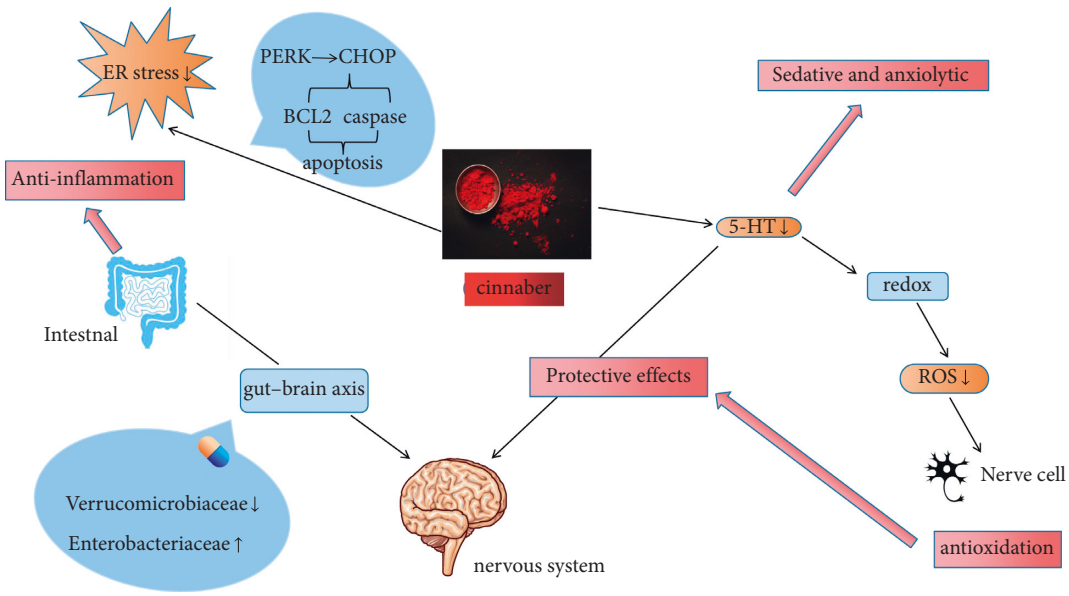


Figure 1: Pharmacological mechanism of cinnabar.

Although long-term use of cinnabar can cause a series of toxic side effects, scholars have found that cinnabar is indeed much less toxic than other mercury-containing compounds, such as $HgCl_2$ [30, 32] and MeHg [21, 31].

This reduced toxicity may be related to the down-regulation of oligopeptide transporter 1 mRNA and protein expression by cinnabar [32], which affects the intestinal absorption and transport of cinnabar; its dissolution, absorption, distribution, and accumulation in vivo; and the molecular configuration of Mercury compounds, particle size, and biological activity of the gastrointestinal tract [52–54].

The toxic effects of cinnabar have varied greatly in different experiments. In addition to disparities in the administered dose, different methods of cinnabar processing may also have contributed to the different toxicities. Ancient traditional Chinese medicine practitioners stressed that the washing (called Shui-Fei) method during cinnabar processing was extremely sensible, as it avoided high temperatures and reduced the levels of soluble toxic elements. However, different researchers have used different medicinal materials in their toxicological studies on cinnabar, and only a few studies have emphasized the use of washing methods, which may be another important reason explaining the inconsistently experimental results. Given that the detoxification methods of cinnabar and realgar are similar, the final statement is unified.

3. Realgar

Realgar contains >90% arsenic sulfide (As_2S_2) and is widely used externally and internally in many traditional medicine recipes in China. The 2020ChP [1] contains more than 30 types of formulas containing realgar with representative formulas including the Niuhuang Jiedu Pill, Niuhuang Qingxin Pill, and Angong Niuhuang Pill. The functions of realgar are mostly "detoxifying and killing insects, drying dampness and removing phlegm, and treating malaria" [1]. Realgar is used for various diseases, such as "carbuncles, swelling, sores, snake bites, ascariasis, epilepsy, and malaria" [1].

Recent studies have found that realgar exhibits significant anticancer activity. The possible antitumor mechanism may be closely related to the regulation of cell cycle arrest, intrinsic and extrinsic apoptosis, induction of differentiation, autophagy, metabolic reprogramming, matrix metalloproteinase-9 (MMP-9) signaling, and ROS generation [4, 10].

However, 90% of realgar is As_2S_2, which exhibits toxic effects characteristic of heavy metals; thus, the clinical application of realgar is controversial. Epidemiological investigations and animal experiments have shown that long-term arsenic exposure can lead to language, cognitive, behavioral, and hearing and motor impairments, as well as mental retardation and neurological deficits in severe cases. Even when arsenic exposure ceases, central nervous system function does not quickly return to normal [55]. Therefore, the pharmacology and toxicology of realgar and traditional medicines containing realgar have attracted extensive attention.

3.1. Pharmacological Research on Realgar

3.1.1. Pharmacological Research on Realgar Alone

(1) Antitumor Mechanism. Induction of tumor cell apoptosis is the main mechanism of action of realgar in the treatment of tumors, and these effects can be achieved through a variety of ways. Studies have demonstrated a link between oxidative stress and tumor cell apoptosis [56]. To improve the activity of realgar, some researchers have used intrinsic biotransformation pathways in microorganisms to obtain

a realgar transformation solution (RTS). RTS activates p53-mediated cell cycle arrest and apoptotic pathways by inhibiting the cellular antioxidant defense system and massive accumulation of ROS in tumor cells and by inducing apoptosis and interrupting G2/M progression in HepG2 cells to inhibit the growth of tumor cells and promote apoptosis [17]. Similar mechanistic studies have shown that the expression of proapoptotic proteins, including caspase-7 and caspase-8, is activated under the action of As_2S_2 to trigger tumor cell apoptosis [10]. The results of another study suggest that As_2S_2 inhibits cell viability and induces apoptosis and cell cycle arrest in both MCF-7 and MDA-MB-231 cell lines by regulating the expression of key proteins involved in related pathways [4].

Cell cycle-regulated gene mutations play important roles in tumorigenesis. Cell cycle arrest provides cells with additional time to repair damage, thereby reducing the occurrence of mutations and tumor development. In a molecular study on the use of As_2S_2 in the treatment of APL, As_2S_2 was found to induce the accumulation of NB4-R1 cells in S and G(2)/M phases [57]. This effect may be related to the regulation of the expression of related proteins, including cyclin B1 and cell division cycle protein 2 [10].

Induction of differentiation is a common strategy in tumor therapy. Realgar induces multilineage differentiation in the human promyelocytic leukemia cell line HL-60, thereby exerting anticancer effects. Myeloid differentiation of HL-60 cells is induced via the p38 mitogen-activated protein kinase-related signaling pathway [58], and monocytic differentiation of HL-60 cells is induced via serine/threonine protein phosphatase-related pathways [59]. Realgar simultaneously induces apoptosis and differentiation in both the all-trans retinoic acid- (ATRA-) sensitive NB4 and ATRA-resistant MR2 cell lines [60]. The following specific mechanisms may be involved: (1) binding of realgar to the pML/RARα fusion protein, making it ineffective; (2) enhancement of the level of cellular ubiquitin by realgar; (3) promotion of pML/RARα fusion protein ubiquitination; (4) formation of a covalent enzyme system; and (5) entry into the protease system for degradation into short peptides and loss of activity [5].

Autophagy is an important cell biological process induced by multiple forms of cellular stress, including nutrient or growth factor deprivation, hypoxia, and ROS [61]. Excessive levels of autophagy damage body tissues and cells and disrupt homeostasis [62]. Realgar can induce autophagy in endometrial cancer JEC cells as one of its anticancer mechanisms [63]. In addition, realgar preparations can induce autophagy via upregulation of LC3 and p62/SQSTM1 [64] and inhibit the Akt/mTOR signaling pathway [14].

Under hypoxic conditions, hypoxia-inducible factor (HIF-1) promotes the metabolic reprogramming of tumor cells and induces acidification of the tumor extracellular environment, which helps tumor cells obtain energy and survive [65]. However, nanorealgar inhibits tumor growth in vitro and in vivo by repressing metabolic reprogramming. This inhibitory effect is potentially related to the downregulation of HIF-1α expression via the PI3K/Akt/mTOR pathway [13].

MMP-9 is one of the most complex matrix metalloproteinases. In tumor cells, MMP-9 contributes to the remodeling of extracellular matrix proteins, providing a microenvironment for tumorigenesis. MMP-9 degrades basal type IV collagen near tumor cells and then invades other normal tissues, thereby inducing cancer invasion and metastasis [66]. However, As_2S_2 treatment inhibits MMP-9 protein expression [10].

Studies have shown that As_2S_2 inhibits the proliferation of colon cancer cells by regulating the nuclear factor of activated T cells (NFAT) pathway [15, 67]. Its inhibitory effect is related to a signaling pathway related to vascular endothelial growth factor 2 [68].

These results suggest that realgar preparations have broad-spectrum scavenging effects in tumor cells. In cells and tissues, regulation is a complex process involving multiple signaling pathways, and the pathways in which realgar is involved require further study.

(2) Immunoregulatory and Antibacterial Mechanisms. Severe systemic lupus erythematosus (SSLE) refers to a manifestation of systemic lupus erythematosus (SLE) involving important organs. Patients with SSLE often experience multiple complications simultaneously, treatment difficulties, and worse prognoses [69]. Studies have shown that realgar can be used to treat severe forms of SLE, such as lupus nephritis, and that the mechanism may involve downregulation of the expression of phosphorylated signal transducer and activator of transcription 1 [70].

Realgar promotes apoptosis of B cells from MRL/lpr mice by increasing Ca^{2+} concentrations, reducing surface molecule activation, stimulating surface factor expression, and restraining its activation to reduce the production of antibodies, reduce MRL/lpr mouse autoimmune injury, and slow down the SLE course [71]. In addition, in *Caenorhabditis elegans*, realgar can alleviate the infection of wild-type N2, *glp*-4 mutant, and *daf*-2 mutant nematode strains by inducing both immune and protective responses and significantly increasing antibacterial effector levels, which leads to pathogen elimination [72, 73].

(3) Prevention and Treatment of Epidemics. Epidemic diseases are highly contagious and dangerous. Historically, realgar has played an important role in the prevention and treatment of epidemics in China in past dynasties. However, pharmacological research on realgar use for epidemic prevention and treatment is scarce. According to a recent literature search, realgar was the drug most frequently used by physicians for epidemic prevention in past dynasties [74]. Wearing colorful sachets containing realgar, drinking realgar wine, and sprinkling realgar on walls and doors during the Dragon Boat Festival represent traditional public uses. Realgar works primarily by killing pathogens (disinfecting) and expelling pathogens (detoxifying) [75].

External use through various methods (i.e., burning, externally coating, wearing, stuffing, sneezing, and tears) can block the transmission of the epidemic pathogen. Realgar can also be taken internally in the form of pills, medicinal liquors, etc. and can be combined with cinnabar for

epidemic prevention. Ruan [76] and others believed that coronavirus disease 2019 (COVID-19) was caused by a "cold-damp pestilential pathogen" invading the human body according to climatic, seasonal, and regional characteristics. Based on syndrome differentiation, realgar and other pungent and warm (Xin Wen) products were chosen to relieve the cold-damp poison. Similarly, Changchun University of Traditional Chinese Medicine also provided guidance that the new strains of coronavirus, mainly Omicron, that broke out in China in 2022 were caused by a cold-damp pestilential pathogen and suggested that pungent and warm (Xin Wen) products should be used. Compared with the epidemic prevention and treatment methods of modern medicine, those of traditional Chinese medicine have broad-spectrum immune effects and strong universality, are forward-looking, and achieve the purpose of disease prevention. Research on the prevention and treatment of diseases with realgar may provide new ideas for the use of TCM for the prevention and treatment of modern epidemics. However, the antiepidemic mechanism of action of realgar requires further study.

(4) Antiviral Mechanism. In an experiment using acyclovir as a positive control, nanorealgar showed good anti-herpes simplex virus type II activity when administered in 3 modes (pretreatment, treatment, and direct inactivation) [77]. The antiviral mechanism may involve destruction of the enzyme system required for virus proliferation by the realgar nanoparticles, so that the virus cannot proliferate in living cells. Realgar nanoparticles may also directly kill the virus by directly destroying the virus envelope protein, thereby reducing virus viability [77]. The pharmacological mechanism of realgar is presented in Figure 2.

3.1.2. Research on Realgar Formulas

(1) Antitumor Mechanisms. Since the 1970s, researchers in the field of blood diseases in China have successively applied realgar-*Indigo naturalis* formula (RIF) as the active ingredient in the clinical treatment of APL, achieving definite curative effects. RIF, a typical representative of realgar anticancer preparations, has now been approved for marketing in China.

A molecular-level study indicated that realgar is the principal component of the formula, whereas tanshinone IIA and indirubin serve as adjuvant ingredients [78]. Clinical trials have confirmed that oral arsenic (RIF) provides an outcome similar to that of intravenous arsenic trioxide (ATO) [79, 80]. More detailed clinical trials have shown that RIF is also safe and effective in newly diagnosed pediatric APL patients [81]. Furthermore, compared with ATO plus all-trans retinoic acid (ATRA), simultaneous use of oral RIF plus ATRA greatly reduces the clinical expenses [82, 83] and hospital stays [84–87] of APL patients during induction and remission therapy. According to long-termfollow-up results, RIF provides excellent long-term survival advantages [88]. The possible mechanisms of action include [78] (1) synergistic effects on human APL cell differentiation in vitro, (2) enhanced ubiquitination and degradation of the PML-RARα oncoprotein, (3) relief of transcriptional suppression, and (4) G1/G0 arrest of APL cells with effects on key regulators of cell cycle progression. The main component realgar cooperates with other components in RIF to increase the absorption rate by inducing upregulation of the transmembrane protein aquaglyceroporin 9. Further studies have shown that RIF can significantly inhibit the growth of APL NB4 cells [89] and the proliferation and viability of the K562 cell line [90].

Clinical studies have shown that the Chinese medicine Qinghuang Powder (QHP) (daily dose of 0.1 g of realgar), the main component of which is realgar, is effective and safe for the treatment of patients with MDS and that reasonable adjustment of the daily dose of realgar can improve the efficacy without increasing the clinical toxicity [8, 9]. In vitro studies have shown that realgar, as the main drug contained in QHP, can induce cell differentiation in AML cells that progress from MDS, which might explain one of the mechanisms of QHP in the treatment of MDS [91].

Different DNA methylation subtypes may be present in MDS-metachromatic leukodystrophy (MLD) patients. A clinical study found that Chinese herbal medicine containing realgar has good effects in the treatment of MDS-MLD patients with the hypomethylation subtype [7]. The therapeutic mechanism may involve an increase in DNA methyltransferase expression in MDS [6]. After genotyping 43 MDS patients via ultradeep targeted sequencing, Zhao et al. [92] found that QHP was effective and safe, especially in those with genetic mutations in SF3B1, DNMT3A, U2AF1, and/or ASXL1. Another study revealed that As_2S_2 can reduce BCR-ABL protein levels in chronic myelogenous leukemia cells without affecting its transcription level. The mechanism of action involves binding of As_2S_2 to the RING finger domain of CBL (a RING-type E3 ligase) to inhibit its self-ubiquitination/degradation, thereby ubiquitinating the BCR-ABL protein [93].

These findings suggest that traditional Chinese medicine products containing realgar represent potential drugs with multiple activities. The active ingredients of realgar can be combined with some antitumor drugs to synergistically enhance the inhibition of tumor cells and reverse antitumor drug resistance [8], thus enhancing therapeutic efficacy [94].

(2) Antiviral and Anti-Inflammatory Mechanisms. Modern physicians have also applied realgar to various conditions. Professor Liangchun, a master of traditional Chinese medicine, used the traditional Chinese medicine formula Duo-Tan-Ding-Jing-San created with realgar to treat viral infectious diseases such as Japanese encephalitis, pneumonia, and meningitis and achieved good clinical results [95].

The realgar-containing Chinese medicine formula Liu Shen Wan (LSW) can significantly inhibit the influenza virus at different stages of viral replication in vitro. The antiviral effect is attributable to downregulation of the expression of inflammatory cytokines induced by the influenza virus via regulation of the activity of the TLR4/NF-κB signaling pathway [96]. Further research has shown that LSW

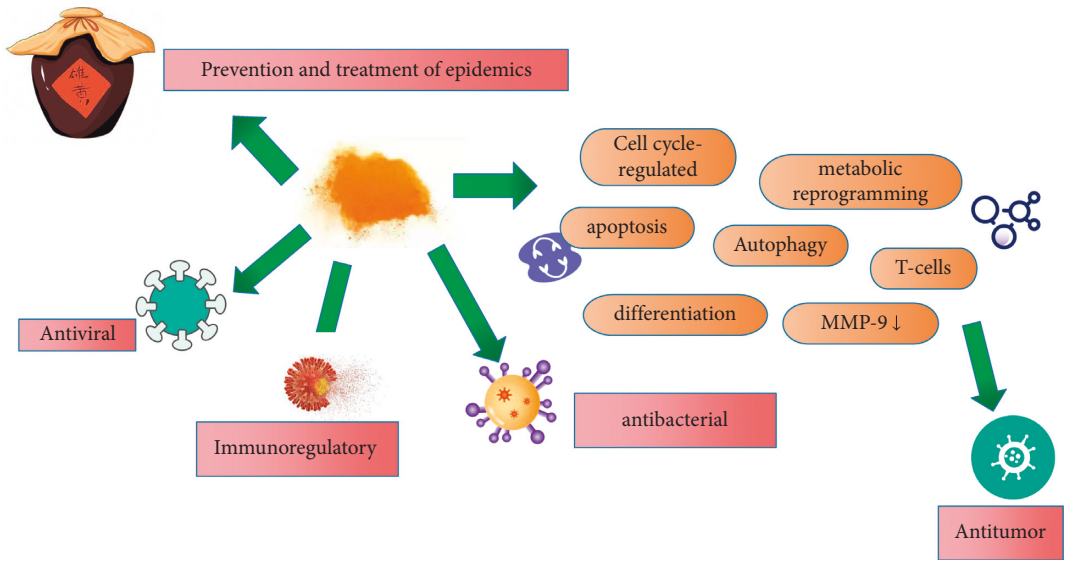


Figure 2: Pharmacological mechanism of realgar.

significantly ameliorates lung injury caused by viral and secondary bacterial infections [97].

3.2. Toxicological Research on Realgar. Studies have shown that the disturbance of the intestinal flora and the severity of intestinal inflammation in realgar-treated mice are related to the realgar dose [98]. Therefore, in clinical applications, realgar should be used at a low dose in combination with other drugs to reduce intestinal inflammation [98].

One study performed untargeted lipidomics and other analyses on realgar-exposed mice to identify markers of the toxicity of realgar exposure to the nervous system [99]. The study found that the arsenic contained in realgar passed through the blood-brain barrier and accumulated in the brain, causing abnormal changes in the cerebral cortex [99]. In addition, increased oxidative damage and lipid dysfunction are also responsible for the neurotoxicity of realgar [99]. The specific mechanism involves excessive activation of Nrf2 regulated by the upstream signaling molecules ERK1/2 and p38MAPK [100].

Metabolomics studies have revealed that oral administration of realgar disrupts endogenous metabolites and related metabolic pathways in mice [101]. Follow-up kidney proteomic studies have revealed that apoptosis and oxidative stress might be related to realgar-induced nephrotoxicity in mice [102].

Cytochrome P450 enzymes are extremely important enzyme transport systems in the body. The main function of these enzymes is to metabolize exogenous substances (such as drugs) and endogenous substances. However, some studies have shown that higher doses of realgar can inhibit the enzymatic activity of cytochrome P450 enzymes, which may be related to the accumulation of arsenic from realgar [103]. Realgar exposure in mice activates innate immune-mediated inflammatory responses and disrupts the homeostasis of bile acids in the liver, plasma, and urine, causing immune-inflammatory infiltration in the liver [104]. A cross-sectional study of 1556 adults from the area surrounding a realgar plant showed that chronic arsenic exposure impairs cognitive function in adults and that this impairment is positively dose-related [105].

Although the toxicity of realgar nanoparticles is lower than that of realgar, studies have shown that realgar nanoparticles can induce free fatty acid and triglyceride accumulation, resulting in hepatotoxicity, and the metabolic markers of nanoparticle-treated subjects are different from those of traditional realgar-treated subjects [106].

Long-term or high-dose consumption of realgar can lead to the accumulation of heavy metals in the body, causing damage to the liver, blood, and nervous system. However, some clinical studies have found that higher concentrations of As_2S_2 in peripheral blood can enhance the clinical efficacy of realgar in the treatment of MDS-MLD patients [107]. Therefore, it is hypothesized that the pharmacological activity and toxicity of realgar may differ according to the activity of different cells. Realgar has a protective effect on nerve cells under pathological conditions but is harmful to normal nerve cells.

4. Research on Formulas Containing Both Cinnabar and Realgar

Compared with single mineral medicines, traditional Chinese medicine formulas containing multiple mineral medicines are more widely used in clinical practice. The mechanisms of action of mineral medicines or mineral-containing medicines in the treatment of diseases are complex but are related to the multicomponent, multitarget, and multichannel treatment characteristics of the medicines; these features also represent the characteristics and advantages of TCM for the treatment of diseases in general. Cinnabar and realgar are often used together in traditional Chinese medicine compounds.

4.1. Basic Medical Research. An-Gong-Niu-Huang Wan (AGNH) is a famous traditional Chinese medicine used to treat cerebral emergencies, such as traumatic brain injury,

hemorrhage, brain infection, ischemia, and stroke, with a history of use of more than 3,000 years [1]. Modern research has suggested that AGNH has neuroprotective effects by reducing infarction volume, preserving blood-brain barrier integrity, and improving neurological functions against cerebral ischemia-reperfusion injury [108].

Based on skepticism about mineral medicines, some scholars have tried to remove cinnabar and realgar from AGNH. Interestingly, removing realgar and/or cinnabar from AGNH abolishes the neuroprotective effects [108]. In one study, histopathological analysis of mice that received AGNH orally showed that AGNH caused mild or no injuries to the liver and kidneys [109].

Wu Jutong, a warm-disease scientist of the Qing Dynasty, called Angong Niuhuang Pill the first of the "three classic antipyretic preparations" and believed that it had good heat-clearing, detoxifying, and sedating effects. Modern research suggests that cinnabar and realgar in the Angong Niuhuang Pill may play the main roles in "clearing heat and detoxifying" by helping inhibit the excessive release of inflammatory mediators [110]. This formula has significant effects against acute cerebrovascular disease, the Japanese epidemics of encephalitis and cerebrospinal meningitis, acute jaundice and primary hepatitis, renal failure, severe pneumonia, and infantile measles [110]. Tang et al.'s study on a fever rat model showed that realgar overinduced stress proteins (HSP70, HO-1) to improve positive stress levels in the body and inhibited overrelease of some inflammatory mediators (IL-1beta) to reduce inflammatory reactions under pathological conditions and inhibit the storm of inflammatory factors, thereby helping remove internal toxins and prevent damage to important organs [111].

Some scholars [112] have conducted similar research on Hua-Feng-Dan (HFD). Traditional HFD (containing 10% cinnabar and 10% realgar) improves behavioral dysfunction and attenuates microglial activation, rescuing the loss of dopamine (DA) neurons. However, a HFD lacking cinnabar and realgar is ineffective. This phenomenon reveals that cinnabar and realgar are active ingredients in HFD that exert useful effects in a neurodegenerative model and on the gut microbiota.

4.2. Clinical Applications. AGNH and HFD are still widely used clinically in their traditional and new forms. These agents can be used alone or in combination with other drugs for synergistic treatment.

Some meta-analyses of clinical studies on AGNH have revealed that AGNH has been widely used for acute cerebral infarction and intracerebral hemorrhage [113], traumatic brain damage [114], persistent vegetative state [115], and other severe cranial brain diseases. Moreover, studies have found that it can effectively promote postoperative recovery and exert respiratory care effects in chronic obstructive pulmonary disease patients after cardiac surgery [116]. However, there are relatively few clinical reports on the therapeutic effects of HFD, and all of the existing reports were published in China. HFD is often used in combination with other drugs in clinical applications and has good therapeutic effects on epilepsy [117], stroke [118], peripheral facial paralysis [119], and idiopathic facial paralysis [120].

With the introduction of modern biopharmaceutical technologies, innovations in the dosage forms of traditional Chinese medicine, such as capsules or liquid forms, have been reported. Based on these innovations, the pharmacological effects of the medicines are relatively clear, and these formulations have achieved outstanding curative effects that have been widely recognized in the medical field in recent years.

Traditional Chinese medicine injections are unique dosage forms in mainland China [121] that are administered by intramuscular injection or intravenous infusion. Qingkailing (QKL) injection is based upon the traditional Chinese medicine formulation AGNH, which is widely used in the treatment of many diseases given its good clinical efficacy. The main target diseases include acute cerebrovascular diseases and respiratory system infections, such as upper respiratory tract infection [122], pneumonia caused by respiratory syncytial virus [123], and acute stroke [124]. In the 2003 SARS incident, QKL was used as the basic drug for the treatment of atypical pneumonia with the integration of traditional Chinese and Western medicines and was combined with other drugs for comprehensive treatment [125]. During the coronavirus disease (COVID)-19 pandemic, Beijing also included QKL capsules in its clinical treatment plan [126].

In a previous study, QKL (or combined treatment) had a better effect on most outcome indicators compared with control, but the occurrence of adverse reactions cannot be ignored [127]. The current evidence, while being weak, indicates that QKL carries a low risk of adverse drug reactions and adverse events; however, some of the adverse events may be ascribed to improper use of the drug [128]. In the future, macromolecular substances should be removed as thoroughly as possible in production [121]. Serious care should be taken when QKL is administered to children, and QKL should not be combined with cephalosporin [121]. In addition, studies have shown that the delivery of Qingkailing by nebulization shows an equivalent or better curative effect with fewer side effects than injection of QKL in the treatment of pneumonia, respiratory tract infection, and tracheitis [129].

Xingnaojing injection (XNJ) is the only Chinese herbal injection approved for emergency treatment of stroke in China [130]. In the treatment of ischemic stroke, XNJ results in a significantly better overall response rate and better improvement of clinical symptoms than conventional treatment alone [130, 131]. In addition, XNJ can be used alone or as an adjuvant therapy to reduce brain damage and improve nerve health for the treatment of acute cerebral hemorrhage [132] and traumatic craniocerebral injury [133, 134]. During the COVID-19 epidemic, the National Health Commission of the People's Republic of China listed XNJ as a recommended traditional Chinese medicine injection in the "Guidelines for the Diagnosis and Management of COVID-19 (8th Edition)" [135]. For patients with

severe symptoms, the Angong Niuhuang Pill is also recommended [135].

The above findings clearly show that the application of new technologies and new processes in development and production processes has enabled innovations and improvements of TCM preparations. This new technology has not only driven the development of traditional Chinese medicine, but also (and more importantly) promoted the internationalization of traditional Chinese medicine. In the Chinese Pharmacopeia (2020) [1], 24 recipes contain both cinnabar and realgar, such as Jufang Zhibao Dan for the treatment of febrile diseases and Qingyu Piwen Dan for heat stroke. Elucidation of the pharmacology, safety, and clinical application of AGNH and HFD and their novel formulations is critical to help assess the benefits and risks of these metal-containing traditional medicines.

5. Safe Application of Cinnabar and Realgar

Chinese materia medica containing cinnabar and realgar are generally relatively nontoxic at therapeutic doses under the pharmacopeia guidelines [1]. Important factors affecting the toxicity of cinnabar and realgar include the processing method, dose, compatibility, and attenuation.

5.1. Processing Method. In TCM, cinnabar and realgar are subjected to grinding and washing (called Shui-Fei) at least 3-4 times, which is crucial for the safe use of cinnabar and realgar in medicine.

Ancient physicians emphasized several important purposes of concocting mineral medicines using the grinding and washing method: to remove impurities, to reduce soluble elements as much as possible, to control the temperature to avoid the formation of new toxic and harmful substances, and to make the drug powder extremely fine and improve its bioavailability. Modern research illustrates that, as a TCM processing method, water grinding reduces toxicity [136, 137].

5.2. Appropriate Doses. The 1995 edition of the Chinese Pharmacopoeia reduced the daily dose of cinnabar from 0.3 to 1.5 g to 0.1~0.5 g, and this standard is still used today [1]. In the 2000–2020 editions of the Pharmacopoeia of the People's Republic of China [1], the dose of realgar was 0.05~0.1 g. Studies have shown that the toxicity of cinnabar [19] and realgar [138] is concentration- and time-dependent. Therefore, mineral medicines should not be used in large quantities, nor should they be used in small amounts for a long time. It is also necessary to pay attention to the administration period and special populations. Use should be limited to special populations, such as children, pregnant women, and those with liver and kidney insufficiency.

5.3. Toxicity-Attenuating Compatibility. In TCM, minerals are not applied alone but mixed with herbs and/or animal products. Chemical reactions may occur due to the mixing of agents, and it is assumed that the various components promote each other to achieve the desired therapeutic effect and reduce toxicity [29]. Studies have confirmed that the toxicity of cinnabar is low and that the herbal medicines in cinnabar formulas can attenuate the damage caused by cinnabar to body systems [29, 139, 140], which may be related to the absorption, distribution, and excretion of Mercury [28, 138]. Similarly, when realgar is used in a formula, the other herbal medicines in the formula can significantly reduce realgar toxicity by reducing blood arsenic levels [29, 141].

Metabolomics studies have confirmed that the other herbs present in AGNH alleviate inflammatory cell infiltration and damage in the liver and kidneys as well as the serum metabolic profile disruptions induced by cinnabar and realgar insults [139, 142]. The mechanism may involve protective effects of AGNH's herbal constituents against the accumulation of Hg and As and the hepatorenal toxicity induced by cinnabar and realgar via downregulation of the expression of uptake transporters and upregulation of the expression of efflux transporters in hepatorenal tissues [141]. Similar studies on LSW [143] and Niuhuang Jiedu tablets [144] have also demonstrated that other herbal ingredients in the formulas can reduce the damage caused by realgar to some extent.

6. Summary and Outlook

The above review demonstrates that, despite great progress in research on the pharmacological and toxicological mechanisms of cinnabar and realgar, limited pharmacological studies have assessed cinnabar's sedative and soothing effects and realgar's anti-inflammatory, antioxidant, and epidemic-preventing effects. Notably, the compositions of metal mineral medicines are complex, as these medicines contain a variety of compounds. However, research on the physiological effects of the other components is relatively limited. In addition, because the body itself contains various trace elements, it is difficult for the current analytical technologies to achieve accurate detection in vivo, which is the key hindrance to in-depth research on the absorption and metabolism of metal-based mineral drugs. Researching pharmacology, toxicology, and mechanisms of action is an important method to study medicinal properties. However, most of the research conducted to date has focused on the overall efficacy and toxicity of cinnabar, realgar, or their formulations. Many researchers are observing and testing some macro-indicators, but research to clarify the mechanisms of action at the molecular-level remains lacking. There is no sufficient scientific basis for rational clinical drug use, and there is an urgent need to conduct more in-depth research on the molecular mechanisms of the pharmacological and toxic effects of cinnabar and realgar.

To date, research on the toxicity of mineral drugs has mostly adopted modern toxicology methods to analyze the toxicity to normal organisms. However, due to neglect of the toxicity-reducing compatibility theory of traditional Chinese medicine, the toxicity of mineral medicines has been exaggerated.

To improve efficacy and reduce adverse effects, TCM often combines mineral medicines with herbs, animal products, and/or other minerals in products called formulas.

Increasing evidence demonstrates that treatment regimens for various illnesses contain multiple drugs with distinct but related mechanisms. These drugs can often amplify the therapeutic efficacy of the other drugs, enabling multi-targeted synergistic therapy and leading to maximal therapeutic efficacy with minimal adverse effects. However, the essential compounds have not been identified in most formulas, and the precise mechanisms of the formulas remain to be addressed using molecular approaches. When the specific mechanisms of action and toxicity mechanisms of two-drug formulas are unclear, the use of each single drug may not completely explain the pharmacodynamic and toxicity mechanisms of the two drugs together. Each drug may need to be combined with other compatible drugs to completely explain the possible mechanisms of action, and pharmacodynamic research may need to be conducted to achieve attenuation and antiviral effects.

For clinical applications, it is necessary to combine theory and clinical experience to guide scientific and rational drug use and achieve the purposes of reducing toxicity and increasing efficacy through the use of modern preparation methods or drug combinations. Processing has a significant effect on the content of toxic impurities in mineral medicines. The processing parameters of the washing method should be further standardized, and the amount of water added and the numbers of repeated grinding operations should be clarified to obtain decoctions of uniform quality. The development of nanopreparations, microbial leaching preparations, new crystal preparations, and compound preparations represents the current focus of research on mineral pharmaceutical preparations. How to effectively improve drug solubility, bioavailability, and targeting, adjust sustained release and controlled release, and reduce the occurrence of adverse reactions is the key issue to be considered with these new processing methods.

The next steps are to explore the effective traditional formulas through the combination of minerals and other drugs, to elucidate the mechanisms of these formulas, to upgrade the formulas into modern dosage forms, and to describe the traditional theories of traditional Chinese medicine, such as synergistic treatments and toxin counteraction with toxin therapy, in the modern language of molecular biology. The complementary combination of systems biology and theoretical research on TCM will greatly promote the pace of traditional Chinese medicine internationalization.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

The authors read and approved the final manuscript.

Acknowledgments

This study was supported by Jilin Province Health Science and Technology Capacity Improvement Project (No. 2021JC070) and the Disciplinary Layout Project of Natural Science Foundation of Jilin Province (No. 20200201412JC) and the National Natural Science Foundation of China (No. 82205190) and the Natural Science Foundation of Henan Province (No.202300410251) and the Henan Province Traditional Chinese Medicine Scientific Research Special Project (No.2019JDZX2033) and the Jilin Province Science and Technology Development Plan Project (No.20190201124JC).

Supplementary Materials

The authors review the accumulating evidence and discuss the pharmacology, toxicology, formulas, and safe use of cinnabar (HgS) and realgar (As_2S_2). (*Supplementary Materials*)

References

[1] *Pharmacopedia of China*, People's Press, Beijing, China, 2020.

[2] X. Zhou, Q. Wang, and X. Yang, "Progresses on mechanisms of pharmacological and toxicological effects of cinnabar," *Zhongguo Zhongyao Zazhi*, vol. 34, no. 22, pp. 2843–2847, 2009.

[3] C. J. Chen, S. K. Wu, Y. B. Wang, J. F. Hou, L. Ma, and X. Y. Sun, "Recent researches of synthetic mercury sulfide in traditional medicine system," *Zhongguo Zhongyao Zazhi*, vol. 37, no. 19, pp. 2968–2970, 2012.

[4] Y. Zhao, B. Yuan, K. Onda et al., "Anticancer efficacies of arsenic disulfide through apoptosis induction, cell cycle arrest, and pro-survival signal inhibition in human breast cancer cells," *American Journal of Cancer Research*, vol. 8, no. 3, pp. 366–386, 2018.

[5] Y. Hai, X. Wang, P. Song et al., "Realgar transforming solution-induced differentiation of NB4 cell by the degradation of PML/RARα partially through the ubiquitin-proteasome pathway," *Archives of Pharmacal Research*, vol. 42, no. 8, pp. 684–694, 2019.

[6] Q. B. Zhou, Z. T. Liu, H. Z. Wang, X. Q. Guo, Y. G. Xu, and X. M. Hu, "Arsenic disulfide promoted hypomethylation by increasing DNA methyltransferases expression in myelodysplastic syndrome," *Drug Design, Development and Therapy*, vol. 14, pp. 1641–1650, 2020.

[7] Q. B. Zhou, Y. Du, S. S. Zhang, Z. T. Liu, R. Ma, and Y. G. Xu, "Clinical response to traditional Chinese herbs containing realgar (As(2)S(2)) is related to DNA methylation patterns in bone marrow DNA from patients with myelodysplastic syndrome with multilineage dysplasia," *Cancer Management and Research*, vol. 13, pp. 55–63, 2021.

[8] Z. Y. Deng, S. R. Zhu, M. J. Wang et al., "Relation of blood arsenic concentration with effect and safety of arsenic-containing Qinghuang powder in patients with myelodysplastic syndrome," *Chinese Journal of Integrative Medicine*, vol. 25, no. 7, pp. 497–501, 2019.

[9] Q. Zhu, Z. Deng, S. Zhu, P. Zhao, M. Wang, and X. Hu, "Study on the clinical safe and effective methods of arsenic-containingcompound-qinghuang powder in the treatment of myelodysplastic syndrome," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 2095682, 6 pages, 2017.

[10] Y. Zhao, K. Onda, K. Sugiyama et al., "Antitumor effects of arsenic disulfide on the viability, migratory ability, apoptosis

and autophagy of breast cancer cells," *Oncology Reports*, vol. 41, no. 1, pp. 27–42, 2019.

[11] X. Xiaoxia, S. Jing, X. Dongbin et al., "Realgar nanoparticles inhibit migration, invasion and metastasis in a mouse model of breast cancer by suppressing matrix metalloproteinases and angiogenesis," *Current Drug Delivery*, vol. 17, no. 2, pp. 148–158, 2020.

[12] W. Ding, T. Ji, W. Xiong, T. Li, D. Pu, and R. Liu, "Realgar, a traditional Chinese medicine, induces apoptosis of HPV16-positive cervical cells through a HPV16 E7-related pathway," *Drug Design, Development and Therapy*, vol. 12, pp. 3459–3469, 2018.

[13] F. R. Yang, Y. F. Zhao, X. W. Hu et al., "Nano-realgar suppresses lung cancer stem cell growth by repressing metabolic reprogramming," *Gene*, vol. 788, Article ID 145666, 2021.

[14] G. Wang, T. Zhang, W. Sun et al., "Arsenic sulfide induces apoptosis and autophagy through the activation of ROS/JNK and suppression of Akt/mTOR signaling pathways in osteosarcoma," *Free Radical Biology and Medicine*, vol. 106, pp. 24–37, 2017.

[15] X. Zhang, T. Kang, L. Zhang, Y. Tong, W. Ding, and S. Chen, "NFATc3 mediates the sensitivity of gastric cancer cells to arsenic sulfide," *Oncotarget*, vol. 8, no. 32, Article ID 52735, 2017.

[16] Z. Bujňáková, M. Kello, J. Kováč et al., "Preparation of As(4)S(4)/Fe(3)O(4) nanosuspensions and in-vitro verification of their anticancer activity," *Materials Science and Engineering: C*, vol. 110, Article ID 110683, 2020.

[17] P. Song, P. Chen, D. Wang et al., "Realgar transforming solution displays anticancer potential against human hepatocellular carcinoma HepG2 cells by inducing ROS," *International Journal of Oncology*, vol. 50, no. 2, pp. 660–670, 2017.

[18] S. Biswas and J. Bellare, "Ayurvedic processing of α-HgS gives novel physicochemistry and distinct toxicokinetics in zebrafish," *Chemosphere*, vol. 251, Article ID 126295, 2020.

[19] L. Wei, R. Xue, P. Zhang, Y. Wu, X. Li, and F. Pei, "(1)H NMR-based metabolomics and neurotoxicity study of cerebrum and cerebellum in rats treated with cinnabar, a traditional Chinese medicine," *OMICS: A Journal of Integrative Biology*, vol. 19, no. 8, pp. 490–498, 2015.

[20] J. J. Chuu, C. J. Hsu, and S. Y. Lin-Shiau, "Abnormal auditory brainstem responses for mice treated with mercurial compounds: involvement of excessive nitric oxide," *Toxicology*, vol. 162, no. 1, pp. 11–22, 2001.

[21] J. J. Chuu, S. H. Liu, and S. Y. Lin-Shiau, "Differential neurotoxic effects of methylmercury and mercuric sulfide in rats," *Toxicology Letters*, vol. 169, no. 2, pp. 109–120, 2007.

[22] Y. Wang, D. Wang, J. Wu et al., "Cinnabar induces renal inflammation and fibrogenesis in rats," *BioMed Research International*, vol. 2015, Article ID 280958, 10 pages, 2015.

[23] W. H. Yu, N. Zhang, J. F. Qi, C. Sun, Y. H. Wang, and M. Lin, "Arsenic and mercury containing traditional Chinese medicine (realgar and cinnabar) strongly inhibit organic anion transporters, Oat1 and Oat3, in vivo in mice," *BioMed Research International*, vol. 2015, Article ID 863971, 7 pages, 2015.

[24] H. Shi, X. Shi, and K. J. Liu, "Oxidative mechanism of arsenic toxicity and carcinogenesis," *Molecular and Cellular Biochemistry*, vol. 255, no. 1-2, pp. 67–78, 2004.

[25] J. Liu and M. P. Waalkes, "Liver is a target of arsenic carcinogenesis," *Toxicological Sciences*, vol. 105, no. 1, pp. 24–32, 2008.

[26] C. R. Tyler and A. M. Allan, "The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review," *Current Environmental Health Reports*, vol. 1, no. 2, pp. 132–147, 2014.

[27] S. F. Xu, Q. Wu, B. B. Zhang et al., "Comparison of mercury sulfides with mercury chloride and methylmercury on hepatic P450, phase-2 and transporter gene expression in mice," *Journal of Trace Elements in Medicine & Biology*, vol. 37, pp. 37–43, 2016.

[28] Y. Sui, H. Yang, X. Z. Tian, J. Liu, and J. Z. Shi, "Effect of Zhusha Anshen pill, cinnabar, HgS, HgCl2 and MeHg on gene expression of renal transporters in mice," *Zhongguo Zhongyao Zazhi*, vol. 40, no. 3, pp. 506–510, 2015.

[29] J. Liu, L. X. Wei, Q. Wang et al., "A review of cinnabar (HgS) and/or realgar (As(4)S(4))-containing traditional medicines," *Journal of Ethnopharmacology*, vol. 210, pp. 340–350, 2018.

[30] Y. Wang, S. Zhou, H. Ma, J. S. Shi, and Y. F. Lu, "Investigation of the differential transport mechanism of cinnabar and mercury containing compounds," *Environmental Toxicology and Pharmacology*, vol. 66, pp. 83–90, 2019.

[31] J. Liu, J. Z. Shi, L. M. Yu, R. A. Goyer, and M. P. Waalkes, "Mercury in traditional medicines: is cinnabar toxicologically similar to common mercurials," *Experimental Biology and Medicine*, vol. 233, no. 7, pp. 810–817, 2008.

[32] Q. Wu, X. He, S. Zhou, F. Shi, and Y. Lu, "Role of PEPT1in the transport of cinnabar in Caco-2 cells," *Toxicology in Vitro*, vol. 63, Article ID 104747, 2020.

[33] Y. F. Lu, Q. Wu, J. W. Yan, J. Z. Shi, J. Liu, and J. S. Shi, "Realgar, cinnabar and An-Gong-Niu-Huang Wan are much less chronically nephrotoxic than common arsenicals and mercurials," *Experimental Biology and Medicine*, vol. 236, no. 2, pp. 233–239, 2011.

[34] C. F. Huang, S. H. Liu, and S. Y. Lin-Shiau, "Neurotoxicological effects of cinnabar (a Chinese mineral medicine, HgS) in mice," *Toxicology and Applied Pharmacology*, vol. 224, no. 2, pp. 192–201, 2007.

[35] Q. Wang, X. Yang, B. Zhang, X. Yang, and K. Wang, "The anxiolytic effect of cinnabar involves changes of serotonin levels," *European Journal of Pharmacology*, vol. 565, no. 1-3, pp. 132–137, 2007.

[36] A. K. Pandey, R. Patnaik, D. F. Muresanu, A. Sharma, and H. S. Sharma, "Quercetin in hypoxia-induced oxidative stress: novel target for neuroprotection," *International Review of Neurobiology*, vol. 102, pp. 107–146, 2012.

[37] Q. He, J. Ma, P. K. Kalavagunta et al., "HgS inhibits oxidative stress caused by hypoxia through regulation of 5-HT metabolism pathway," *International Journal of Molecular Sciences*, vol. 20, no. 6, p. 1364, 2019.

[38] H. Hu, M. Tian, C. Ding, and S. Yu, "The C/EBP homologous protein (CHOP) transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection," *Frontiers in Immunology*, vol. 9, p. 3083, 2018.

[39] H. H. Ma, Y. N. Ding, A. Wang et al., "Cinnabar protects serum-nutrient starvation induced apoptosis by improving intracellular oxidative stress and inhibiting the expression of CHOP and PERK," *Biochemistry and Biophysics Reports*, vol. 27, Article ID 101055, 2021.

[40] A. L. Hu, S. Song, Y. Li et al., "Mercury sulfide-containing Hua-Feng-Dan and 70 W (Rannasangpei) protect against LPS plus MPTP-induced neurotoxicity and disturbance of gut microbiota in mice," *Journal of Ethnopharmacology*, vol. 254, Article ID 112674, 2020.

[41] L. B. Guo Wencheng, L. W. Y. Zhang, and L. I. T. Huang Lili, "Effect of ZhushaAnshen pill on expression of monoamine neurotransmitters and c-Fos protein in neurons of rats with conditioned fear," *Journal of Guangdong Pharmaceutical University*, vol. 37, no. 01, pp. 84–90, 2021.

[42] L. Yue and C. J. S. Tingli, "Effect of Zhusha Anshen pill on synaptic plasticity of hippocampus in rats with conditional fear," *Shanghai Journal of Traditional Chinese Medicine*, vol. 54, no. 02, pp. 97–101+107, 2020.

[43] J. Yang, W. Guangwei, and L. Tingli, "Influence of decoction of "Zhusha anshen pill" on sleep phase in insomia rats," *Shanghai Journal of Traditional Chinese Medicine*, vol. 42, no. 12, pp. 74–76, 2008.

[44] L. B. Tingli, "The effect of Zhusha Anshen Pill on monoamine neurotransmitters in rat VLPO brain region," *Chinese Journal of Drug Dependence*, vol. 27, no. 06, pp. 425–430, 2018.

[45] Y. Tie, C. Hanyu, and C. Fengli, "Effect of aqueous decoction of Zhusha Anshin Pill on sleep phase and γ-aminobutyric acid content in the brain of mice," *Guangdong Medical Journal*, vol. 37, no. 03, pp. 351–353, 2016.

[46] T. Ding, J. Y. Luo, X. Han, S. H. Yang, R. C. Lin, and M. H. Yang, "Advances of toxicity evaluation of cinnabar and compatibility necessity analysis," *Zhongguo Zhongyao Zazhi*, vol. 41, no. 24, pp. 4533–4540, 2016.

[47] L. Wei, P. Liao, H. Wu et al., "Toxicological effects of cinnabar in rats by NMR-based metabolic profiling of urine and serum," *Toxicology and Applied Pharmacology*, vol. 227, no. 3, pp. 417–429, 2008.

[48] M. Yang, L. Wang, T. Zhang et al., "Different proteomic profiles of cinnabar upon therapeutic and toxic exposure reveal distinctive biological manifestations," *Journal of Ethnopharmacology*, vol. 253, Article ID 112668, 2020.

[49] J. J. Chuu, Y. H. Young, S. H. Liu, and S. Y. Lin-Shiau, "Neurotoxicity of mercury sulfide in the vestibular ocular reflex system of Guinea pigs," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 364, no. 3, pp. 249–258, 2001.

[50] Q. Q. Shen, J. J. Wang, D. Roy et al., "Organic anion transporter 1 and 3 contribute to traditional Chinese medicine-induced nephrotoxicity," *Chinese Journal of Natural Medicines*, vol. 18, no. 3, pp. 196–205, 2020.

[51] X. Zhou, L. Wang, X. Sun et al., "Cinnabar is not converted into methylmercury by human intestinal bacteria," *Journal of Ethnopharmacology*, vol. 135, no. 1, pp. 110–115, 2011.

[52] W. Dong, J. Liu, L. Wei, Y. Jingfeng, M. Chernick, and D. E. Hinton, "Developmental toxicity from exposure to various forms of mercury compounds in medaka fish (*Oryzias latipes*) embryos," *PeerJ*, vol. 4, Article ID e2282, 2016.

[53] Y. Lu, D. Yang, X. Song, S. Wang, M. Song, and T. Hang, "Bioaccessibility and health risk assessment of mercury in cinnabar containing traditional Chinese medicines," *Journal of Trace Elements in Medicine & Biology*, vol. 44, pp. 17–25, 2017.

[54] Z. Y. Zheng, C. Li, M. Zhang et al., "Dissolution, absorption and bioaccumulation in gastrointestinal tract of mercury in HgS-containing traditional medicines Cinnabar and Zuotai," *Zhongguo Zhongyao Zazhi*, vol. 40, no. 12, pp. 2455–2460, 2015.

[55] R. Manju, A. M. Hegde, P. Parlees, and A. Keshan, "Environmental arsenic contamination and its effect on intelligence quotient of school children in a historic gold mining area hutti, north Karnataka, India: a pilot study," *Journal of Neurosciences in Rural Practice*, vol. 8, no. 3, pp. 364–367, 2017.

[56] C. L. Guo, L. J. Wang, Y. Zhao et al., "A novel bromophenol derivative BOS-102 induces cell cycle arrest and apoptosis in human A549 lung cancer cells via ROS-mediated PI3K/Akt and the MAPK signaling pathway," *Marine Drugs*, vol. 16, no. 2, p. 43, 2018.

[57] Y. Wang, P. C. He, J. Qi, Y. F. Liu, and M. Zhang, "Tetra-arsenictetra-sulfide induces cell cycle arrest and apoptosis in retinoic acid-resistant acute promyelocytic leukemia cells," *Biomedical Reports*, vol. 3, no. 4, pp. 583–587, 2015.

[58] N. Wang, L. W. Wang, B. D. Gou, T. L. Zhang, and K. Wang, "Realgar-induced differentiation is associated with MAPK pathways in HL-60 cells," *Cell Biology International*, vol. 32, no. 12, pp. 1497–1505, 2008.

[59] L. Y. Luo, J. Huang, B. D. Gou, T. L. Zhang, and K. Wang, "Induction of human promyelocytic leukemia HL-60 cell differentiation into monocytes by arsenic sulphide: involvement of serine/threonine protein phosphatases," *Leukemia Research*, vol. 30, no. 11, pp. 1399–1405, 2006.

[60] S. Chen, Y. Fang, L. Ma, S. Liu, and X. Li, "Realgar-induced apoptosis and differentiation in all-trans retinoic acid (ATRA)-sensitive NB4 and ATRA-resistant MR2 cells," *International Journal of Oncology*, vol. 40, no. 4, pp. 1089–1096, 2012.

[61] G. Kroemer, G. Mariño, and B. Levine, "Autophagy and the integrated stress response," *Molecular Cell*, vol. 40, no. 2, pp. 280–293, 2010.

[62] L. Murrow and J. Debnath, "Autophagy as a stress-response and quality-control mechanism: implications for cell injury and human disease," *Annual Review of Pathology: Mechanisms of Disease*, vol. 8, no. 1, pp. 105–137, 2013.

[63] Z. Liu, K. Xu, Y. Xu et al., "Involvement of autophagy in realgar quantum dots (RQDs) inhibition of human endometrial cancer JEC cells," *PeerJ*, vol. 8, Article ID e9754, 2020.

[64] X. Wang, B. Chen, L. Zhao et al., "Autophagy enhanced antitumor effect in K562 and K562/ADM cells using realgar transforming solution," *Biomedicine & Pharmacotherapy*, vol. 98, pp. 252–264, 2018.

[65] C. Sebastian, "Tracking down the origin of cancer: metabolic reprogramming as a driver of stemness and tumorigenesis," *Critical Reviews in Oncogenesis*, vol. 19, no. 5, pp. 363–382, 2014.

[66] S. Mondal, N. Adhikari, S. Banerjee, S. A. Amin, and T. Jha, "Matrix metalloproteinase-9 (MMP-9) and its inhibitors in cancer: a minireview," *European Journal of Medicinal Chemistry*, vol. 194, Article ID 112260, 2020.

[67] W. Ding, Y. Tong, X. Zhang, M. Pan, and S. Chen, "Study of arsenic sulfide in solid tumor cells reveals regulation of nuclear factors of activated T-cells by PML and p53," *Scientific Reports*, vol. 6, no. 1, Article ID 19793, 2016.

[68] P. Song, Y. Hai, X. Wang et al., "Realgar transforming solution suppresses angiogenesis and tumor growth by inhibiting VEGF receptor 2 signaling in vein endothelial cells," *Archives of Pharmacal Research*, vol. 41, no. 4, pp. 467–480, 2018.

[69] V. Signorini, E. Elefante, D. Zucchi, F. Trentin, A. Bortoluzzi, and C. Tani, "One year in review 2020: systemic lupus erythematosus," *Clinical & Experimental Rheumatology*, vol. 38, no. 4, pp. 592–601, 2020.

[70] W. Xu, Z. Chen, X. Shen, and C. Pi, "Reno-protective effect of realgar nanoparticles on lupus nephritis of MRL/lpr mice through STAT1," *Iranian Journal of Immunology*, vol. 16, no. 2, pp. 170–181, 2019.

[71] X. Y. L. Wei-Dong, L. X. L. Zheng-Feng, and S. J. Y. Xiao-Dong, "Realgar nano-particlesdown-regulated the expression of CD40 in B cells from MRL/lpr mice and promote B cell apoptosis through rising their Ca2+ concentration," *Chinese journal of immunology*, vol. 29, no. 12, pp. 1249–1253, 2013.

[72] W. Ma, J. Yue, S. Liang et al., "Realgar increases defenses against infection by *Enterococcus faecalis* in *Caenorhabditis elegans*," *Journal of Ethnopharmacology*, vol. 268, Article ID 113559, 2021.

[73] D. Liu, D. Zhi, T. Zhou et al., "Realgar bioleaching solution is a less toxic arsenic agent in suppressing the Ras/MAPK pathway in *Caenorhabditis elegans*," *Environmental Toxicology and Pharmacology*, vol. 35, no. 2, pp. 292–299, 2013.

[74] J. R. Meng-Na, F. Q. Liu Zheng, M.-J. L. Y. Zhang, and Q. Yan-hong, "Data mining of preventing pestilence by aromatic therapy in dictionary of traditional Chinese medicine prescriptions," *Guiding Journal of Traditional Chinese Medicine and Pharmacy*, vol. 27, no. 11, pp. 217–221, 2021.

[75] X. Q. Wu, W. N. Zhang, M. Z. Hao et al., "How Chinese herbal medicine prevents epidemics: from ancient pestilences to COVID-19 pandemic," *The American Journal of Chinese Medicine*, vol. 49, no. 5, pp. 1017–1044, 2021.

[76] R. Y. F. D. X. Y. Z. Xiaomin, "Discussion on thoughts for treatment of coronavirus disease 2019 based on cold-damp epidemics," *Journal of Guangzhou University of Traditional Chinese Medicine*, vol. 37, no. 6, pp. 1003–1007, 2020.

[77] D. Wang, L. Wang, R. Xu, X. Wu, and Y. Li, "Antiviral activity of nano-realgar against herpes simplex virus type II in vitro," *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, vol. 44, no. 10, pp. 1143–1150, 2019.

[78] L. Wang, G. B. Zhou, P. Liu et al., "Dissection of mechanisms of Chinese medicinal formula Realgar-Indigo naturalis as an effective treatment for promyelocytic leukemia," *Proceedings of the National Academy of Sciences of the USA*, vol. 105, no. 12, pp. 4826–4831, 2008.

[79] H. H. Zhu, D. P. Wu, X. Du et al., "Oral arsenic plus retinoic acid versus intravenous arsenic plus retinoic acid for non-high-risk acute promyelocytic leukaemia: a non-inferiority, randomised phase 3 trial," *The Lancet Oncology*, vol. 19, no. 7, pp. 871–879, 2018.

[80] H. H. Zhu and X. J. Huang, "Oral arsenic and retinoic acid for non-high-risk acute promyelocytic leukemia," *New England Journal of Medicine*, vol. 371, no. 23, pp. 2239–2241, 2014.

[81] L. Zhang, X. M. Yang, J. Chen et al., "Population pharmacokinetics and safety of oral tetra-arsenictetra-sulfide formula in pediatric acute promyelocytic leukemia," *Drug Design, Development and Therapy*, vol. 15, pp. 1633–1640, 2021.

[82] H. H. Zhu, J. Hu, and X. F. Gu, "Arsenic as traditional Chinese medicine provides new hope for overcoming high treatment costs of acute promyelocytic leukemia," *Journal of Global Oncology*, vol. 2, no. 6, pp. 442-443, 2016.

[83] H. Jiang, G. W. Liang, X. J. Huang et al., "Reduced medical costs and hospital days when using oral arsenic plus ATRA as the first-line treatment of acute promyelocytic leukemia," *Leukemia Research*, vol. 39, no. 12, pp. 1319–1324, 2015.

[84] M. H. Yang, W. Q. Wan, J. S. Luo et al., "Multicenter randomized trial of arsenic trioxide and realgar-Indigo naturalis formula in pediatric patients with acute promyelocytic leukemia: interim results of the SCCLG-APL clinical study," *American Journal of Hematology*, vol. 93, no. 12, pp. 1467–1473, 2018.

[85] Y. Lou, Y. Lu, X. Ye et al., "PML-RARA monitoring in newly diagnosed acute promyelocytic leukemia treated with an entirely oral chemotherapy-free postremission approach: a multiple institution experience," *Hematological Oncology*, vol. 38, no. 4, pp. 618–621, 2020.

[86] H. H. Zhu, Z. P. Guo, J. S. Jia, Q. Jiang, H. Jiang, and X. J. Huang, "The impact of oral arsenic and all-trans-retinoic acid on coagulopathy in acute promyelocytic leukemia," *Leukemia Research*, vol. 65, pp. 14–19, 2018.

[87] X. Shi, S. Li, S. Tang, and Y. Lu, "Successful treatment of acute promyelocytic leukemia in a 92-year-old man using all-trans retinoic acid combined with oral arsenic: a case report," *Medicine*, vol. 100, no. 22, Article ID e26144, 2021.

[88] H. H. Zhu, D. P. Wu, J. Jin et al., "Long-term survival of acute promyelocytic leukaemia patients treated with arsenic and retinoic acid," *British Journal of Haematology*, vol. 174, no. 5, pp. 820–822, 2016.

[89] Q. Xie, L. Yu, X. Wang et al., "A novel realgar-indigo naturalis formula more effectively induces apoptosis in NB4 cells," *Pakistan journal of pharmaceutical sciences*, vol. 32, no. 3, pp. 957–962, 2019.

[90] M. Zhang, L. Guo, L. F. Lin et al., "Absorption characteristics of combination medication of realgar and indigo naturalis: in vitro transport across MDCK-MDR1 cells and in vivo pharmacokinetics in mice after oral administration," *Evidence-Based Complementary and Alternative Medicine*, vol. 2018, Article ID 6493630, 10 pages, 2018.

[91] X. M. Hu, B. Yuan, S. Tanaka et al., "Involvement of oxidative stress associated with glutathione depletion and p38 mitogen-activated protein kinase activation in arsenic disulfide-induced differentiation in HL-60 cells," *Leukemia and Lymphoma*, vol. 55, no. 2, pp. 392–404, 2014.

[92] P. Zhao, J. B. Liang, Z. Y. Deng et al., "Association of gene mutations with response to arsenic-containing compound Qinghuang powder in patients with myelodysplastic syndromes," *Chinese Journal of Integrative Medicine*, vol. 25, no. 6, pp. 409–415, 2019.

[93] J. H. Mao, X. Y. Sun, J. X. Liu et al., "As4S4 targets RING-type E3 ligase c-CBL to induce degradation of BCR-ABL in chronic myelogenous leukemia," *Proceedings of the National Academy of Sciences of the USA*, vol. 107, no. 50, Article ID 21683, 2010.

[94] W. Fang, Z. L. Peng, Y. J. Dai, D. L. Wang, P. Huang, and H. P. Huang, "(-)-Epigallocatechin-3-gallate encapsulated realgar nanoparticles exhibit enhanced anticancer therapeutic efficacy against acute promyelocytic leukemia," *Drug Delivery*, vol. 26, no. 1, pp. 1058–1067, 2019.

[95] Z. Liangchun, "Duotan dingjing powder," 2012, https://www.google.com/search?rlz=1C1GCEB_enIN1005IN1005&q=Dotan+Dingjing+Powder&spell=1&sa=X&ved=2ahUKEwj47fTJ_P_5AhUYSGwGHZe5DHUQBSgAegQIARA1&biw=1366&bih=600&dpr=1.

[96] Q. Ma, W. Huang, J. Zhao, and Z. Yang, "Liu Shen Wan inhibits influenza a virus and excessive virus-induced inflammatory response via suppression of TLR4/NF-κB signaling pathway in vitro and in vivo," *Journal of Ethnopharmacology*, vol. 252, Article ID 112584, 2020.

[97] J. Zhao, Y. Wang, X. Huang et al., "Liu Shen Wan inhibits influenza virus-induced secondary *Staphylococcus aureus* infection in vivo and in vitro," *Journal of Ethnopharmacology*, vol. 277, Article ID 114066, 2021.

[98] Y. T. Sun, H. H. Xu, Y. Nie et al., "Preliminary study of Realgar and arsenic trioxide on gut microbiota of mice," *Zhongguo Zhongyao Zazhi*, vol. 45, no. 1, pp. 142–148, 2020.

[99] W. Zhang, T. Huo, A. Li et al., "Identification of neurotoxicity markers induced by realgar exposure in the mouse cerebral cortex using lipidomics," *Journal of Hazardous Materials*, vol. 389, Article ID 121567, 2020.

[100] Y. Meng, R. Feng, Z. Yang, T. Liu, T. Huo, and H. Jiang, "Oxidative stress induced by realgar in neurons: p38 MAPK and ERK1/2 perturb autophagy and induce the p62-Keap1-Nrf2 feedback loop to activate the Nrf2 signalling pathway," *Journal of Ethnopharmacology*, vol. 282, Article ID 114582, 2022.

[101] S. Zhang, C. Li, T. Feng et al., "A metabolic profiling study of realgar-induced acute kidney injury in mice," *Frontiers in Pharmacology*, vol. 12, Article ID 706249, 2021.

[102] S. Zhang, C. Li, T. Feng et al., "Proteomics analysis in the kidney of mice following oral feeding Realgar," *Journal of Ethnopharmacology*, vol. 275, Article ID 114118, 2021.

[103] H. H. Xu, F. R. Hao, M. X. Wang et al., "Influences of realgar-indigo naturalis, a traditional Chinese medicine formula, on the main CYP450 activities in rats using a cocktail method," *Evidence-Based Complementary and Alternative Medicine*, vol. 2017, Article ID 2374624, 9 pages, 2017.

[104] A. Li, X. Wu, J. Yang et al., "Sub-chronic exposure to realgar induces liver injury via upregulating the TXNIP/NLRP3 pathway and disturbing bile acid homeostasis in mice," *Journal of Ethnopharmacology*, vol. 281, Article ID 114584, 2021.

[105] X. Wang, X. Huang, L. Zhou et al., "Association of arsenic exposure and cognitive impairment: a population-basedcross-sectional study in China," *Neurotoxicology*, vol. 82, pp. 100–107, 2021.

[106] M. H. Zhang, J. Q. Chen, H. M. Guo et al., "Combination of LC/MS and GC/MS based metabolomics to study the hepatotoxic effect of realgar nanoparticles in rats," *Chinese Journal of Natural Medicines*, vol. 15, no. 9, pp. 684–694, 2017.

[107] D. X. Wang, Y. G. Xu, Y. Du et al., "Arsenic concentration in peripheral blood is correlated with efficacy of a traditional Chinese medicine regimen containing realgar for the treatment of myelodysplastic syndrome," *Journal of Traditional Chinese Medicine*, vol. 41, no. 4, pp. 630–635, 2021.

[108] B. Tsoi, S. Wang, C. Gao et al., "Realgar and cinnabar are essential components contributing to neuroprotection of Angong Niuhuang Wan with no hepatorenal toxicity in transient ischemic brain injury," *Toxicology and Applied Pharmacology*, vol. 377, Article ID 114613, 2019.

[109] Y. F. Lu, J. W. Yan, Q. Wu, J. Z. Shi, J. Liu, and J. S. Shi, "Realgar- and cinnabar-containing an-gong-niu-huang wan (AGNH) is much less acutely toxic than sodium arsenite and mercuric chloride," *Chemico-Biological Interactions*, vol. 189, no. 1-2, pp. 134–140, 2011.

[110] P. Y. Lin, Y. S. Tang, and N. S. Wang, "Effects of cinnabar and realgar in angong niuhuang powder on heat shock protein, nitric oxide synthase and inflammatory cytokines in contusion cerebral edema," *Zhong Yao Cai*, vol. 29, no. 5, pp. 458–461, 2006.

[111] Y. S. Tang, N. S. Wang, Y. Q. Zhang, S. M. Ye, and W. P. Ou, "Effects of realgar and prescription containing realgar on stress response proteins, inflammatory mediators and complements in fever rat models," *Zhong Yao Cai*, vol. 32, no. 1, pp. 73–78, 2009.

[112] C. Chen, B. B. Zhang, A. L. Hu, H. Li, J. Liu, and F. Zhang, "Protective role of cinnabar and realgar in Hua-Feng-Dan against LPS plus rotenone-induced neurotoxicity and disturbance of gut microbiota in rats," *Journal of Ethnopharmacology*, vol. 247, Article ID 112299, 2020.

[113] H. Liu, Y. Yan, P. Pang et al., "Angong Niuhuang Pill as adjuvant therapy for treating acute cerebral infarction and intracerebral hemorrhage: a meta-analysis of randomized controlled trials," *Journal of Ethnopharmacology*, vol. 237, pp. 307–313, 2019.

[114] P. C. Zhao, Z. S. Huang, S. N. Xu, and Y. M. Zhang, "Adjuvant treatment with Angong Niuhuang pills in treating traumatic brain damage: a meta-analysis of randomized controlled trials," *Annals of Palliative Medicine*, vol. 10, no. 2, pp. 1569–1577, 2021.

[115] H. Song, X. Chen, Y. Yu, and L. Zhang, "Xingnao Kaiqiao acupuncture combined with Angong Niuhuang Wan for a patient under persistent vegetative state: a case report," *Frontiers of Medicine*, vol. 12, no. 3, pp. 334–339, 2018.

[116] L. Lv, J. Zheng, Y. Zhang et al., "Respiratory nursing care with Angong Niuhuang pill for patients with chronic obstructive pulmonary disease following cardiac surgery," *Japan Journal of Nursing Science*, vol. 18, no. 1, Article ID e12344, 2021.

[117] Y. X. Fan, D. Q. Guo, and M. L. Wang, "Clinical study of Huafengdan combined with sodium valproate in treatment of epilepsy," *Drugs & Clinic*, vol. 37, no. 5, pp. 999–1003, 2022.

[118] Y. Xu, S. Y. Li, Y. N. Li et al., "Efficacy and safety of huafeng dan combined with basic therapy in the treatment of stroke: A meta-analysis," *China Pharmaceuticals*, vol. 31, no. 8, pp. 112–116, 2022.

[119] J. Chen, M. Dai, and Y. Gui, "Clinical observation of acupuncture combined with Huafeng Elixir (化风丹)in treatment of 35 cases of peripheral facial paralysis of wind-cold type," *Journal of Traditional Chinese Medicine*, vol. 62, no. 15, pp. 1332–1337, 2021.

[120] J. L. Wang, Y. Tang, and C. Y. Wang, "Clinical observation on Huafeng Dan (化风丹) combined with mecobalamine and acupuncture in treating 36 cases of idiopathic facial paralysis of wind-phlegm obstructing collateral type," *Journal of Traditional Chinese Medicine*, vol. 60, no. 17, pp. 1488–1492, 2019.

[121] Y. Li, J. Duan, H. Xia, B. Shu, and W. Duan, "Macromolecular substances as a dangerous factor in traditional Chinese medicine injections were determined by size-exclusive chromatography," *Toxicology Research*, vol. 9, no. 3, pp. 323–330, 2020.

[122] S. C. Zhao, R. L. Wei, Y. M. Xie, L. X. Wang, Q. Wang, and D. H. Yi, "Analysis of Qingkailing Injection in treatment of combined medication features of 2 147 cases of upper respiratory tract infection," *Zhongguo Zhongyao Zazhi*, vol. 44, no. 23, pp. 5207–5216, 2019.

[123] S. He, W. S. Li, Y. J. Luo, C. L. Ye, and Z. Y. Zhang, "Qingkailing injection for treatment of children pneumonia induced by respiratory syncytial virus: a meta-analysis of randomized controlled trials," *Chinese Journal of Integrative Medicine*, vol. 24, no. 4, pp. 288–295, 2018.

[124] J. Wu, X. Zhang, and B. Zhang, "Qingkailing injection for the treatment of acute stroke: a systematic review and meta-analysis," *Journal of Traditional Chinese Medicine*, vol. 34, no. 2, pp. 131–139, 2014.

[125] Edition RTSnPDO, "Progress in research on prevention and treatment of sars with traditional chinese medicine,"

2003, https://scholar.google.co.in/scholar?q=Progress+in+Research+on+Prevention+and+Treatment+of+SARS+with+Traditional+Chinese+Medicine&hl=en&as_sdt=0&as_vis=1&oi=scholart.

[126] X. H. Li and Q. Q. Liu, "Chinese medicine prevention and treatment scheme for COVID-19 in Beijing (trial fifth edition)," *Beijing Journal of Traditional Chinese Medicine*, vol. 39, no. 07, pp. 655-656, 2020.

[127] B. L. Wu, W. X. He, M. Ke, X. F. Shang-Guan, G. F. He, and R. Huang, "A retrospective analysis on 1330 adverse event reports of Qingkailing in China: further perception of its risks and rational use," *Current Medical Science*, vol. 38, no. 6, pp. 1103–1108, 2018.

[128] J. Zhang, S. Wang, L. Xu et al., "Multiple perspectives of qingkailing injection-fraction-single compound in revealing the hepatotoxicity of baicalin and hyodeoxycholic acid," *Journal of Ethnopharmacology*, vol. 215, pp. 147–155, 2018.

[129] X. Miao, J. Zhou, J. Li, Y. Liao, and Y. Zheng, "Chinese medicine in inhalation therapy: a review of clinical application and formulation development," *Current Pharmaceutical Design*, vol. 21, no. 27, pp. 3917–3931, 2015.

[130] Z. Y. Tian, L. D. Feng, Y. Xie et al., "Chinese herbal medicine xingnaojing injection for acute ischemic stroke: an overview of systematic reviews and meta-analyses," *Frontiers in Pharmacology*, vol. 12, Article ID 659408, 2021.

[131] L. Wang, X. Fan, Y. Chen, X. Liang, W. Shen, and Y. Zhang, "Efficacy and safety of xingnaojing injection for emergency treatment of acute ischemic stroke: a systematic review and meta-analysis," *Frontiers in Pharmacology*, vol. 13, Article ID 839305, 2022.

[132] Z. R. Zhou, Y. H. Zhao, R. Sun, and Y. R. Zhang, "Effects of Xingnaojing on serum high-sensitivityC-reactive protein and neuron-specific enolase in patients with acute cerebral hemorrhage: a protocol of systematic review and meta-analysis," *Medicine*, vol. 99, no. 45, Article ID e21379, 2020.

[133] Z. An, Y. Yin, L. Zhang et al., "Effect of ulinastatin combined with xingnaojing injection on severe traumatic craniocerebral injury and its influence on oxidative stress response and inflammatory response," *BioMed Research International*, vol. 2022, Article ID 2621732, 6 pages, 2022.

[134] L. Chen, H. Jiang, G. Xing et al., "Effects of Yunanan Baiyao adjunct therapy on postoperative recovery and clinical prognosis of patients with traumatic brain injury: a randomized controlled trial," *Phytomedicine*, vol. 89, Article ID 153593, 2021.

[135] Y. Wu and P. Zhong, "Clinical progress on management of pneumonia due to COVID-19 with Chinese traditional patent medicines," *Frontiers in Pharmacology*, vol. 12, Article ID 655063, 2021.

[136] T. Han, H. Zhang, W. Xu et al., "Study on the mechanism of reducing hepatotoxicity of water-grinding realgar by metabolomics, morphology, and chemical analysis," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 8538287, 14 pages, 2021.

[137] G. J. Y. T. J. J. X. H. H. Tingguo, "The impact of water processing on the content of soluble sulphur and mercury in cinnabar," *Chinese Archives of Traditional Chinese Medicine*, vol. 33, no. 5, pp. 1113–1115, 2015.

[138] F. Kang, K. Wu, H. He et al., "Comparative toxicology study of Cinnabar, Zhusha Anshenwan, methylmercury and mercuric chloride," *Zhongguo Zhongyao Zazhi*, vol. 35, no. 4, pp. 499–503, 2010.

[139] F. Xia, A. Li, Y. Chai et al., "UPLC/Q-TOFMS-Based metabolomics approach to reveal the protective role of other herbs in an-gong-niu-huang wan against the hepatorenal toxicity of cinnabar and realgar," *Frontiers in Pharmacology*, vol. 9, p. 618, 2018.

[140] G. Su, G. Chen, X. An, H. Wang, and Y. H. Pei, "Metabolic profiling analysis of the alleviation effect of treatment with baicalin on cinnabar induced toxicity in rats urine and serum," *Frontiers in Pharmacology*, vol. 8, p. 271, 2017.

[141] S. Wang, X. Xiao, A. Li, and P. Li, "The herbal constituents in an-gong-niu-huang wan (AGNH) protect against cinnabar- and realgar-induced hepatorenal toxicity and accumulations of mercury and arsenic in mice," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 5566078, 9 pages, 2021.

[142] A. Li, J. Y. Zhang, X. Xiao et al., "Hepatorenal protective effects of medicinal herbs in An-Gong-Niu-Huang Wan (AGNH) against cinnabar- and realgar-induced oxidative stress and inflammatory damage in mice," *Food and Chemical Toxicology*, vol. 119, pp. 445–456, 2018.

[143] J. Wang, L. Ding, J. Zhou et al., "Target lipidomics approach to reveal the resolution of inflammation induced by Chinese medicine combination in Liu-Shen-Wan against realgar overexposure to rats," *Journal of Ethnopharmacology*, vol. 249, Article ID 112171, 2020.

[144] W. Xu, Y. Pei, S. Xu, H. Wang, and P. Jin, "Metabolic profiling analysis of the alleviation effect of the fractions of Niuhuang Jiedu tablet on realgar induced toxicity in rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2018, Article ID 2154603, 13 pages, 2018.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 3520336, 10 pages
https://doi.org/10.1155/2022/3520336

Research Article

Antioxidant and Teratogenic Activities of Formulated Agar Extracted from Brown Seaweed *Turbinaria conoides* against Zebrafish Larvae

Thabitha Aavula,[1] Vignesh Narasimman,[1] Saravanan Ramachandran,[1] Radajurai Murugan,[2] Murugavel Ponnusamy,[3] Gururaja Perumal Pazhani,[4] and Sivaleela G[5]

[1]*Native Medicine and Marine Pharmacology Laboratory, Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education, Kelambakkam 603 103, Tamil Nadu, India*
[2]*Department of Food Technology, M S Ramaiah University of Applied Sciences, Bangaluru, Karnataka, India*
[3]*Center for Developmental Cardiology, Qingdao University, Qingdao 266021, China*
[4]*SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur 603203, Tamil Nadu, India*
[5]*Marine Biology Reginal Centre, Zoological Survey of India, Chennai 600 028, Tamil Nadu, India*

Correspondence should be addressed to Saravanan Ramachandran; saran_prp@yahoo.com

Received 16 February 2022; Accepted 4 May 2022; Published 11 July 2022

Academic Editor: Amjad Iqbal

This study examines the antioxidant and teratogenic effects of two different type's methods of formulating agar from *Turbinaria conoides* (*T. conoides*) using a zebrafish model. The agar was extracted using the aqueous extraction method and developed in two different formulations using separate procedures. Formulated agar1 (FA1) used a higher concentration of the ingredients while formulated agar 2 (FA2) had a lesser concentration. The two unique formulated agars (FAs) were studied using biochemical composition, Fourier infrared (FT-IR) spectroscopy, gas chromatography-mass spectroscopy (GC-MS), and scanning electron microscopy (SEM). The antioxidant activities of both FAs in vitro were shown to be significantly different ($P < 0.05$) at various concentrations (60–180 μl/ml) in the study. The toxicity of the FAs was dose-dependent, with FA1 having the least teratogenic activity when compared to FA2. In comparison to FA2, FA1 was found to have higher antioxidant activity. At various concentrations (0.5, 0.25, and 0.125 μg/ml), the teratogenic activity of two FAs was examined in zebrafish embryos (ZFE) at 24, 48, 72, and 96 hours post fertilization (hpf). Both FAs exhibit dose-dependent toxicity and increased antioxidant activity, and this can be utilized as an alternative for standard antioxidants, according to this study.

1. Introduction

Seaweeds/marine algae are a major part of the structurally diverse marine flora with several properties like antibacterial, anti-inflammatory, antiaging and anticarcinogenic. Seaweeds are rich in antioxidants and have potentially higher cosmetic effects due to the presence of several active ingredients. Cosmetic products derived from seaweed are increasingly preferred over synthetic compounds, with no harmful chemical compounds [1, 2]. Polysaccharides extracted from seaweeds play a pivotal role as humectants and moisturizers in cosmetic formulations, and can also be used as thickeners, gelling agents, film formers and emulsifiers [3]. *Turbinaria conoides* (*T. conoides*), brown algae which belong to the Sargassaceace family and the Fucales order, are a rich source of polysaccharides. The ethyl acetate extract of *Turbinaria conoides* was reported to have several properties like antibacterial, anticancer and higher antioxidant activity. Antioxidants are vital to reduce oxidative stress by removing the excess free radicals which have been associated with the emergence of degenerative processes in molecular biology, which encourage oxidative activities that

are harmful to the body. The capacity of antioxidant chemicals in plants (carotenoids, polyphenols, polysaccharides, unsaturated fatty acids, vitamins, enzymes, and cofactors) to trap free radicals has inspired interest in employing them in preventative and curative phytotherapy [4]. *T. conoides* are a rich source of several phytochemical components such as sulphated polysaccharides, glucuronic acid, and alginic acid, and also contain compounds such as digestible proteins, mineral salts (K, Ca, and Fe), and polyunsaturated fatty acids [5]. Our laboratory had previously investigated the anti-skin cancer and antioxidant effects of FA from the brown seaweed *Laminaria digitata* [6], but this finding has not been reported using the standard FA method. Drug screening with zebrafish has several advantages, like increased efficiency, lower operating costs, shorter testing periods, easily controllable experimental conditions, and most significantly, increased genetic similarities between zebrafish and humans (approximately 70%) which makes the zebrafish a successful animal model [7]. Hence, the current study compared the antioxidant capabilities and toxicity of FA in zebrafish embryos (ZFEs).

2. Materials and Methods

2.1. Seaweed Collection. The brown seaweeds (*T. conoides*) were collected from the Gulf of Mannar (9.1278°N, 79.4662°E), Tamil Nadu, India, during the period of December 2020–January 2021. The brown seaweeds were known and confirmed by the Central Marine Fisheries Research Institute (CMFRI). Brown seaweeds were collected and then washed in fresh water to remove any undesired elements, such as other seaweed species. These samples were meticulously cleaned with seawater before being rinsed in double-distilled water. The seaweeds were dried in the shade and finely powdered with a blender before being stored at −20°C for subsequent investigation.

2.2. Extraction of Agar. To extract water-soluble polysaccharides, 20 g fine powdered seaweed samples were combined with 200 ml Milli-Q water and autoclaved at 121°C for 1 h. To remove seaweed debris, the extract was then filtered through Whatman No. 1 filter paper and centrifuged at 4500 rpm for 20 min. To produce a gel, the extract was maintained at room temperature [8].

2.3. Preparation of FAs. Agar processing methods have been developed in several countries, as illustrated in Figure 1, the extracted agar was transformed into a formulation using two distinct processes [9, 10].

2.4. Characterization of FAs. The colour, odour, consistency, sterility, and homogeneity were all employed to identify the characteristics of both FAs [11–13]. pH, vibrational testing, centrifugation tests, and physicochemical properties were all looked into [14, 15]. The information presented here pertains to three different determinations.

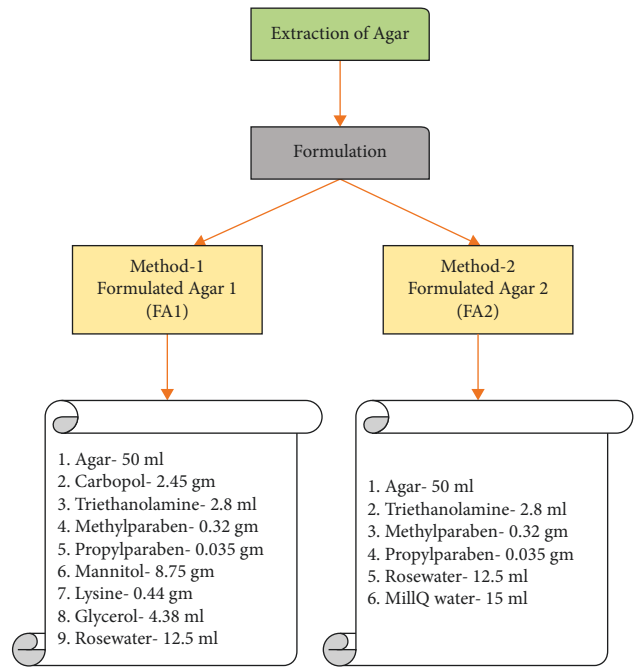


Figure 1: Extraction and flow chart of FAs.

2.5. Biochemical Composition of FAs by Flame Emission Atomic (FEA) Spectroscopy. 0.2 g of two FAs were mixed with $HClO_4$ and left undisturbed for 5 min. 5 ml of conc. HNO_3 was mixed and incubated for 5 min followed by adding 5 ml of conc. HCl. The mixture was allowed to evaporate. An FEA spectrophotometer was used to analyze the filtrate. The minerals analyzed were calcium and phosphorus. The results were expressed as ppm and percentage [16].

2.6. Structural Characterization

2.6.1. Fourier Transform Infrared Spectroscopy (FT-IR) of FAs. FT-IR qualitative analysis of two FAs was performed by using a Bruker Alpha instrument (USA) with the spectrum recorded between 4000 to 400 cm^{-1} [17].

2.6.2. Gas Chromatography-Mass Spectrometry (GC-MS) of FAs. The GC-MS spectroscopic analysis was carried out (Agilent, Santa Clara, CA, USA). The detection of an electron ionization system with an activity force of 70 eV and a mass range of 30 to 650 m/z was utilized [18].

2.6.3. Scanning Electron Microscope (SEM) of FAs. The structural characteristic of both FAs were designed with a JSM-5600 LV, SEM, (Jeol, Tokyo, Japan). The samples were analyzed at increasing temperatures (200°C, 400°C, 600°C, and 1200°C) with magnifications of 5.00 KX, 2.50 KX, 2.52 KX, and 1.00 KX [19].

2.7. In Vitro Antioxidant Assay

2.7.1. (1,1,Diphenyl-2,picrylhydrazyl) DPPH Scavenging Activity. The DPPH radical scavenging assay was performed

Table 1: Yield and biochemical composition of FAs.

S. no.	Sample	Yield (%)	Sulphate content (%)	Na (%)	K (%)	Mn (%)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Co (ppm)
1	FA 1	84.2	41.78	2.6 ± 0.3	2.7 ± 0.2	20 ± 0.3	51.1 ± 1.0	32.0 ± 1.1	76.01 ± 0.4	3.12 ± 1.3
2	FA 2	80.6	37.25	2.4 ± 0.2	2.5 ± 0.1	18.9 ± 0.2	48.2 ± 1.5	30.3 ± 1.3	73.2 ± 0.5	1.92 ± 1.5

$P < 0.05$, statistical significance.

in increasing sample concentration of 50 μl-100 μg/ml. 200 μl of methanolic DPPH solution was added and mixed thoroughly using a vortex mixer. The absorbance was measured at 517 nm in UV spectroscopy and the state was measured by using the following formula [20]:

$$DPPH \text{ scavenging assay } (\%) = [(A\,control - A\,sample)/A\,control]x100, \quad (1)$$

where A control = absorbance of the control sample; A sample = absorbance of FA.

2.7.2. H_2O_2 Radical Scavenging Activity. 0.5 ml of FA solutions was added with 600 μl H_2O_2 solution (40 Mm) to determine the H_2O_2 radical scavenging activity. The absorbance was then calculated at 230 nm in UV spectroscopy [16].

H_2O_2 scavenging activity (%) = [(A control−A sample)/A control] x 100,where A control = absorbance of the control sample; A sample = absorbance of FA.

2.7.3. Total Antioxidant Activity (TAA). The TAA of FAs was determined by using a total antioxidant capacity (TAC) solvent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). 0.2 ml FAs were mixed with 3 ml of the TAC reagent solution and then incubated in a water bath for 90 min. The readings of the reaction were then measured at 695 nm by using a UV-visible spectrophotometer [4].

$$TAA\,(\%) = [(A\,control - A\,sample)/A\,control]x100, \quad (2)$$

where A control = absorbance of the control sample and A sample = absorbance of FA.

2.7.4. 2,2-Azino-is-3-ethylbenzothiazoline-6-sulfonic Acid (ABTS) Activity. The ABTS solution absorbance was checked using 1 ml ABTS solvent with 60 ml methanol to attain the readings of 0.73 at 734 nm. The ABTS activity of FAs was compared with standard vitamin *E* at 734 nm in a UV-visible spectrophotometer [20].

$$ABTS\,(\%) = [(A\,control - A\,sample)/A\,control]x100, \quad (3)$$

where A control = absorbance of the control sample and A sample = absorbance of FA.

2.8. Zebrafish Husbandry. At a temperature of 28°C, adult wild zebrafish were kept in dark and light for 10 to 14 hrs at a time. Natural spawning was used to gather viable eggs, which were then washed in distilled water the next morning. The E3 medium (34.8 g NaCl, 1.6 g KCl, 5.8 g $CaCl_2{\cdot}2H_2O$, 9.78 g $MgCl_2{\cdot}6H_2O$) was used to harvest and store embryos. For the teratogenic FAs investigation, coagulated and damaged eggs were removed and viable eggs were chosen [21].

2.9. Teratogenic Activities of FAs. At varied diluted concentrations (0.5, 0.25, and 0.125 μg/ml), the teratogenic activity of the two distinct FAs was tested. A stereomicroscope was used to investigate the ZEF at various phases of growth [22].

2.10. Statistical Analysis. Each test was carried out in a triplicate manner, with the findings reported as the mean SD. Significance was defined as a *P* value of <0.05. IBM SPSS Statistics for Windows, version 22, was used for statistical analysis (IBM Corp., Armonk, N.Y., USA).

3. Results and Discussion

The wet weights of FAs obtained by FA1 84.2% and FA2 80.6% are mentioned in (Table 1). The chemicals and quantity have an impact on the yield and rate of an FA. Marine pharmacologist creates methods in the manufacturing business that maximize the yield and production rate of FA. They also want to cut waste and energy expenses at every step of the experiment [23]. The agar formulation methods and yield used for developing antioxidant properties for both the FAs from brown seaweed have been successful (*T. conoides*). The macroelements of FA1 were found to be high in Na (3.3%) when compared to FA2 being low in Na (2.23%). The macro elements of FA1 were found to be high in sodium Na (2.6 ± 0.3) compared to FA2 being low in Na (2.4 ± 0.2). The microelements in FA1 contained more copper (76.01 ± 0.4) than in FA2 (73.2 ± 0.5). The FA1 has higher sulphate content (39.78%) than the FA2 (37.25%), as shown in (Table 1).

Marine algae are rich sources of macroelements such as K, Ca, Na, and Mg. The results of FA were similar to *Sargassum wightii*, which also showed higher Na ions concentration. In *L. digitata,* higher Na, Ca, and Fe were also associated with increased antioxidant capacity, and a lower level had adverse consequences. Brown algae, like *Saccharina latissima*, were reported to be used as an alternative to Na content. Similarities in higher concentrations of Na, Ca, and Fe were found. As shown in earlier studies, the increased levels of K, Zn, Cu, and Co were associated with higher antioxidant levels [16]. The variation in agar yield has been reported to differ from species to species, depending on the harvest season.

Two distinct agar formulations were created to ensure agar quality, effectiveness, safety, and stability. Table 2 shows

Table 2: Stability of FAs.

S. no.	Parameters/tests	FA1 (25°C ± 2°C) months (0–3)	FA2 (25°C ± 2°C) months (0–3)
1	Colour	Brown colour	Brown colour
2	Odour	No odour change	No odour change
3	pH	6	6.5
4	Consistency	Smooth	Smooth
5	Viscosity (m^2/s)	0.413	0.61
6	Homogeneity	Good	Good
7	Sterility	No microbial contamination	No microbial contamination
8	Vibrational test	No phase separation	No phase separation
9	Centrifugation test	No phase separation	No phase separation

both the formulation procedures, FA1 is better for *T. conoides* agar formulation. Stability may be influenced by environmental factors such as pH, light, temperature, air, and movement. No change in colour or odour, as well as robust consistency, smooth homogeneity, no microbiological contamination, and sterility, were determined as stability parameters in two different types of FAs. These findings suggest that the two FA gels would be suitable for topical use. There was no evidence pertaining to the phase separation in the two types of FAs during the three-month investigation.

The FT-IR spectrum of FAs extracted from seaweed confirmed the presence of functional groups and bands absorbed in the 4000–500 cm^{-1} ranges are clearly displayed as shown in Figure 2. FAs are confirmed by FT-IR spectra, which showed peaks around 3283.4 cm^{-1} (OH group), 2922.2 cm^{-1} (alkenes), 1829.7 cm^{-1} (C–N stretch), 1496.2 cm^{-1} (alcohols and carboxylic acids), 1038 cm^{-1} (aliphatic amines), and 892 cm^{-1} (aromatics). A considerable absorption band is discovered in 1260 cm^{-1}. The existence of S=O bonds in sulphate ester groups explains this. The FT-IR analysis of FAs confirmed the presence of galactopyranose unit and sulphate content indicating the presence of polysaccharides contents which are compared with the standard. All of the FT-IR spectra indicated a broad band ranging from 2900 to 3300 cm^{-1}, which is attributed to the O–H stretching vibration from the hydroxyl group of polysaccharides, which corresponded to the hydrophilic property of seaweed [4].

When compared to FA2 (346 kDa), FA1 had a lower molecular weight (205 kDa). The fragmentation spectra of the ions generated from each of the three protonated HexNAcs (m/z 150) were compared first. Despite, the fact that both the HexNAcs have the same composition, GalNAc and GalNAc differ from GlcNAc (Figure 3(a) and B) at a single stereocenter [24]. Agaropectin from red algae has a lower molecular weight and sulphate concentration than other agaropectins, with m/z = 149 for pentoses, 163 for desoxy-hexoses, and 179 for hexoses. [25].

SEM is used to evaluate the morphology and gel surface of the FAs. SEM images of the plain's exterior surface show it

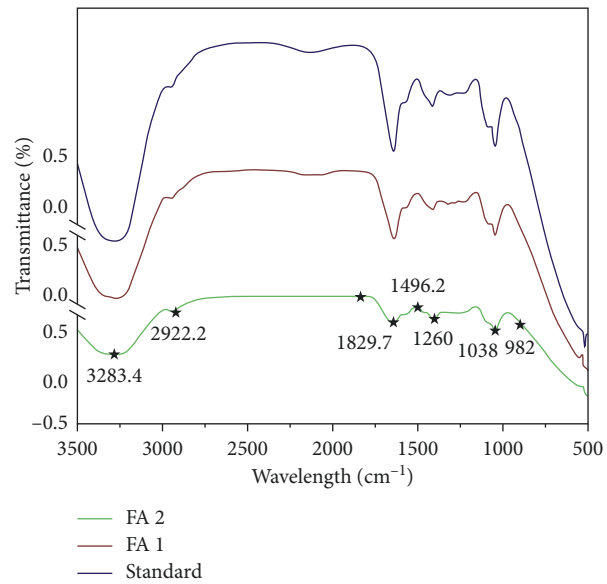
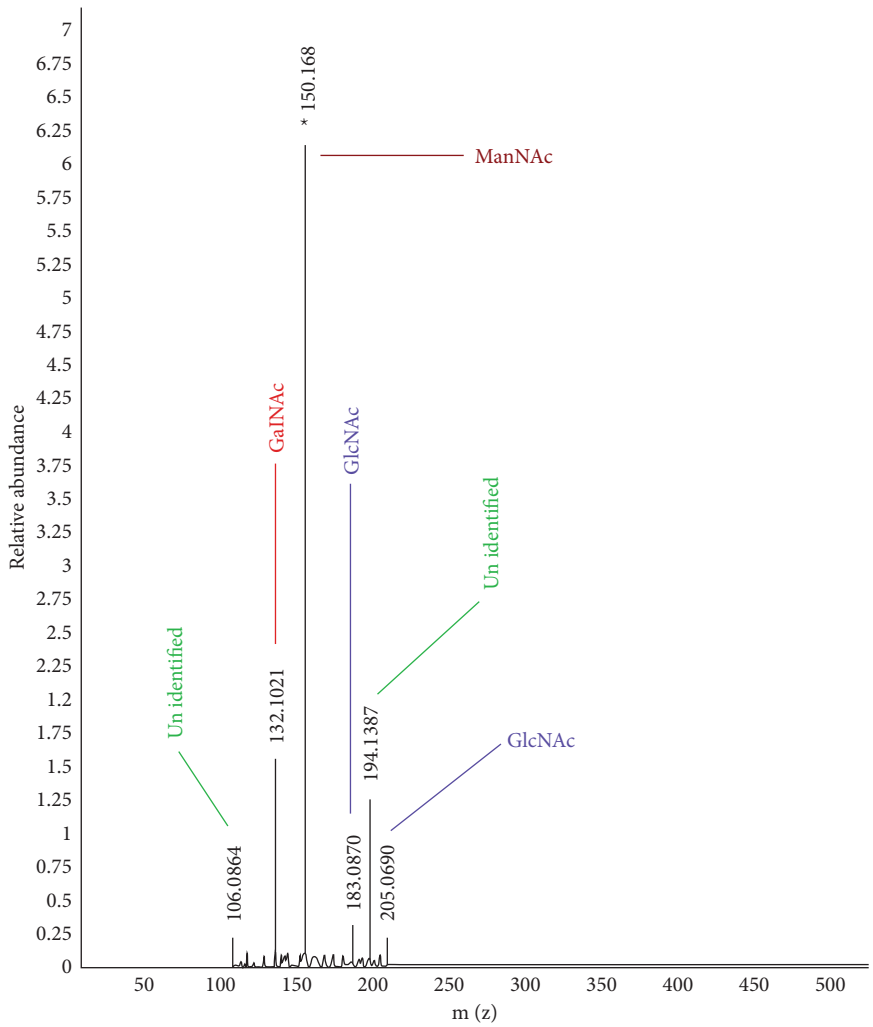


Figure 2: FT-IR spectrum of FAs.

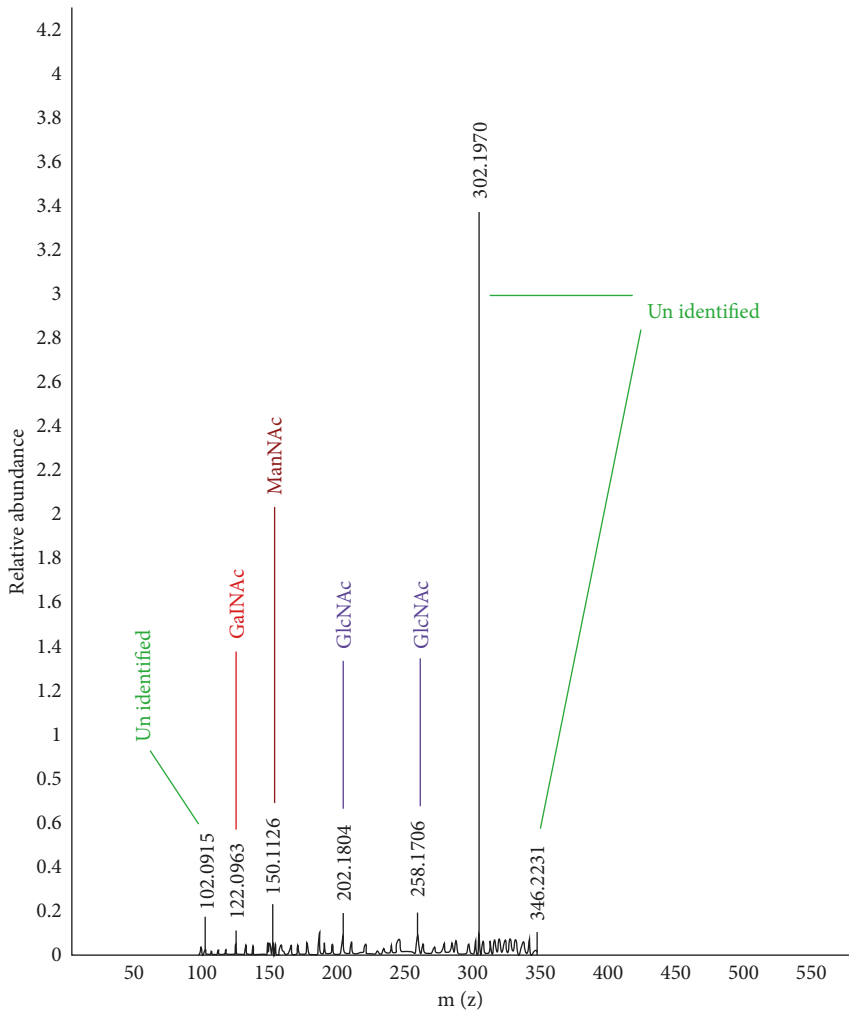
to be flattened, rough, and exceedingly uneven, with a porous structure (Figure 4(a) & 4(b)). The FA1 characteristics' results are present in (Figure 4(a)). It appears to be a smooth, stratified, and ordered flat continuous sheet. Whereas FA2 displayed an irregular, continuous network sheet (Figure 4(b)). According to the findings of Syad et al. [19], the morphological analysis (SEM) extracted from green and brown seaweed has a smooth, flat, and continuous sheet appearance.

The ability of the FA to produce radicals scavenging activities are investigated, as shown in (Table 3). When compared to the antioxidant activities of FA2, FA1 has a significant ($P < 0.05$) increase in antioxidant activities such as DPPH, H_2O_2, total antioxidant, and ABTS activities. FA1 has higher sulphate content (41.78%) than FA2(37.25%), which contributes to its high antioxidant activity. Furthermore, when compared to low dose (60 μg/ml) and standard vitamin E, all of the above antioxidant parameters are significantly increased at a high-dose (180 μg/ml) concentration of FA1 and FA2. As a result, significant differences in the in vitro antioxidant, scavenging abilities of phytochemicals isolated from seaweed could be due to differences in the type of extraction and/or the collection zone of the seaweeds [26]. The 180 μl concentration of FA 1 from *T. conoides* showed significant ($P < 0.05$) increase in equal scavenging (82.28 ± 1.09) activity when compared to the concentration of FA2 of 180 μl (80.11 ± 0.13). The variations in H_2O_2 scavenging percentages in this analysis may be due to differences in extraction techniques or species-specific differences reported in [4]. The ability of the FA to produce total antioxidant activity was investigated, as shown in (Table 3). The 180 μl concentration of FA1 from *T. conoides* showed significant ($P < 0.05$) increase equal scavenging (69.65 ± 3.5) activity when compared to the concentration of FA2 of 180 μl (64.67 ± 1.41). The capability of the FA to produce ABTS radicals was



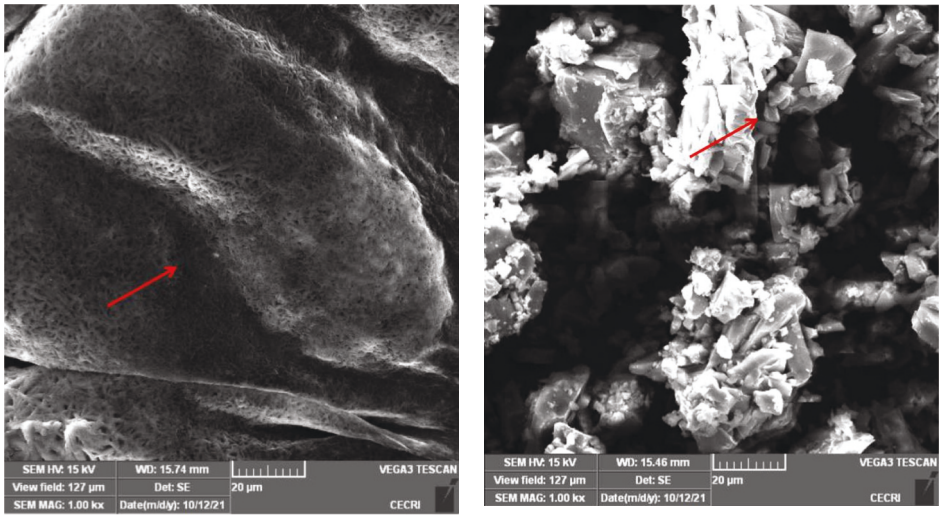
(a)

Figure 3: Continued.



(b)

Figure 3: (a, b) GC-MS spectrum of FAs.



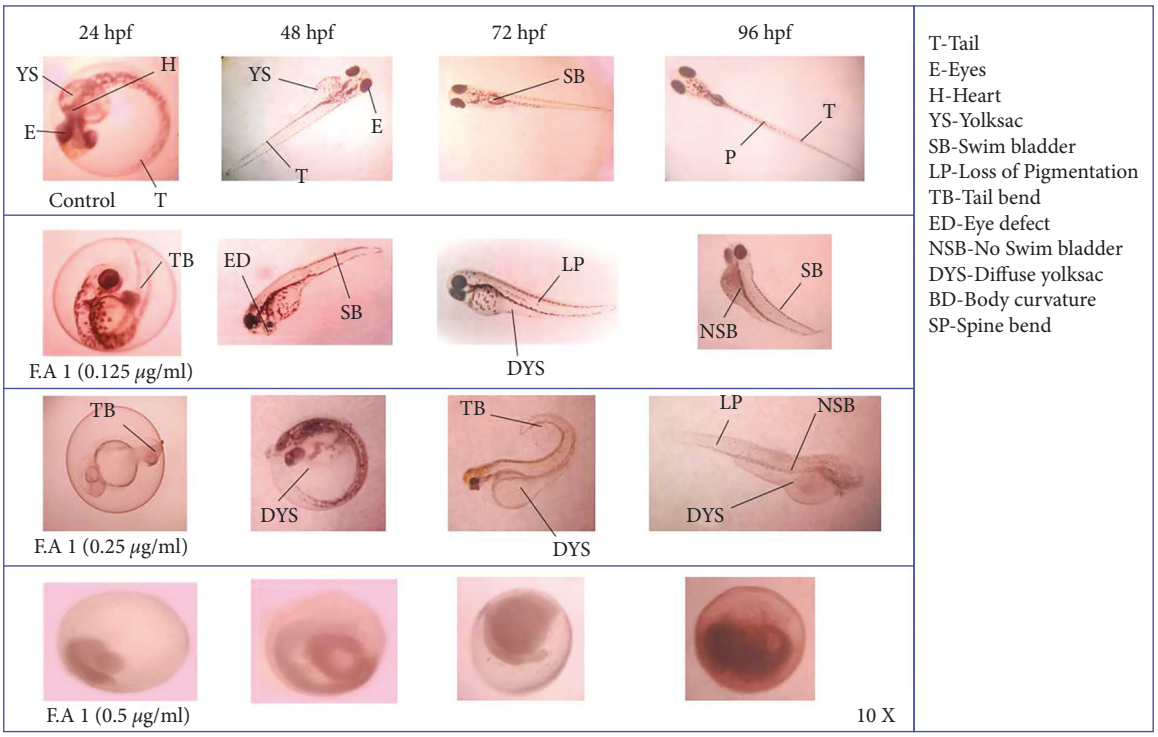
(a) (b)

Figure 4: (a, b) SEM analysis of FAs.

Table 3: *In vitro* antioxidant activities of FAs.

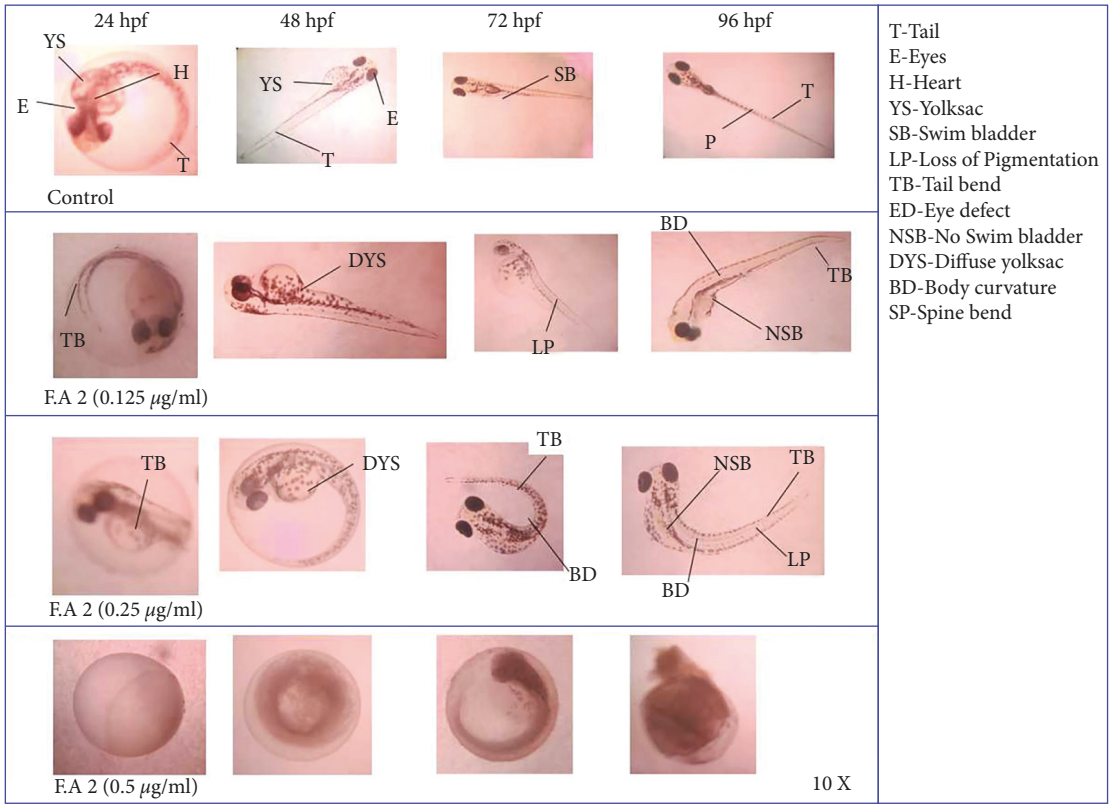
Samples	DPPH scavenging activity (%)	H_2O_2 activity (%)	Total antioxidant activity (%)	ABTS activity (%)
FA1 (60 μl)	*36.12 ± 0.87	*40.18 ± 1.04	*20.95 ± 0.83	*65.6 ± 0.43
FA1 (120 μl)	69.05 ± 0.9	66.52 ± 2.44	41.31 ± 1.27	66.79 ± 0.16
FA1 (180 μl)	*85.14 ± 1.1	*82.28 ± 1.09	*69.65 ± 3.5	*80.66 ± 0.64
FA2 (60 μl)	*29.62 ± 0.53	*39.40 ± 1.1	*20.87 ± 0.09	*58.71 ± 1.1
FA2 (120 μl)	65.14 ± 0.9	62.79 ± 0.47	39.17 ± 0.5	64.03 ± 1.67
FA2 (180 μl)	*79.7 ± 0.5	*80.11 ± 0.13	*64.67 ± 1.41	*72.41 ± 0.35
Vitamin E (180 μl)	*79.86 ± 2.25	*77.13 ± 1.91	*76.11 ± 3.71	*78.50 ± 1.23

∗Statistical significance: $P < 0.05$ (DMRT); *Comparison was made between 180 μl of sample and standard.



(a)

Figure 5: Continued.



(b)

Figure 5: (a, b) Embryotoxicity of FAs.

investigated, as shown in (Table 3). The 180 μl concentration of FA1 from *T. conoides* showed significantly ($P < 0.05$) increase equal scavenging (80.66 ± 0.64) activity when compared to the concentration of FA2 of 180 μl (72.41 ± 0.35). Considering the absence of phytochemical components, the overall antioxidant activity of *Hypericum olympicum* was found to be very high. This is most likely owing to the presence of other compounds mentioned in the study [20].

The toxicity effects of different concentrations of two FAs with different doses of 0.5 μg/ml, 0.25 μg/ml, and 0.125 μg/ml were evaluated in various aspects. In a low concentration of 0.125 μg/ml, FA1 was less teratogenic when compared with FA2 [22]. It shows the development of embryos for different concentrations of two FAs (control, 0.5 μg/ml, 0.25 μg/ml, and 0.125 μg/ml) at various time periods for different concentrations of two FAs (24 hpf, 48 hpf, 72 hpf, and 96 hpf) (Figure 5(a) and 5(b)). The higher toxicity level resulted in deformities such as yolk sac diffusion, delayed hatching, poor development, and death rate. The lowest concentrations, 0.125 μg/ml and 0.25 μg/ml, showed low pigmentation, tail bending, and body curvature, similarly to the control under constant observation in (Figures 5(a) and 5(b)), and when compared to FA2, the teratogenic activity of the sulphated chitosan, FA1, showed less toxicity in zebrafish larvae [27].

4. Conclusion

The agar isolated from *T. conoides* was made into a formulation and FA1 made with more ingredient concentration was found to have higher antioxidant, scavenging activity, and less teratogenicity compared to FA2 made with less ingredient concentration. After clinical trials, the skin permeability of the agar separated may be examined, which could be useful in cosmetic production.

Data Availability

All the data generated or analyzed during this study are included in this published article.

Ethical Approval

Human subjects, human material, or human data are not used in this study. The Institutional Animal Ethics Committee, Chettinad Academy of Research and Education (Deemed to be University), Kelambakkam-603 103, Tamil Nadu, India, approved this study, which followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Conflicts of Interest

All the authors declare that there are no conflicts of interest.

Authors' Contributions

TA and VN carried out general investigations, assays, and in vivo tests. AT assisted with data analysis as well as a variety of other parts of the manuscript development and preparation process. MR, MP, GPP, and SG aided in the research process. SR gave feedback on data analyses after thoroughly reviewing the final manuscript text. RS performed the conceptualization, framework, manuscript preparation, and critical editing/evaluation. The final manuscript was read and approved by all writers.

Acknowledgments

The corresponding author gratefully acknowledges the Department of Biotechnology (BT/PR15676/AAQ/03/794/2016), Ministry of Science and Technology, Govt. of India, New Delhi, for their financial support.

References

[1] K. Chakraborty, F. Makkar, A. Maneesh, and F. Makkar, "Antioxidant activity of brown seaweeds," *Journal of Aquatic Food Product Technology*, vol. 26, no. 4, pp. 406–419, 2017.

[2] I. S. Fernando, M. Kim, K. T. Son, Y. Jeong, and Y. J. Jeon, "Antioxidant activity of marine algal polyphenolic compounds: a mechanistic approach," *Journal of Medicinal Food*, vol. 19, no. 7, pp. 615–628, 2016.

[3] E. D. Goddard and J. V. Gruber, *Principles of Polymer Science and Technology in Cosmetics and Personal Care*, CRC Press, New York USA, 1999.

[4] M. Venkatesan, V. Arumugam, R. Pugalendi et al., "Antioxidant, anticoagulant and mosquitocidal properties of water soluble polysaccharides (WSPs) from Indian seaweeds," *Process Biochemistry*, vol. 84, pp. 196–204, 2019.

[5] P. Leelapornpisid, L. Mungmai, and B. Sirithunyalug, "A novel moisturizer extracted from freshwater macroalga [Rhizoclonium hieroglyphicum (C. Agardh) Kützing] for skin care cosmetic," *Chiang Mai Journal of Science*, vol. 41, pp. 1195–1207, 2014.

[6] A. Ponnan, K. Ramu, and M. Marudhamuthu, "Antibacterial, antioxidant and anticancer properties of Turbinaria conoides," *J. Agardh) Kuetz. Clinical Phytoscience*, vol. 3, no. 1, pp. 1–10, 2017.

[7] K. Benchoula, A. Khatib, F. M. C. Quzwain et al., "Optimization of hyperglycemic induction in zebrafish and evaluation of its blood glucose level and metabolite fingerprint treated with *Psychotria malayana* jack leaf extract," *Molecules*, vol. 24, no. 8, p. 1506, 2019.

[8] X. Wang, D. Duan, and X. Fu, "Enzymatic desulfation of the red seaweeds agar by Marinomonas arylsulfatase," *International Journal of Biological Macromolecules*, vol. 93, pp. 600–608, 2016.

[9] E. A. Yapar, Ö. Ýnal, and M. S. Erdal, "Design and in vivo evaluation of emulgel formulations including green tea extract and rose oil," *Acta Pharmaceutica*, vol. 63, no. 4, pp. 531–544, 2013.

[10] A. P. Mishra, S. Saklani, L. Milella, and P. Tiwari, "Formulation and evaluation of herbal antioxidant face cream of Nardostachys jatamansi collected from Indian Himalayan region," *Asian Pacific Journal of Tropical Biomedicine*, vol. 4, pp. 679–682, 2014.

[11] V. C. K. N. Deuschle, R. A. N. Deuschle, M. R. Bortoluzzi, and M. L. Athayde, "Physical chemistry evaluation of stability, spreadability, in vitro antioxidant, and photo-protective capacities of topical formulations containing *Calendula officinalis* L. leaf extract," *Brazilian Journal of Pharmaceutical Sciences*, vol. 51, no. 1, pp. 63–75, 2015.

[12] M. Emeje, S. Boyi, I. Obidike, C. Isimi, O. Kunle, and S. Ofoefule, "Natural antidiabetic compound for the therapeutic management of diabetes mellitus and its drug delivery system," *Journal of Dietary Supplements*, vol. 8, no. 3, pp. 266–279, 2011.

[13] J. P. Jadhav, S. S. Phugare, R. S. Dhanve, and S. B. Jadhav, "Rapid biodegradation and decolorization of Direct Orange 39 (Orange TGLL) by an isolated bacterium *Pseudomonas aeruginosa* strain BCH," *Biodegradation*, vol. 21, no. 3, pp. 453–463, 2010.

[14] A. Bouranen, *Determination of the Stability of Cosmetic Formulations with Incorporation of Natural Products*, pp. 10–50, Doctoral dissertation), 2017.

[15] R. Aiyalu, A. Govindarjan, and A. Ramasamy, "Formulation and evaluation of topical herbal gel for the treatment of arthritis in animal model," *Brazilian Journal of Pharmaceutical Sciences*, vol. 52, no. 3, pp. 493–507, 2016.

[16] R. Karthik, V. Manigandan, R. Sheeba, R. Saravanan, and P. R. Rajesh, "Structural characterization and comparative biomedical properties of phloroglucinol from Indian brown seaweeds," *Journal of Applied Phycology*, vol. 28, no. 6, pp. 3561–3573, 2016.

[17] M. Tomczykowa, M. Wróblewska, K. Winnicka et al., "Novel gel formulations as topical carriers for the essential oil of *bidens tripartita* for the treatment of candidiasis," *Molecules*, vol. 23, no. 10, p. 2517, 2018.

[18] S. S. Shitole, A. K. Balange, and S. S. Gangan, "Use of seaweed (*Sargassum tenerrimum*) extract as gel enhancer for lesser sardine (*Sardinella brachiosoma*) surimi," *International Aquatic Research*, vol. 6, no. 1, p. 45, 2014.

[19] A. N. Syad, K. P. Shunmugiah, and P. D. Kasi, "Seaweeds as nutritional supplements: analysis of nutritional profile, physicochemical properties and proximate composition of *Gelidiella acerosa* and *Sargassum wightii*," *Biomedicine & Preventive Nutrition*, vol. 3, no. 2, pp. 139–144, 2013.

[20] D. Zheleva-Dimitrova, P. Nedialkov, and G. Kitanov, "Radical scavenging and antioxidant activities of methanolic extracts from *Hypericum* species growing in Bulgaria," *Pharmacognosy Magazine*, vol. 6, no. 22, p. 74, 2010.

[21] J. Prakash, M. Venkatesan, J. Sebastian Prakash J et al., "Investigations on the in-vivo toxicity analysis of reduced graphene oxide/TiO2 nanocomposite in zebrafish embryo and larvae (*Danio rerio*)," *Applied Surface Science*, vol. 481, pp. 1360–1369, 2019.

[22] C. R. David, A. Angeles, and R. C. Angoluan, "*Moringa oleifera* (Malunggay) water extracts exhibit embryo-toxic and teratogenic activity in zebrafish (*Danio rerio*) embryo model," *Der Pharmacia Lettre*, vol. 8, pp. 163–168, 2016.

[23] N. A. Chudasama, R. A. Sequeira, K. Moradiya, and K. Prasad, "Seaweed polysaccharide based products and materials: an assessment on their production from a sustainability point of view," *Molecules*, vol. 26, no. 9, p. 2608, 2021.

[24] A. Mookherjee, S. S. Uppal, and M Guttman, "Dissection of fragmentation pathways in protonated *N*-acetylhexosamines," *Analytical Chemistry*, vol. 90, no. 20, pp. 11883–11891, 2018.

[25] Y. Zhang, X. Fu, D. Duan, J. Xu, and X. Gao, "Preparation and characterization of agar, agarose, and agaropectin from the red alga *Ahnfeltia plicata*," *Journal of Oceanology and Limnology*, vol. 37, no. 3, pp. 815–824, 2019.

[26] S. M. Shanab, S. S. Mostafa, E. A. Shalaby, and G. I. Mahmoud, "Aqueous extracts of microalgae exhibit antioxidant and anticancer activities," *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 8, pp. 608–615, 2012.

[27] V. Gomathy, V. Manigandan, N. Vignesh, A. Thabitha, and R. Saravanan, "Evaluation of antibacterial, teratogenicity and antibiofilm effect of sulfated chitosans extracted from marine waste against microorganism," *Journal of Bioactive and Compatible Polymers*, vol. 36, no. 3, Article ID 088391152110142, 2021.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7990508, 32 pages
https://doi.org/10.1155/2022/7990508

Review Article

Medicinal Uses, Phytochemistry, Pharmacology, and Toxicology of *Mentha spicata*

Naoual El Menyiy,[1] Hanae Naceiri Mrabti,[2] Nasreddine El Omari,[3] Afaf EI Bakili,[4] Saad Bakrim,[5] Mouna Mekkaoui,[6] Abdelaali Balahbib,[7] Ehsan Amiri-Ardekani,[8] Riaz Ullah,[9] Ali S. Alqahtani,[9] Abdelaaty A. Shahat,[9] and Abdelhakim Bouyahya[10]

[1]*Laboratory of Pharmacology, National Agency of Medicinal and Aromatic Plants, Taounate 34025, Morocco*
[2]*Laboratory of Pharmacology and Toxicology, Bio Pharmaceutical and Toxicological Analysis Research Team, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Rabat, Morocco*
[3]*Laboratory of Histology, Embryology and Cytogenetic, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco*
[4]*Mohammed V University in Rabat, LPCMIO, Materials Science Center (MSC), Ecole Normale Supérieure, Rabat, Morocco*
[5]*Molecular Engineering, Valorization and Environment Team, Department of Science and Technology, Polydisciplinary Faculty of Taroudant, Ibn Zohr University, Taroudant, Morocco*
[6]*Pharmacodynamics Research Team, Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy, University Mohammed V in Rabat, Morocco*
[7]*Laboratory of Biodiversity, Ecology and Genome, Faculty of Sciences, Mohammed V University, Rabat 10106, Morocco*
[8]*Department of Phytopharmaceuticals (Traditional Pharmacy), Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran*
[9]*Department of Pharmacognosy (Medicinal Aromatic and Posnous Plants Research Center, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[10]*Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences and Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Rabat, Morocco*

Correspondence should be addressed to Riaz Ullah; rullah@ksu.edu.sa and Abdelhakim Bouyahya; boyahyaa-90@hotmail.fr

Received 26 October 2021; Accepted 3 February 2022; Published 12 April 2022

Academic Editor: Saravanan Ramachandran

Mentha spicata, also called *Mentha viridis*, is a medicinal plant of the Lamiaceae family characterized by its potency to synthesize and secret secondary metabolites, essentially essential oils. Different populations use the aerial parts of this plant for tea preparation, and this tisane has shown several effects, according to ethnopharmacological surveys carried out in different areas around the world. These effects are attributed to different compounds of *M. spicata*, in which their biological effects were recently proved experimentally. Pharmacological properties of *M. spicata* extracts and essential oils were investigated for different health benefits such as antioxidant, anticancer, antiparasitic, antimicrobial, and antidiabetic effects. In vitro and in vivo studies showed positives effects that could be certainly related to different bioactive compounds identified in *M. spicata*. Indeed, volatile compounds seem to be efficient in inhibiting different microbial agents such as bacteria, fungi, and parasites through several mechanisms. Moreover, *M. spicata* exhibited, according to some studies, promising antioxidant, antidiabetic, anti-inflammatory, and anticancer effects, which show its potential to be used as a source for identifying natural drugs against cellular oxidative stress and its related diseases. Importantly, toxicological investigations of *M. spicata* show the safety of this species at different doses and several periods of use which justify its use in traditional medicines as tisane with tea. Here, we report, explore, and highlight the data published on *M. spicata* concerning its botanical description and geographical distribution, its phytochemical compounds, its pharmacological properties, and its toxicological investigations of *M. spicata*.

1. Introduction

The use of *M. spicata* is importantly characterized in several populations, including Moroccan population, which has used the aerial parts (with tea) of this plant since time against several diseases including diabetes, digestive and respiratory disorders, throat ailments, and skin disease [1, 2].

Certainly, *M. spicata* contains molecules biologically active having biological effects, and effective spectroscopic analysis of extracts and essential oils of *M. spicata* using GC-MS, HPLC, HPLC-MS, and RMN revealed the presence of several phytochemical bioactive compounds belonging to different classes of secondary metabolites in particularly the classes of flavonoids, phenolic acids, and terpenes [3, 4]. Indeed, the distribution of these chemical compounds between different plant parts and collection regions is variable, which explains different traditional uses (with efficacy) of this species according to each region. In addition, the extraction of these chemical compounds depends on used methods and therefore can justify the difference in traditional applications according to used methods of pharmaceutical formulations preparation.

In vitro and in vivo experimental explorations showed that *M. spicata* extracts and essential oils exhibit remarkable biological activities, including antimicrobial, antiparasitic, antidiabetic, anti-inflammatory, and anticancer effects. Indeed, different organic extracts (rich in bioactive compounds) revealed important antifungal activity by their potency to inhibit the growth of some strains involved in human infections such as *Aspergillus niger, Candida albicans, Cryptococcus neoformans*, and *Microsporum audouinii* [5]. Moreover, *M. spicata* showed antibacterial properties against various bacterial strains, either clinical or reference [6, 7]. It was also revealed that *M. spicata* extracts target some human complex diseases, including chronic inflammatory diseases, diabetes, and cancers. Plant extracts inhibit or activate targets and/or pathways involved in these pathologies, including membrane receptors, signaling pathways, and molecular targets [8, 9].

To the best of our knowledge, despite numerous investigations that have been carried out until now showing remarkable results, there are now literature reviews exploring *M. spicata* as a source of potential lead compounds. Therefore, this review aims to explore, discuss, and highlight all data concerning *M. spicata* and give suggestions about its exploitation as a source for developing bioactive compounds in the pharmaceutical and cosmetic fields.

2. Research Methodology

3. Results and Discussion

3.1. Taxonomy, Botanical Description, Geographic Distribution, and Ecological Factors. Mentha spicata (ID: 29719) is also known as spearmint. There are a couple of heterotypic synonyms for this species including *Mentha cordifolia, Mentha crispa* var. *crispata f. reticulata, Mentha viridis* (L.) L., *Mentha × cordifolia*, and *Mentha × villosa* var. *cordifolia.* It is an aromatic plant that belongs to the genus *Mentha*, family Lamiaceae, subfamily Nepetoideae, placed in Magnoliopsida class, and belongs to order Lamiales. The genus *Mentha*, one of the most important members of the Lamiaceae family, is represented by 19 species and 13 natural hybrids, and Lamiaceae family consists of over 7000 species and around 260 genera of trees and shrubs [10]. The spearmint, *M. spicata*, is a hybrid of *M. longifolia* and *M. rotundifolia.* This species is widely grown in Europe, North America, and Asia, but nowadays cultivated throughout all regions of the world [11].

M. spicata L. (spearmint) is a creeping rhizomatous, glabrous, and perennial herb with a strong aromatic odor, growing up to 30–100 cm tall with variably hairless to hairy stems and foliage, and a wide spreading fleshy underground rhizome [12]. The leaves are ovate to lancolate, 5–9 cm long and 1.5–3 cm broad, with a serrated margin. Spearmint produces flowers in slender spikes, each flower pink or white, and 2.5–3 mm long and broad. The stem is square-shaped, a trademark of the mint family of herbs [13]. *M. spicata* L is well adapted to climatic conditions in tropical and subtropical areas. It can be cultivated in wide range of soils and found in back gardens of homesteads [14].

3.2. Medicinal Uses. Mentha viridis is widely used in a variety of applications [15]. Since ancient times, Western and Eastern cultures have practiced *Mentha viridis* as a medicinal and aromatic plant against several diseases (Table 1) [15]. Ethnobotanical investigations into *Mentha viridis* have suggested its potential medical applications in different disorders. It has beneficial effects on diabetes, digestive, skin, and respiratory disorders [1, 2, 16–23].

In Morocco, *Mentha viridis* is a medicinal plant most used in the treatment of throat ailments. The use of this plant to treat throat ailments has been demonstrated by Orch et al. [20], who reported the use of aerial parts' infusion of *Mentha viridis* in Moroccan oriental folklore. The leaves of *M. viridis* are also administered as a decoction to treat diabetes in the Al Haouz-Rhamna region (Morocco) [1]. Idm' hand et al. [17] showed that the leaves and stems of *M. viridis* are also used as a decoction and infusion to treat diabetes; on the other hand, El-hilaly et al. [16] showed that these parts were used to treat headache and tiredness. The leaves and flowers of *M. viridis* have also been widely used to treat asthma, bronchitis, chest pain, lung disorders, kidney problems, and diuretics by decoction or infusion [18]. In addition, the leaves of *M. viridis* have been used against gastric disorders by decoction, and the stems are used against ailments of intestines [2, 23]. *M. viridis* whole plant infusions are also used to treat aphrodisiac, cold, flatulence, headache, tonic, and toothache [19, 22]. In another study in Morocco, the powder from the leaves of *M. viridis* is used to treat skin diseases [21].

Table 1: Medicinal use of *M. spicata*.

Used part	Dosage form	Traditional use	References
Leaf	Decoction	Diabetes	[1]
Leaf	Decoction	Against stomach disorders	[2]
Leaf, stem	Infusion	Headache, tiredness	[16]
Leaf, stem	Infusion and decoction	Diabetes	[17]
Leaf, flower	Infusion and decoction	Asthma, bronchitis, chest pain, lungs disorder, kidney problems, diuretic	[18]
Leaf	Infusion	Cold and flu, toothache	[19]
Aerial parts	Infusion	Throat affection	[20]
Leaf	Powder	Skin diseases	[21]
Whole plant	Infusion	Aphrodisiac, cold, flatulence, headache, tonic, toothache	[22]
Leaf, stem	Decoction	Against the ailments of intestines	[23]

3.3. Phytochemical Compounds. Extracts and essential oils extracted from *M. spicata* (*viridis*) are considered as valuable source phytochemicals, including natural phenolics and EO_S. These volatile compounds are complex mixtures of substances that have been found to create different chemotypes distinguished based on the dominant compound in the essential oil, which depends on the plant species, and within the same variety, the essential oil composition can vary according to the geographical region [24]. In terms of phytochemical content, terpenes and terpenoids are the major components of EOs obtained from aerial parts of *M. spicata*. Thus, more detailed discussion regarding chemical aspects of EOs of these species is described (Table 2). Previous studies reported the existence of different chemotypes in the chemical composition of *M. spicata*, naturally grown as cultivated, around the world, and the essential oil mainly composed of carvone, carvacrol, trans-carveol, piperitone oxide, limonene, 1,8-cinéole, camphene, p-cymene, dihydrocarvone, pulegone, β-caryophyllene, germacrene D, menthone, α-pinene, and linalool [3, 5, 26, 27]; whereas, carvone is mentioned as the absolute predominant constituent of *M. spicata* oil as well as monoterpenes including linalool, piperitone, piperitone oxide, menthone, isomenthone, and pulegone (Figure 1 and Table 2). The composition of *M. spicata* EO_S from Morocco is relatively stable and has strong homogeneity [7, 40, 53, 56]. No significant difference between samples was observed; whatever the locality (region), the main essential oil compounds are carvone and trans-carveol, showing variation in a narrow range of 29–47.3% and 14–20%, respectively [34, 46, 47, 51, 52]. Various chemotypes of *M. spicata* were also identified for plants cultivated in Italy and Turkey. In plants from Italy, carvone (39.13–59.26%) was detected as the main compound [29], while for the species from Turkey, piperitenone oxide (25.84%), pulegone (24.72%), cis-piperitenone oxide (12.55%), and limonene 1.59% were the principal constituents of the EO_S [31]. It is worth noting that chemotype carvone represented the most variation, 79.70% in spearmint *M. spicata* EO_S [24]. Other examples of *M. spicata* producing EOs with high piperitone oxide content (above 70%) are samples from India [36]. As established in the literature, such compound is one of the most abundant components of *M. spicata* EO, which offers spearmint its unique smooth characteristic scent [57], and it also varies according to the spearmint oil grown in different countries. Similarly, EOs from *Cyprus* is reported to possess a higher carvone content (69.23–74.27%) [55].

However, four chemotypes of *M. spicata* were found in Brazil, characterized by the dominant occurrence of carvone which vary from 39.42% to 72.28% and piperitone presented high level 81.18% [7, 56], Although carvone was constantly present as a chief component among spearmint species, there was one landrace with linalool content up to 58.51%. Since all the studies were carried out in the same environmental conditions, this variation may be triggered by their different genetic backgrounds, having evolved due to complex geographic-environmental differences across Brazil. Interestingly, in most *M. spicata* EOs, carvone is the major constituent, notably found in quantities above 50% in EOs extracted from plants cultivated in Hungary, Iran, Bangladesh, Serbia, Czech Republic, and Pakistan [3, 5, 40, 46, 47, 52, 53].

Furthermore, the occurrence of huge chemical variations among *Mentha* accessions collected from diverse countries seems to be due to the divergent climatological and geographical conditions; existing variations in oil content and composition may be attributed to factors related to ecotype and the environment including temperature, relative humidity, irradiance, and photoperiod [34]. Additionally, the reported yields of carvone for *M. spicata* range from 39.21% to 75.53%, being the highest value found for plants cultivated in Tunisia [51].

As given in Table 2, plants cultivated in several states in Iran usually produce EOs with high (>50%) 1,8-cineole content [39]. Similarly, *M. spicata* populations in China also show certain stability in essential oils, with carvone chemotype affording high yield 46.7–65.4% above, while dihydrocarveol acetate (0.2–7%) observed in Chinese spearmint is the only oxygenated sesquiterpenes [46]. Also, a large chemical variability is observed among *M. spicata* essential oil extracted by different methods. Such variation can be attributed to several factors, including genetic, environmental, and their interaction effects, such as plant part, harvest time, extraction method, ecotype, and geographic origin (climate, edaphic, elevation, and topography) [4].

M. spicata has a broad spectrum of bioactive compounds; preliminary screening of *M. spicata* revealed the presence of polyphenols, flavonoids, tannins, sterols, triterpenes, and glycosides [58]. Besides, the chemical composition of *M. spicata* methanolic extracts harvested from different regions of India confirmed the presence of alcohols, phenols, alkanes, alkenes, carbonyl, carboxylic acids, and aromatic compounds [35]. Besides, Bimakr et al. identified

Table 2: Chemical compounds of *M. spicata*.

Country	Part used	Compounds	Reference
Morocco	Aerial parts	Carvone (33.14%) trans-Carveol (20.06%) β-Caryophyllene (4.41%) 1,8-Cineole (3.99%) Germacrene D (3.14%) Menthone (2.19%) α-Pinene (1.06%)	[25]
	Aerial parts	Carvone (47.30–69.19%) Limonene (4.48–15.43%) trans-4-Caranone (0.82–4.63%) iso-Dihydrocarveol acetate (0.06–2.66%) ρ-Mentha-3,8-diene (0.85–1.32%)	[26]
	Aerial parts	Carvone (57.11%) Limonene (27.77%) 3-Carene (1.01%) Germacrene D (0.65%)	[27]
	Aerial parts	Carvone (29.00%) trans-Carveol (14.00%) 1,8-Cineole (7.30%) Dihydrocarveol (l4.50%) Carvyl acetate-Z (6.70%) Germacrene D (3.90%)	[28]
Italy	Aerial parts	Carvone (39.13 to 59.26%) 1,8-Cineole (1.07–9.02%) Dihydrocarveol (2.36–5.94%) Germacrene D (1.79–4.11%) Limonene (5.9–11.40%) trans-Carvyl acetate (0.72–5.90%)	[29]
	Aerial parts	p-Cymene (33.9%) iso-Piperitone (23.7%) Piperitone (6.9%) Menthone (21.8) p-Cymen-8-ol (19.6) β-Linalool (15.2%)	[30]
Czech Republic	Aerial parts	Carvone (0.7–59.1%) Menthol (1.1%–14.9%) p-Menthone (1.1%–4.4%) Piperitone oxide (34.1%) Germacrene D (14.6%) β-Caryophyllene (2.2–3%) Dihydrocarvone (11.8–12.7%) cis-Jasmone (1.6–1.8%)	[3]

Table 2: Continued.

Country	Part used	Compounds	Reference
Turkey	Aerial parts	Piperitenone oxide (25.84%) Pulegone (24.72%) cis-Piperitenone oxide (12.55%) Limonene (1.59%)	[31]
	Aerial parts	Carvone (34.70 to 79.70%) 1,8-Cineole (3.40–33.80%) β-Pinene (0.87–5.29%) Limonene (1.10–22.10%) Menthone (0.20–2.73%)	[24]
	Aerial parts	Pulegone (1.70–9.94%) Carvone (48.6–57.9%) ρ-Cymene (9.6–20.5%) 1,8-Cineole (14.6–19.3%) Carvacrol (0.1–3.5%) α-Pinene(2.3–4.3%)	[32]
	Phenolic acids	Rosmarinic acid derivatives (88%) Salvianolic acids (5.6%) Caffeoylquinic acids (1.2%) Hydroxycinnamic (1.1%)	[30]
	Fatty acids	Palmitic acid (5.11 0.41%) Stearic acid (1.92 ± 0.21%) Oleic acid (8.19%) Linoleic acid (31.14%) α-Linolenic acid (48.17%) γ-Linolenic acid(2.07%) Stearidonic acid (3.02%)	[33]
India	Aerial parts	Carvone (49.62–76.65%) Limonene (9.57–22.31%) 1,8-Cineole (1.32–2.62%) trans-Carveol (0.3–1.52%)	[34]
	Phenolics	Pentadecanoic acid (7.47%) 7-Oxabicyclo[4.1.0] heptane (9.56%) 3-Penten-2-one,4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0] hept-1-yl)-,(E)-(12.20%) stigmast-4-EN-3-one (18.99%)	[35]
	Aerial parts	trans-Muurola-4 (14%) 5-Diene (27.28%) Piperitenone oxide (22.22%) β-Caryophyllene (10.48%) Geranyl propanoate (6.55%) Sibirene (3.45%) Borneol (1.98%) Allo-ocimene (1.71%) β-Elemene (1.34%) Germacrene D-4-ol (1.02%)	[36]
	Aerial parts	Carvone (57.49–72.47%) Limonene (10.70–24.81%) Myrcene (0.25–4.36%) 1,8-Cineole (0.2–2.02%)	[37]
	Aerial parts	Carvone (48.60%) Limonene (11.30%) cis-Carveol (21.30%) Linalool (1.30%) 1,8-Cineole (2.55%) cis-Carvyl acetate (2.10%) cis-Dihydrocarvone (1.30%)	[38]

Table 2: Continued.

Country	Part used	Compounds	Reference
Iran	Aerial parts	Carvone (65.15–74.21%) Limonene (12.22–20.55%) cis-Dihydrocarvone (2.34–11.13%) Caryophyllene (1.13–5.06%)	[39]
	Aerial parts	Carvone (42.74–54.34%) trans-Dihydrocarvone (21.58%) 1,8-Cineole (8.41–21.78%) Pulegone (6.83%) Limonene (5.2–6.1%) β-Caryophyllene (3.05%) Linalool (5.82%) trans-Dihydrocarvone (3.18%)	[4]
	Aerial parts	Carvone (49.91%–56.92%) Piperitone oxide (10.69%–11.72%) 1,8-Cineole (3.78–3.34%) Limonene (7.33–6.61%) Germacrene D (6.26–1.90%)	[40]
	Aerial parts	Carvone (54.34%) 1,8-Cineole (8.41–22.71%) Piperitenone oxide (58.87%) 3,8-Menthadiene (21.58%) α-Pinene (0.95–1.68%) 2-Cyclohexen (42.74%) Borneol (5.82%) DL-Limonene (5.2%) Pulegone (6.83%)	[41]
Malaysia	Flavonoids leaves	Catechin (14–14.4%) Epicatechin (15.6–16.3%) Rutin 1 (4.8–16.1%) Myricetin (4.1–11.7%) Luteolin (9.3–65.7%) Apigenin (27–39.2%) Naringenin (5.4–24.9%)	[42]
Algeria	Leaves	Carvone (59.40%) Limonene (6.12%) 1,8-Cineole, germacrene D (04.66%) β-Caryophyllene (2.969%) β-Bourbonene (2.796%) α-Terpineol (1.986%) Terpinene-4-ol (1.120%)	[7]
Brazil	Aerial parts	Carvone (39.42–72.28%) Pulegone (5.53–10.48%) Carveol (3.30–4.98%) Cineol (1.49%)	[43]
	Leaves	Linalool (58.51%) Carvone (15.1%) α-Terpineol (1.43%) β-Caryophyllene (2.02%) Eucalyptol (1.04%)	[6]
	Leaves	Terpinen-4-ol (5.73%) Piperitone (81.18%) Piperitenone (14.75%) α-Pinene (0.51%) Limonene (1.47%)	[44]
	Aerial parts	Limonene (2.04–19.91%), Isomenthone (0.46–11.60%) Menthone (0.46–11.60%) 1,8-Cineole (eucalyptol) (2.98–8.10%) d-Carvone (31.35–60.07%) β-Pinene (2.41–4.27%) Isomenthone (4.46%) Pulegone (6.68–53.65%)	[45]

Table 2: Continued.

Country	Part used	Compounds	Reference
China	Aerial parts	Carvone (46.7–65.4%) Limonene (0.3–1.8%) Linalool (0.6 6.9%) Menthone (1.5–4.7%) Dihydrocarvone (0.8–15.7%) Dihydrocarveol acetate (0.2–7%)	[46]
China	Aerial parts	Carvone (65.33%) Limonene (18.19%) Dihydrocarvone (2.97%) Camphene (2.34%)	[11]
Hungary	Leaves	Carvone (35.9–60.5%) Citronellol (10.1–13.4%) Limonene (1.6–9.4%) Menthone (3.2–4.4%) α-Terpineol (2.1–3%) cis-Dihydrocarvone (1.5–2.2%) (e)-b-Caryophyllene (1.5–2.1%)	[47]
Jordan	Aerial parts	Carvone (49.5%) Limonene (16.1%) 1,8-Cineole (8.7%) cis-Dihydrocarvone (3.9%) β-Caryophyllene (2.7%) Germacrene D (2.1%) β-Pinene (1.1%)	[48]
Abu Dhabi	Leaves	Carvone (14.79–87.11%) Dihydrocarvone (0.09–0.19%) Cineole (0.2–0.6%) Limonene (1.94–9.72%) Menthol (0.06–0.19%) Linalool (0.09–0.23%) α-Pinene (0.05–0.3%)	[49]
Romania	Phenolics, 70% ethanol	Ferulic acid (27.32%) Sinapic acid (6.60%) p-Coumaric acid (15.24%) Luteolin (4.68%)	[50]
Tunisia	Aerial parts	Carvone (39.21–75.53%) 1,8-Cineole (7.24–12.49%) Limonene (6.07–18.45%) cis-Dihydrocarveol (1.17–6.56%) trans-Carveol (0–5.22%) Pulegone (38.74%) Menthone (28.56%) Menthol (5.64%)	[51]
Bangladesh	Aerial parts	Carvone (73.29%) D-Limonene (7.59%) Dihydrocarvone (3.83%) α-Bourbonene (1.67%) trans-Sabinene hydrate (1.57%) trans-Carveol (1.25%) Dihydrocarveol (1.12%) Eucalyptol (1.01%)	[52]
Serbia	Aerial parts	Carvone (49.5%) Menthone (21.9%) Piperitone 0.6%) β-Bourbonene (26.8%) β-Caryophyllene (0.7%) Germacrene A(0.5%)	[53]

Table 2: Continued.

Country	Part used	Compounds	Reference
Palestine	Aerial parts	Limonene (6.23–9.79%) Carvone (36.9–76.82%) Sabinene (0.14–5.51%) cis-Dihydrocarvone (0.65–4.59%) β-Caryophyllene (0.81–3.87%) Dihydrocarveol (2.27–13.76%)	[54]
Cyprus	Aerial parts	Carvone (ketone: 69.23–74.27%) Limonene (alkene: 10.42–11.39%) 1,8-Cineole (alcohol: 5.28–5.99%) β-Pinene (alkene: 1.13–1.25%) β-Caryophyllene (alkene: 0.80–1.29%) Germacrene D (alkene: 2.09–3.13%) Bicyclogermacrene (0.60–1.01%)	[55]
Pakistan	Aerial parts	Carvone (51.7%) cis-Carveol (24.3%) Limonene (5.3%) 1,8-Cineol (4.0%) cis-Dihydrocarvone (2.2%) Carvyl acetate (2.1%) cis-Sabinene hydrate (1.0%)	[5]

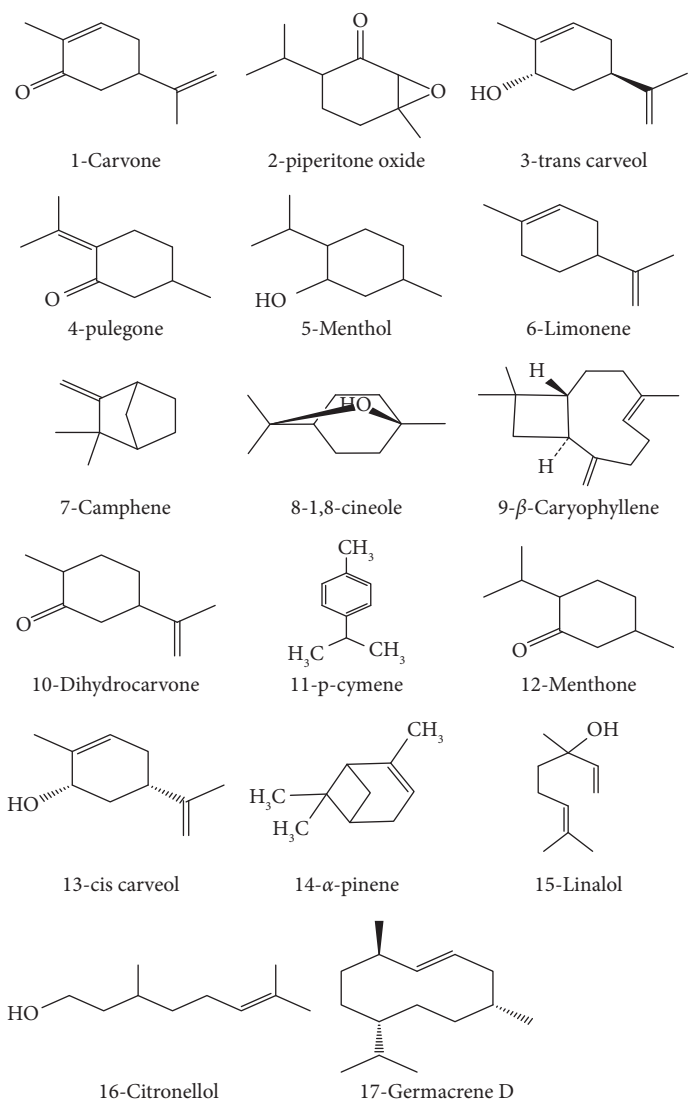


Figure 1: Chemical structures of terpenoids identified.

the flavonoid content from *M. spicata* leaves by using conventional Soxhlet extraction (CSE) and supercritical carbon dioxide (SC-CO2) extraction [42]. The highest content was obtained with methanol solvent, which extracted seven flavonoids. The highest recovery was recorded for the free aglycone apigenin 27–39.2%, followed by naringenin 5.4–24.9%, epicatechin 15.6–16.3%, catechin 14–14.4%, rutin 14.8–16.1%, myricetin 4.1–11.7%, and luteolin 9.3–65.7% (Figure 2 and Table 2); the same study also identified apigenin as the major isolated flavonoid (6.14 ± 0.76%) from ethanolic and hydroethanolic fractions. Interestingly, supercritical carbon dioxide extract was found to have more main flavonoid compounds and high recovery comparing to the 70% ethanol Soxhlet extraction [42].

The ethanolic extracts of *M. spicata* contain a large amount of phenolic compounds (polyphenols, flavonoids, and caffeic acid derivatives); ferulic acid was determined in the highest concentration (27.32%), followed by p-coumaric acid (15.24%) and sinapic acid (6.60%). Caftaric acid, caffeic acid, and chlorogenic acid were also identified in low quantities (Figure 2). In addition, luteolin was identified and quantified (4.68%) in *M. spicata* extract (Figure 2, Table 2) [50]. For fatty acids composition, the EOs produced by *M. spicata* are the most widely investigated among all *Mentha* species. Alpha-linolenic acid (48.17%) has been found to be the major polyunsaturated fatty acid of *M. spicata.* Linoleic acid (31.14%) is the second major polyunsaturated fatty acid in the present study. In comparison, oleic acid (8.19%) and palmitic acid (5.11%) are determined as the major monounsaturated fatty acids, stearidonic acid (3.02%), γ-linolenic acid (2.07%), and stearic acid (1.92%) (Figure 3, Table 2). Various phytosterols including ergosterol (51.42%), stigmasterol (7.6%), and beta-sitosterol (2.86%) (Figure 4) have been found in *M. spicata* [33]. Moreover, *M. spicata* contains r-tocopherol (6.11%) and vitamin D3 (31.74%) as lipide-soluble vitamins (Table 2). On the other hand, naringenin (55.44%), naringin (25%), and quercetin (19.38%) have been identified as the major flavonoids in the seeds of *M. spicata*; while, myricetin and catechin constituents are not detected [33] (Figure 5 and Table 2). Polar extracts of spearmint leaves are characterized mainly by a high content of phenolic compounds; the sum of rosmarinic acid and its derivatives was about 88% of the total amount of detected phenolics, followed by salvianolic acids (5.6%) and caffeoylquinic acids (1.2%). Hydroxycinnamic acids, including caftaric acid, represented about 1.1% of total phenolics. All other detected phenolic groups, such as flavonols, flavanones, flavones, hydroxybenzoic acids, and hydroxyphenyl propanoic acids, represented approximately 1% [30].

3.4. Mineral and Heavy Metal Contents. Mint tea may be an important source of macro and micrometallic elements, which are essential for human health. However, literature reflects enormous variability in determined concentrations. Indeed, Subramanian et al. [59] revealed that total metal concentrations of Fe, Na, Mg, Mn, Pb, Cd, Cu, and Zn in *Mentha spicata* were 395.74 ± 4.09 mg/kg, 808.09 ± 1.64 mg/kg, 532.72 ± 0.93 mg/kg, 85.72 ± 1.13 mg/kg, 9.89 ± 0.36 mg/kg, 0.74 ± 0.07 mg/kg, 29.83 ± 3.16 mg/kg, and 49.76 ± 4.12 mg/kg, respectively. In another study, Choudhury et al. [60] analyzed ten *Mentha spicata* leaves samples collected from four different locations in Northwest India for minor and trace elements including heavy toxic metals using thermal neutron activation analysis (TNAA) and atomic absorption spectrophotometry (AAS). The authors revealed that the most elements were found in widely varying amounts depending on the location: Na (0.21–0.86 mg/g), K(12.4–53.3 mg/g), and Ca (5.82–16.8 mg/g); whereas, mean contents of other nutrient elements in mint were as follows: Fe (108 ± 22 μg/g), Mg (4.83 ± 0.92 mg/g), Mn (53.5 ± 9.6 μg/g), P (3.88 ± 0.94 mg/g), Cu (16.9 ± 1.8 μg/g), Zn (21.0 ± 4.7 μg/g), and Se (0.18 ± 0.03 μg/g). The toxic heavy metals such as Hg (97–983 ng/g), Sb (1.8–315 ng/g), Ni (0.37–3.22 ng/g), Cd (15–772 ng/g), and As (98–320 ng/g) are all found at ng/g level only but vary in a wide range. Moreover, aerial parts *M. spicata* from Iran contains 129.76 μg/g of Fe, 8.52 μg/g of Zn, and 6.8 μg/g of Mn [61].

3.5. Pharmacological Properties of M. spicata. M. spicata essential oils and extracts exhibit different biological and pharmacological properties (Figure 6). These properties will be discussed in the following sections.

3.5.1. Antifungal Activity. Several studies investigated the antifungal activity of *Mentha spicata* extracts using different parts of the plant and different methods such as the disc diffusion method, microdilution method, agar well diffusion method, spots method, and microdilution broth susceptibility assay [5, 11, 62, 63].

Table 3 provides all studies that examined the antifungal potential of *M. spicata* extracts, showing the type of extract, plant part used, used method, tested strains, and key results. Using the disc diffusion method, Alaklabi et al. [62] assessed the antifungal activity of hexane, chloroform, ethyl acetate, methanol, ethanol, toluene, n-butanol, n-propanol, isopropanol, and water extracts from the root of *M. spicata* against *Aspergillus niger, Candida albicans, Cryptococcus neoformans*, and *Microsporum audouinii*. Water extract showed the highest activity against *M. audouinii* (MIC: 16 μg/mL). It revealed a remarkable antifungal response against other fungal species, *A. niger* (MIC = 32 μg/mL), *C. albicans* (MIC = 64 μg/mL), and *C. neoformans* (MIC = 32 μg/mL). Hexane, chloroform, and ethyl acetate extracts exhibited high antifungal activity against *M. audouinii* with a MIC equal to 32 μg/mL, 64 μg/mL, and 32 μg/mL, respectively. In contrast, the same extracts did not show a significant effect against the other fungal strains tested. Moreover, *C. albicans* was significantly inhibited by toluene and n-butanol extracts (MIC = 64 μg/mL), whereas the fungal activity of *A. niger* was highly reduced by using methanol and ethanol extracts (MIC = 64 μg/mL). Using the same method to screen the antifungal activity of *M. spicata* root extracts, isopropanol

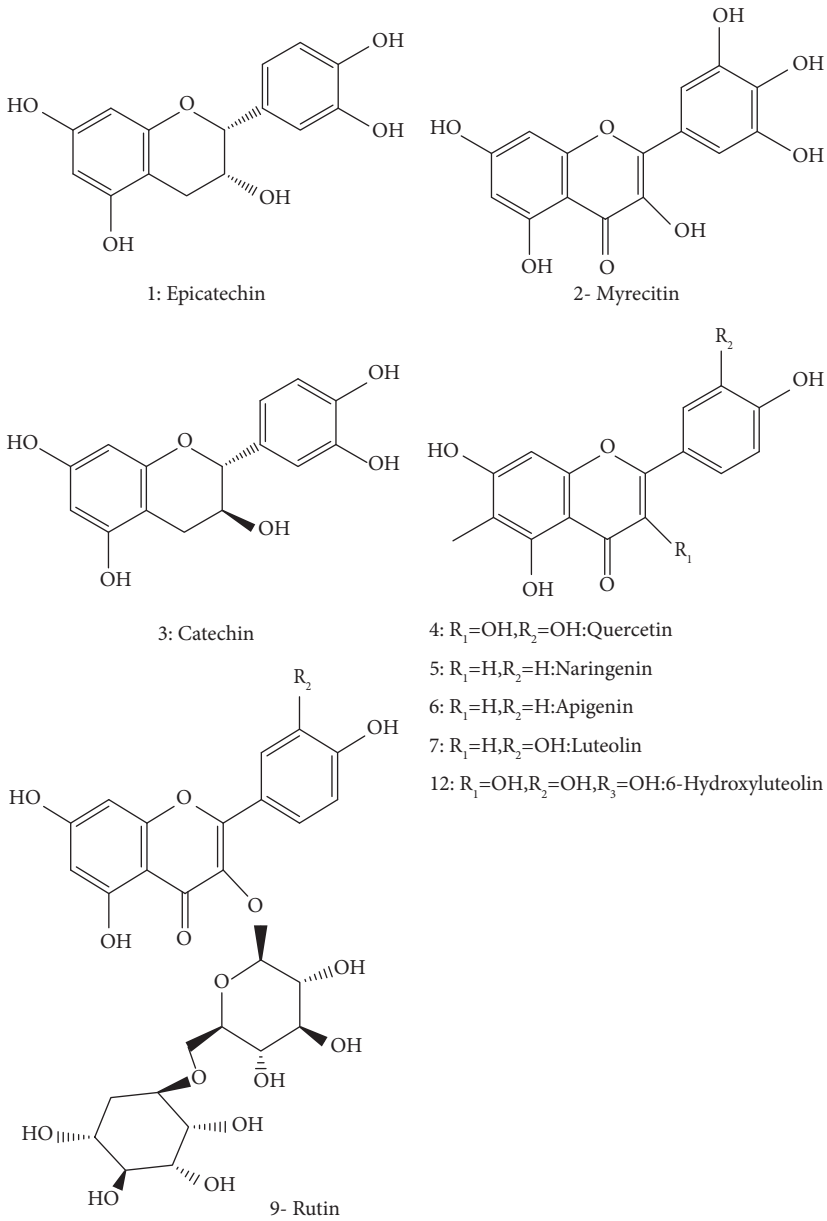


Figure 2: Structures of some flavonoids identified in *M. spicata*.

extract was found to be less active for the four fungal strains evaluated [62].

To investigate the antifungal properties of essential oil isolated from the aerial parts of *M. spicata* cultivated in the Algerian Saharan Atlas, the results published by Bardaweel et al. [48] showed a lower activity of essential oil of *M. spicata* against *Candida glabrata* (MIC = 256 μg/mL) by employing the microdilution method. Nevertheless, in the Turkish study conducted by Bayan et al. [64], the volatile oil from *M. spicata* extracted of aerial parts exhibited a strong fungitoxicity effect with 100% of inhibition of mycelium growth in *F. oxysporum* f.sp. *radicis-lycopersici* (FORL), *Verticillium dahliae* Kleb (*V. dahliae*), *Alternaria solani* (*A. solani*), and *Rhizoctonia solani* J.G. Kühn. (*R. solani*) at a dose of 12 μL petri^{-1} by using the agar well diffusion method.

In another study from Pakistan, Hussain et al. [5] evaluated the antifungal activity of essential oil of spearmint (*Mentha spicata* L.) isolated from dried aerial parts against five fungal strains. The results showed that *Aspergillus niger* was the most responsive fungal species presenting the largest zone of inhibition (26.9 mm) with the MIC value of 0.07 mg/mL, followed by *Mucor mucedo* ($\Phi = 26.2 \pm 0.8$ mm and MIC = 0.08 ± 0.00 μg/mL), *Rhizopus solani* ($\Phi = 26.3 \pm 0.8$ mm and MIC = 0.09 ± 0.00 μg/mL), and

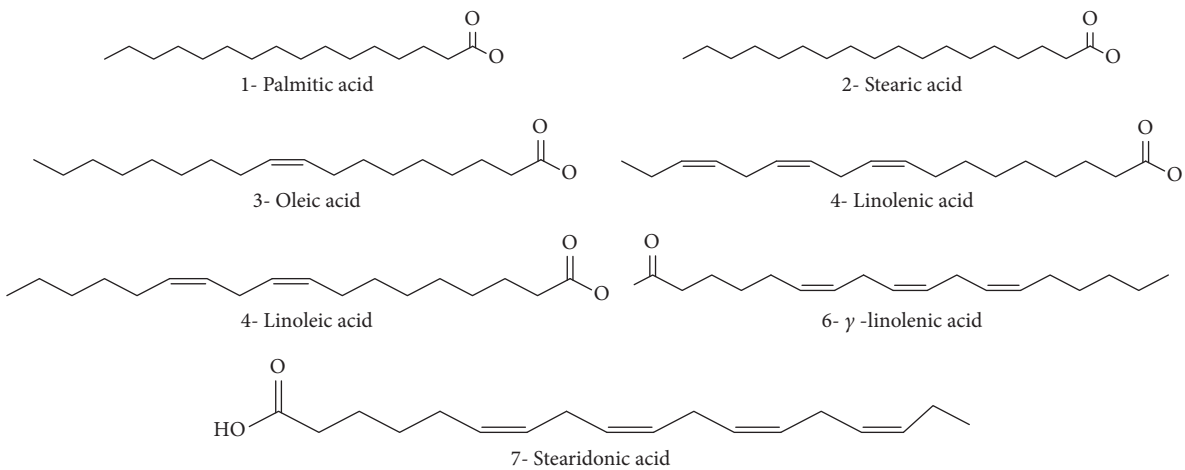


Figure 3: Chemical structures of fatty acids identified in *M. spicata*.

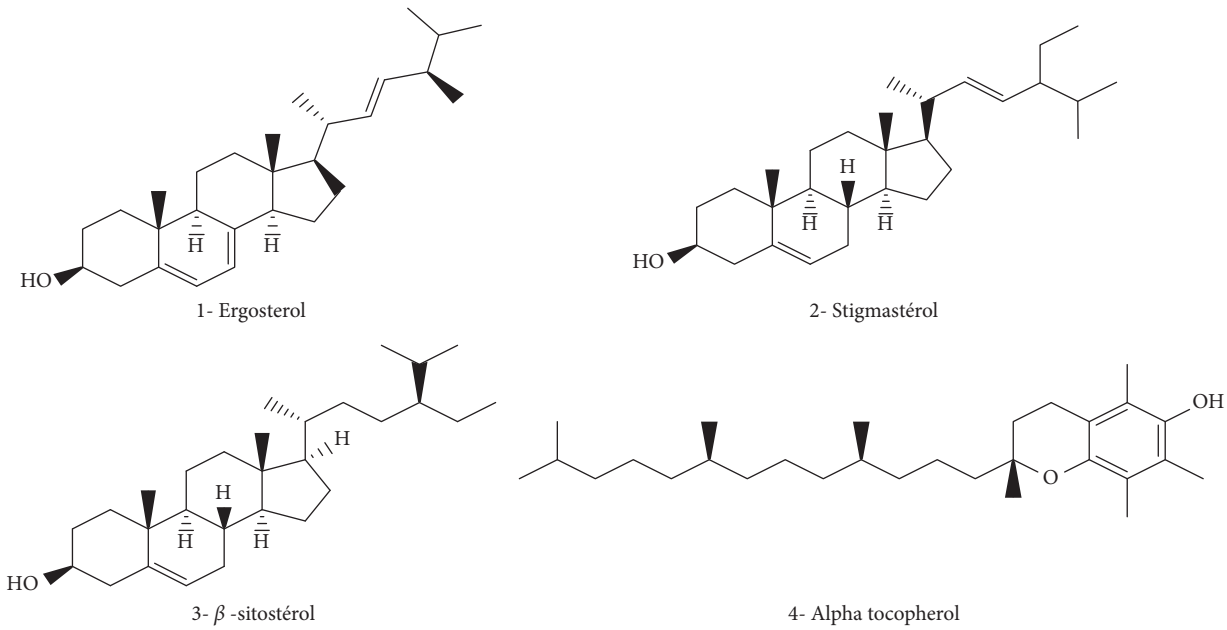


Figure 4: Chemical structures of some sterols identified in *M. spicata* essential oils.

Fusarium solani ($\Phi = 25.2 \pm 1.0$ mm and MIC $= 0.09 \pm 0.00\,\mu$g/mL). However, *B. theobromae* was observed to be the most resistant fungus with the smallest inhibition zone (23.0 mm) and a MIC value equal to 0.11 mg/mL by using microdilution broth susceptibility assay.

Additionally, Kedia et al. [66] tested the antifungal potency of essential oil of spearmint against 19 food-deteriorating molds using the poisoned food assay. The fundings showed that the oil of *M. spicata* has a notable potential to inhibit the fungal growth of all fungi species, causing 100% of mycelial inhibition at $1.0\,\mu\text{L ml}^{-1}$ excluding *Aspergillus luchuensis* and *Aspergillus terreus*, where the percentage of mycelial inhibition was $91.72 \pm 0.36\%$ and $75.67 \pm 0.74\%$, respectively. The results of testing the nature toxicity of the oil from *M. spicata* revealed that spearmint essential oil possessed a fungicidal effect in *Cladosporium cladosporioides*, *Mycelia sterilia*, *Alternaria alternata*, and *Curvularia lunata* at $1.0\,\mu\text{L mL}^{-1}$. In their study, Liu et al. [11] investigated the biological properties of the essential oil isolated from aerial parts of *M. spicata* from China. Using the disc diffusion method, the results of this study showed quite strong antifungal potency against *A. niger* with an MIC value of $6.25\,\mu$g/mL and an MBC value of $12.50\,\mu$g/mL. Compared to a study carried out by Şarer et al. [67] from eastern Turkey, the oil of *M. spicata* subsp. *spicata* exhibited high antifungal activity against *Candida albicans* and *Candida tropicalis* with an MIC value less than $3.19\,\mu$g/mL.

Regarding testing the potential antimicrobial effects of *M. spicata*, [45] investigated the essential oil extracted from air-dried leaves of Algerian spearmint against *Candida albicans* (ATCC 1024) strain and two *Aspergillus* species (*flavus* NRRL 391 and *niger* 2CA 936). Using the spots

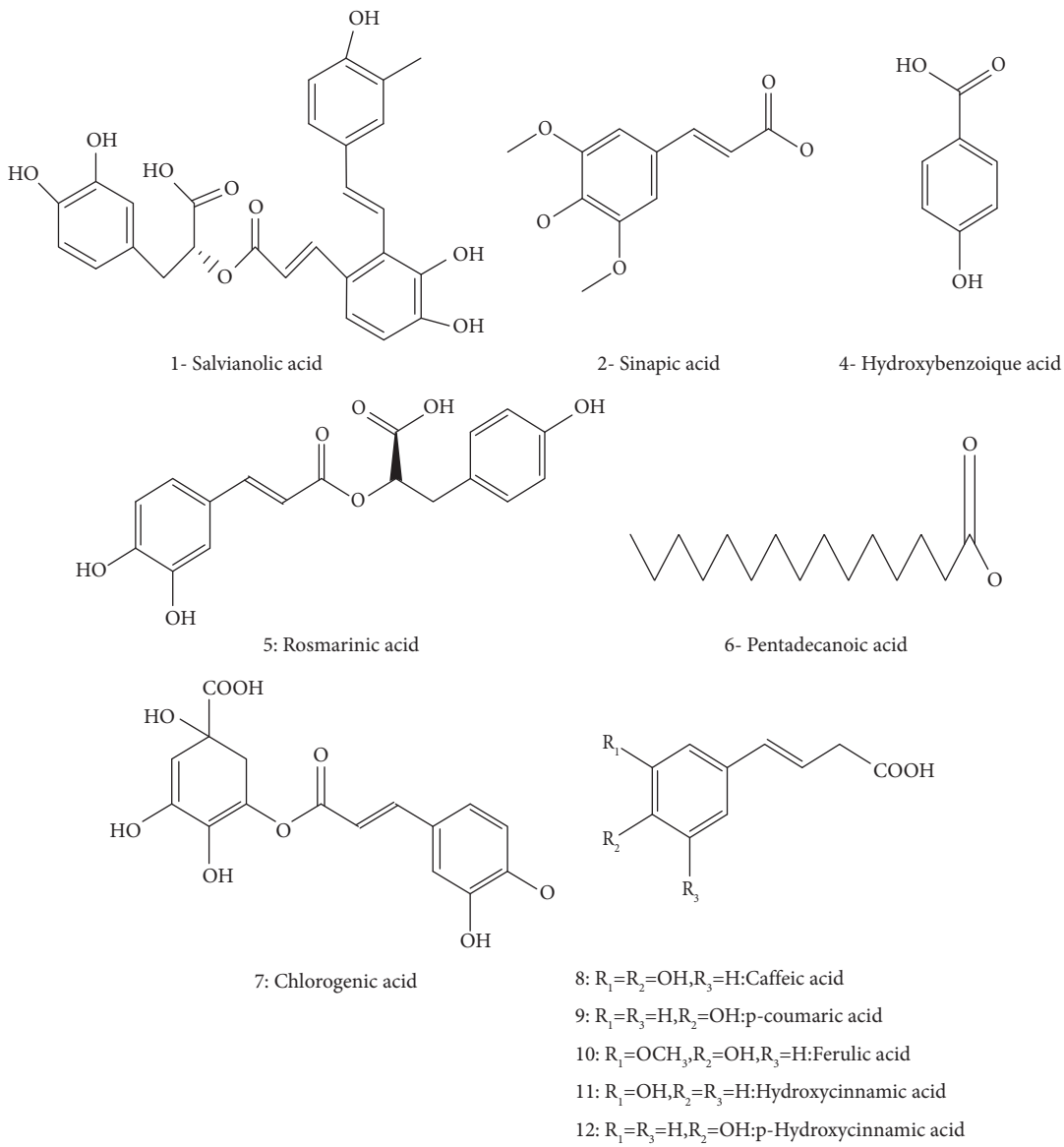


Figure 5: Chemical structures of phenolic acids identified in *M. spicata*.

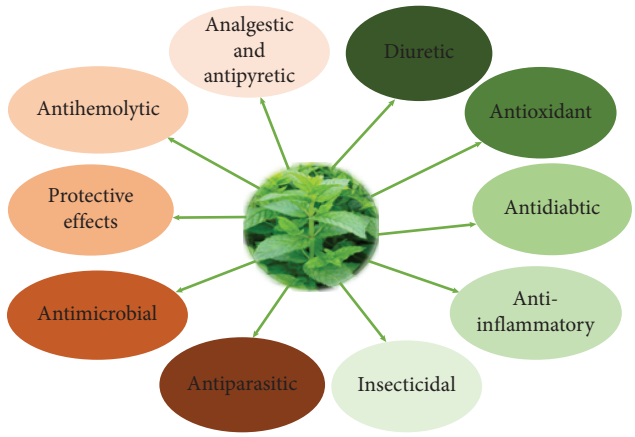


Figure 6: Biological and pharmacological properties of *Mentha spicata*.

Table 3: Antifungal activity of *Mentha spicata*.

Used part	Extracts	Used method	Tested strains	Key results	References
Root	Hexane extract	Disc diffusion method	*A. niger*	MIC > 356 μg/mL	[62]
			C. albicans	MIC > 356 μg/mL	
			C. neoformans	MIC > 356 μg/mL	
			M. audouinii	MIC = 32 μg/mL	
Root	Chloroform extract		*A. niger*	MIC > 356 μg/mL	[62]
			C. albicans	MIC > 356 μg/mL	
			C. neoformans	MIC = 64 μg/mL	
			M. audouinii	MIC = 64 μg/mL	
Root	Ethyl acetate extract		*A. niger*	MIC = 128 μg/mL	[62]
			C. albicans	MIC = 128 μg/mL	
			C. neoformans	MIC = 128 μg/mL	
			M. audouinii	MIC = 32 μg/mL	
Root	Methanol extract		*A. niger*	MIC = 64 μg/mL	[62]
			C. albicans	MIC = 128 μg/mL	
			C. neoformans	MIC = 128 μg/mL	
			M. audouinii	MIC > 356 μg/mL	
Root	Ethanol extract		*A. niger*	MIC = 64 μg/mL	[62]
			C. albicans	MIC = 128 μg/mL	
			C. neoformans	MIC = 128 μg/mL	
			M. audouinii	MIC > 356 μg/mL	
Root	Toluene extract		*A. niger*	MIC > 356 μg/mL	[62]
			C. albicans	MIC = 64 μg/mL	
			C. neoformans	MIC > 356 μg/mL	
			M. audouinii	MIC = 128 μg/mL	
Root	N-butanol extract		*A. niger*	MIC = 128 μg/mL	[62]
			C. albicans	MIC = 64 μg/mL	
			C. neoformans	MIC > 356 μg/mL	
			M. audouinii	MIC = 128 μg/mL	
Root	N-propanol extract		*A. niger*	MIC = 64 μg/mL	[62]
			C. albicans	MIC = 64 μg/mL	
			C. neoformans	MIC = 32 μg/mL	
			M. audouinii	MIC = 128 μg/mL	
Root	Isopropanol extract		*A. niger*	MIC = 128 μg/mL	[62]
			C. albicans	MIC = 128 μg/mL	
			C. neoformans	MIC > 356 μg/mL	
			M. audouinii	MIC > 356 μg/mL	
Root	Water extract		*A. niger*	MIC = 32 μg/mL	[62]
			C. albicans	MIC = 64 μg/mL	
			C. neoformans	MIC = 32 μg/mL	
			M. audouinii	MIC = 16 μg/mL	
Aerial parts	Essential oil	Microdilution method	*Candida glabrata*	MIC = 256 μg/mL	[48]
Aerial parts	Volatile oil	Agar well diffusion method	*R. solani*	Inhibition = 100% at dose of 12 μL	[64]
			A. solani	Inhibition = 100% at dose of 12 μL	
			F. oxysporum f.sp. *radicis-lycopersici*	Inhibition = 100% at dose of 12 μL	
			V. dahliae	Inhibition = 100% at dose of 12 μL	
Leaves	Essential oil	Spots method	*A. niger* 2CA 936	$\Phi = 36.0 \pm 1.0$ mm	[65]
			A. flavus NRRL 391	$\Phi = 43.7 \pm 0.6$ mm	
			C. albicans (ATCC 1024)	$\Phi = 44.3 \pm 1.1$ mm	
Leaves	Essential oil	Disc method	*A. niger* 2CA 936	$\Phi = 32.0 \pm 1.0$ mm	[65]
			A. flavus NRRL 391	$\Phi = 36.0 \pm 2.0$ mm	
			C. albicans (ATCC 1024)	$\Phi = 23.3 \pm 0.6$ mm	

Table 3: Continued.

Used part	Extracts	Used method	Tested strains	Key results	References
Aerial parts	Essential oils	Disc diffusion method Microdilution broth susceptibility assay	*Aspergillus niger*	Φ = 26.9 ± 1.2 mm MIC = 0.07 ± 0.00 μg/mL	[5]
			Mucor mucedo	Φ = 26.2 ± 0.8 mm MIC = 0.08 ± 0.00 μg/mL	
			Fusarium solani	Φ = 25.2 ± 1.0 mm MIC = 0.09 ± 0.00 μg/mL	
			Botryodiplodia theobromae	Φ = 23.0 ± 1.1 mm MIC = 0.11 ± 0.01 μg/mL	
			Rhizopus solani	Φ = 26.3 ± 0.8 mm MIC = 0.09 ± 0.00 μg/mL	
Aerial parts	Essential oil		*Absidia ramosa*	Mycelial inhibition = 100%	[66]
			Alternaria alternata	Mycelial inhibition = 100%	
			Aspergillus fumigatus	Mycelial inhibition = 100%	
			Aspergillus glaucus	Mycelial inhibition = 100%	
			Aspergillus luchuensis	Mycelial inhibition = 91.72 ± 0.36%	
			Aspergillus niger	Mycelial inhibition = 100%	
			Aspergillus terreus	Mycelial inhibition = 75.67 ± 0.74%	
			Aspergillus unguis	Mycelial inhibition = 100%	
			Cladosporium cladosporioides	Mycelial inhibition = 100%	
			Curvularia lunata	Mycelial inhibition = 100%	
			Fusarium oxysporum	Mycelial inhibition = 100%	
			Mucor spp.	Mycelial inhibition = 100%	
			Mycelia sterilia	Mycelial inhibition = 100%	
			Penicillium citrinum	Mycelial inhibition = 100%	
			Penicillium italicum	Mycelial inhibition = 100%	
			Penicillium luteum	Mycelial inhibition = 100%	
			Penicillium purpurogenum	Mycelial inhibition = 100%	
			Rhizopus stolonifer	Mycelial inhibition = 100%	
			Spondylocladium australe	Mycelial inhibition = 100%	
	Essential oils	Disc diffusion method	*A. niger*	MIC = 6.25 μg/mL MBC = 12.50 μg/mL	[11]
Leaves	Hexane	Agar well diffusion techniques	*Saccharomyces cerevisiae*	Φ = 25	[63]
			Aspergillus niger	Φ = 26 mm	
Leaves	Petroleum ether	Agar well diffusion techniques	*Saccharomyces cerevisiae*	Φ = 24 mm	[63]
			Aspergillus niger	Φ = 27 mm	
Aerial parts	Essential oils	Disc diffusion method	*Candida albicans*	MIC < 3.19 μg/mL	[67]
			Candida tropicalis	MIC < 3.19 μg/mL	
Leaves	Ethanol extract		*Fusarium oxysporum* f.sp. *lentis*	Inhibition = 1oo%	[68]
Leaves	Essential oil	Agar diffusion method	*Aspergillus niger* (ATCC 9763)	Φ = 19 mm	[69]
			Candida albicans (ATCC 7596)	Φ = 18 mm	
	Essential oil	Agar well diffusion method	*Aspergillus niger*	Φ = 15.7 ± 0.09 mm	[70]
			Aspergillus spp.,	Φ = 13 ± 0.13 mm	
			Candida albicans	Φ = 11.8 ± 0.10 mm	
			Rhizopus nigricans	No inhibition	
Leaves	Essential oil	Agar diffusion method	*Candida albicans*	Φ = 16 mm at concentration of 100 mg/mL	[71]
Leaves	Essential oil	Agar well diffusion method	*Mucor ramamnianus* (ATCC 9314)	Φ = 40 mm	[72]
			Aspergillus ochraceus (NRRL 3174)	Φ = 43 mm	
			Candida albicans (IPA 200)	Φ = 21 mm	
			Saccharomyces cerevisiae (ATCC 4226 A)	Φ = 25 mm	

method, their finding indicates that *Candida albicans* (ATCC 1024) was the most sensitive species with a diameter of growth inhibition zones equal to 44.3 ± 1.1 mm, followed by *A. flavus* NRRL 391 (Φ = 43.7 ± 0.6 mm), and *A. niger* 2CA 936 (Φ = 36.0 ± 1.0 mm). The disc diffusion method also showed high activity against *Aspergillus* species, *A. flavus* NRRL 391 (Φ = 36.0 ± 2.0 mm) and *A. niger* 2CA 936 (Φ = 32.0 ± 1.0 mm) than *C. albicans* (ATCC 1024) (Φ = 23.3 ± 0.6 mm).

On the other hand, Ojewumi et al. [63] demonstrated the antimicrobial role of the leaf oil extract of *M. spicata* from Nigeria by using two types of petroleum ether and hexane extract. They found that the hexane extract showed higher activity against *Aspergillus niger* (Φ = 26 mm) followed by *Saccharomyces cerevisiae* (Φ = 25). In addition, they observed that petroleum ether extract showed potent activity against *Aspergillus niger* (Φ = 27 mm) followed by *S. cerevisiae* (Φ = 27). Therefore, it was noted that the effectiveness of the two extracts was significantly comparable as the inhibitory zone values are very similar. Furthermore, the ethanolic extract exhibited 100% of inhibition against *Fusarium oxysporum* f.sp. *lentis* in the investigation performed by Singh et al. [68] that aimed to study the antifungal activity of *M. spicata*. The results found were supported by the study conducted in Sudan by Sulieman et al. [69]; they indicated that spearmint oil leaves have demonstrated potent activity against *Aspergillus niger* (ATCC 9763) with an inhibition zone equal to 19 mm at a high concentration (20%) and (15 mm) at low concentration (5%). In addition, the oil of *M. spicata* exhibited considerable inhibition capacity against *C. albicans* with an inhibition zone diameter of 18 mm at higher concentration (20%) and 14 mm at lower concentration (5%). Similarly, the concentration of 100 mg/mL was able to inhibit *C. albicans* with a diameter of growth inhibition zone reached 16 mm using the agar diffusion method [71].

Zaidi et al. [70] evaluated the antifungal efficiency of oil leaves from *M. spicata* against four fungal species including *A. niger* and *Aspergillus* spp., *C. albicans,* and *Rhizopus nigricans*, using the agar well diffusion method. The results showed that *Mentha spicata* oil exhibited an excellent potential against fungal strains tested but with differing sensitivity. *A. niger* showed a strong inhibition zone of 15.7 ± 0.09 mm compared to *C. albicans*, which possessed an inhibition zone of 11.8 ± 0.10 mm. However, *M. spicata* oil was not able to inhibit the growth of *R. nigricans* strain. The oil also exhibited an antifungal effect against *Aspergillus* spp. (13 ± 0.13 mm). In another study, using the agar well diffusion method, essential oil isolated from spearmint was observed to act as a stronger bioactive source against fungal species with a different zone of inhibition. Indeed, inhibition zone diameters for *Aspergillus ochraceus* (NRRL 3174) (Φ = 43 mm) and *Mucor ramamnianus* (ATCC 9314) (Φ = 40 mm) were higher than inhibition zone diameters for *S. cerevisiae* (ATCC 4226 A) (Φ = 25 mm) and *C. albicans* IPA 200 (Φ = 21 mm) [72].

3.5.2. Antibacterial Activity. For over 60 years, antimicrobial agents have been used to treat infections in humans, animals, and plants. Currently, they are among the most widely used therapeutic agents in human and veterinary medicine [73]. At the start of antibiotic therapy, as resistant strains were low and highly effective antimicrobial agents of different classes were detected, antimicrobial resistance was not considered a major problem. This has forced sensitive bacteria living in close contact with antimicrobial producers to develop mechanisms to bypass the inhibitory effects of antimicrobial agents (Table 4). In the context of this study, several in vitro studies have determined the antibacterial activity of *M. spicata* essential oils and solvent extracts against various bacterial strains, either clinical or reference, using the agar diffusion methods (disks or well) and the agar and broth dilution methods [5, 74, 89]. In most of these studies, qualitative inhibition was determined by the dilution method, which is used to assess minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values [5, 74, 89]. Indeed, the increased selective pressure imposed by the widespread use of antimicrobial agents has clearly accelerated the development and spread of bacterial resistance to antimicrobial agents [5, 74, 89]. These observations underscore the enormous flexibility of bacteria to resist less favorable environmental conditions by constantly developing new survival strategies.

3.5.3. Antiparasitic Activity. Table 5 provides investigations interested in the antiparasitic effect of spearmint [90, 91]. Zandi-Sohani and Ramezani [90] investigated the antiparasitic effect of essential oil isolated from spearmint leaves collected from southwestern Iran against *Tetranychus turkestani*. They discovered that the essential oil of spearmint exhibited acaricidal potential and can be employed to protect against *Tetranychus turkestani*, which showed to cause 100% adult mortality at a concentration of 20 μL/L. The lethal concentration values (LC_{50} and LC_{95}) for essential oil spearmint were estimated to be 15.3 $\mu L.L^{-1}$ and 23.4 $\mu L.L^{-1}$, respectively. However, the study conducted by Koumad and Berkani [91] demonstrated that spearmint leaves revealed the lowest acaricidal activity against *Varroa destructor* by smoke. Results showed that spearmint killed 26.20% of *Varroa destructor* and reduced the infestation rate by 2.35%. The mortality rate was estimated at 30.65%, and infestation rate was 13.18%.

3.5.4. Insecticidal Activity. Several investigations reported that extracts and essential oils from *M. spicata* have insecticidal activities against some pathogenic microorganisms [3, 92, 93] (Table 6).

Brahmi et al. [65] studied the impact of essential oil from *M. spicata* leaves against *Rhyzopertha dominica*. This study revealed that the essential oil from *M. spicata* leaf was effectively toxic against *Rhyzopertha dominica* adults. At a high concentration of 2 μL/mL, *M. spicata* oil showed high repellent activity against *Rhyzopertha dominica* (56.2% at 30 minutes), and the mortality rate was 43% after 96 hours of treatment. Furthermore, the toxicity contact assay showed that spearmint oil showed a low insecticidal effect with DL_{50} equal to 6.1 μL/mL. In another study, Kedia et al. [66]

Table 4: Antibacterial activity of *Mentha spicata*.

Parts used	Extracts	Methods used	Strains tested	Key results	References
Leaves	Essential oils	Broth microdilution method	*Staphylococcus aureus* (ATCC 14458)	MIC = 3.2 μL/mL	[6]
			Staphylococcus epidermidis (ATCC 12228)	MIC = 1.6 μL/mL	
			Bacillus cereus (ATCC 11778)	MIC = 1.6 μL/mL	
			Listeria monocytogenes (ATCC 7644)	MIC = 3.2 μL/mL	
			Escherichia coli (ATCC 11229)	MIC = 3.2 μL/mL	
			Salmonella enterica subsp. *enterica serovar typhimurium* (ATCC 13311)	MIC = 1.6 μL/mL	
			Salmonella. enterica subsp. *enterica serovar typhi* (ATCC 19214)	MIC = 1.6 μL/mL	
			Shigella flexneri (ATCC 12022)	MIC = 3.2 μL/mL	
Aerial parts	Essential oils	Disc diffusion assay	*P. aeruginosa* (ATCC 27853)	No inhibition	[7]
			Escherichia coli (ATCC 25922)	Φ = 9 mm	
			Staphylococcus aureus (ATCC 25923)	Φ = 11 mm	
			Staphylococcus epidermidis	Φ = 10 mm	
			Streptococcus pneumoniae	Φ = 13 mm	
			Streptococcus pyogenes	Φ = 16 mm	
			Klebsiella pneumoniae	Φ = 8 mm	
			Salmonella typhi	Φ = 8 mm	
			Shigella sonnei	Φ = 9 mm	
Leaves	Ethanol extract	Disc diffusion assay	*Salmonella paratyphi*	$\Phi = 17.00 \pm 2.00$ mm	[74]
			Shigella boydii	$\Phi = 31.67 \pm 1.53$ mm	
			Staphylococcus aureus	$\Phi = 23.00 \pm 1.00$ mm	
			Escherichia coli	$\Phi = 9.00 \pm 1.00$ mm	
			Vibrio cholerae	$\Phi = 12.00 \pm 1.00$ mm	
			Pseudomonas aeruginosa	Trace activity	
			Enterococcus faecalis	No activity	
			Salmonella typhi	Trace activity	
			Proteus vulgaris	No activity	
			Klebsiella pneumoniae	No activity	
Leaves	Hexane fraction	Disc diffusion assay	*Salmonella paratyphi*	$\Phi = 25.67 \pm 2.08$ mm	[74]
			Shigella boydii	$\Phi = 36.00 \pm 1.00$ mm	
			Staphylococcus aureus	$\Phi = 22.33 \pm 1.53$ mm	
			Escherichia coli	$\Phi = 10.67 \pm 2.52$ mm	
			Vibrio cholerae	$\Phi = 18.67 \pm 0.58$ mm	
			Pseudomonas aeruginosa	Trace activity	
			Enterococcus faecalis	No activity	
			Salmonella typhi	No activity	
			Proteus vulgaris	No activity	
			Klebsiella pneumoniae	No activity	
Leaves	Chloroform	Disc diffusion assay	*Salmonella paratyphi*	$\Phi = 22.67 \pm 2.52$ mm	[74]
			Shigella boydii	$\Phi = 34.00 \pm 1.00$ mm	
			Staphylococcus aureus	$\Phi = 24.00 \pm 1.00$ mm	
			Escherichia coli	$\Phi = 18.67 \pm 1.53$ mm	
			Vibrio cholerae	$\Phi = 16.00 \pm 1.00$ mm	
			Pseudomonas aeruginosa	$\Phi = 12.33 \pm 1.53$ mm	
			Enterococcus faecalis	$\Phi = 8.33 \pm 0.58$ mm	
			Salmonella typhi	No activity	
			Proteus vulgaris	No activity	
			Klebsiella pneumoniae	No activity	

Table 4: Continued.

Parts used	Extracts	Methods used	Strains tested	Key results	References
Leaves	Ethyl acetate fraction	Disc diffusion assay	*Salmonella paratyphi*	$\Phi = 20.67 \pm 1.53$ mm	[74]
			Shigella boydii	$\Phi = 32.67 \pm 2.52$ mm	
			Staphylococcus aureus	$\Phi = 25.33 \pm 0.58$ mm	
			Escherichia coli	$\Phi = 18.33 \pm 1.53$ mm	
			Vibrio cholerae	$\Phi = 17.33 \pm 1.53$ mm	
			Pseudomonas aeruginosa	$\Phi = 8.00 \pm 1.00$ mm	
			Enterococcus faecalis	No activity	
			Salmonella typhi	No activity	
			Proteus vulgaris	No activity	
			Klebsiella pneumoniae	No activity	
Leaves	Aqueous fraction	Disc diffusion assay	*Salmonella paratyphi*	$\Phi = 22.33 \pm 2.52$ mm	[74]
			Shigella boydii	$\Phi = 36.00 \pm 1.00$ mm	
			Staphylococcus aureus	$\Phi = 31.00 \pm 1.00$ mm	
			Escherichia coli	$\Phi = 21.00 \pm 1.00$ mm	
			Vibrio cholerae	$\Phi = 20.33 \pm 0.58$ mm	
			Pseudomonas aeruginosa	$\Phi = 10.00 \pm 1.00$ mm	
			Enterococcus faecalis	No activity	
			Salmonella typhi	Trace activity	
			Proteus vulgaris	No activity	
			Klebsiella pneumoniae	No activity	
Aerial parts	Essential oil	Microdilution method	*Staphylococcus epidermidis*	MIC = 32 μg/mL	[48]
			Escherichia coli	MIC = 64 μg/mL	
Aerial parts	Volatile oil	Disk diffusion method	*Xanthomonas* spp. ZI378	$\Phi = 14$ mm	[64]
			Xanthomonas spp. ZI376	$\Phi = 14$ mm	
			Xanthomonas spp. ZI375	$\Phi = 13$ mm	
			Xanthomonas spp. ZI373	$\Phi = 13$ mm	
			Xanthomonas spp. ZI370	$\Phi = 13$ mm	
			Xanthomonas spp. ZI368	$\Phi = 13$ mm	
			Xanthomonas spp. ZI366	$\Phi = 12$ mm	
			Xanthomonas spp. ZI365	$\Phi = 16$ mm	
Leaves	Essential oil	Agar disc diffusion method Microbroth dilution	*Staphylococcus aureus* (ATCC 29213)	$\Phi = 19 \pm 1.73$ mm MIC = 1.25 μg/mL MBC = 1.25 μg/mL	[75]
			Escherichia coli (ATCC 25922)	$\Phi = 13.66 \pm 1.1$ mm MIC = 1.25 μg/mL MBC = 2.5 μg/mL	
			Pseudomonas aeruginosa (ATCC 27853)	$\Phi = 9.5 \pm 0.70$ mm MIC > 10 MBC > 10	
Leaves	Essential oil	Disc method	MRSA (ATCC 43300)	$\Phi = 24.0 \pm 1.0$ mm	[65]
			Bacillus subtilis (ATCC 6633)	$\Phi = 17.7 \pm 0.6$ mm	
			Staphylococcus aureus (NCC B9163)	$\Phi = 14.3 \pm 1.5$ mm	
			Escherichia coli (ATCC 25922)	$\Phi = 11.0 \pm 1.0$ mm	
			Pseudomonas aeruginosa (ATCC 27853)	$\Phi = 6.0 \pm 0.0$ mm	
			Klebsiella pneumonia E47	$\Phi = 10.3 \pm 0.6$ mm	
Leaves	Essential oil	Spots method	MRSA (ATCC 43300)	$\Phi = 22.3 \pm 1.5$ mm	[65]
			Bacillus subtilis (ATCC 6633)	$\Phi = 32.7 \pm 0.6$ mm	
			Staphylococcus aureus (NCC B9163)	$\Phi = 20.3 \pm 0.6$ mm	
			Escherichia coli (ATCC 25922)	$\Phi = 22.0 \pm 1.0$ mm	
			Pseudomonas aeruginosa (ATCC 27853)	$\Phi = 6.0 \pm 0.0$ mm	
			Klebsiella pneumonia (E47)	$\Phi = 17.3 \pm 0.6$ mm	
Aerial parts	Essential oil	Disc diffusion method	*Escherichia coli*	$\Phi = 14 \pm 0.6$ mm	[76]
			Salmonella enterica subsp. *enterica*	$\Phi = 10 \pm 0.8$ mm	
			Pasteurella multocida	$\Phi = 12 \pm 1.0$ mm	
			Staphylococcus aureus	$\Phi = 9 \pm 1.1$ mm	

Table 4: Continued.

Parts used	Extracts	Methods used	Strains tested	Key results	References
	Essential oils	Disc diffusion assay Microwell dilution assay	*Escherichia coli* (O157H7)	$\Phi = 10$ mm MIC = 2.26 ± 0.11 μg/mL MBC = 3.66 ± 0.11 μg/mL	[77]
			Listeria monocytogenes	$\Phi = 11$ mm MIC = 1.33 ± 0.11 μg/mL MBC = not observed	
Leaves	Essential oils	Disc diffusion technique Checker board technique	*Staphylococcus aureus* 29737	$\Phi = 10.0$ mm MIC = 10 μg/mL	[78]
			Staphylococcus aureus ML 267	$\Phi = 10.0$ mm MIC = 10 μg/mL	
			Suillus luteus 9341	$\Phi = 11.0$ mm MIC = 5 μg/mL	
			Bacillus pumilus 8241	$\Phi = 10.0$ mm MIC = 10 μg/mL	
			Bacillus subtilis (ATCC)	$\Phi = 11.0$ mm MIC = 10 μg/mL	
			Escherichia coli (ATCC 10536)	$\Phi = 9.0$ mm MIC = 50 μg/mL	
			Escherichia coli VC Sonawave 3 : 37 C	$\Phi = 9.0$ mm MIC = 50 μg/mL	
			Escherichia coli (CD/99/1)	$\Phi = 9.5$ mm MIC = 50 μg/mL	
			Escherichia coli (RP4)	$\Phi = 9.0$ mm MIC = 25 μg/mL	
			Escherichia coli (18/9)	$\Phi = 9.0$ mm MIC = 25 μg/mL	
			Escherichia coli (K88)	$\Phi = 8.5$ mm MIC = 25 μg/mL	
			Shigella dysenteriae L.	$\Phi = 10.0$ mm MIC = 10 μg/mL	
			Shigella sonnei 1	$\Phi = 10.0$ mm MIC = 10 μg/mL	
			Shigella sonnei BCH 217	$\Phi = 12.0$ mm MIC = 5 μg/mL	
			Shigella flexneri type 6	$\Phi = 9.0$ mm MIC = 10 μg/mL	
			Shigella boydii 937	$\Phi = 9.5$ mm MIC = 10 μg/mL	
			Pseudomonas aeruginosa (ATCC 25619)	$\Phi = 11.0$ mm MIC = 10 μg/mL	
			Vibrio cholerae 2	$\Phi = 10.0$ mm MIC = 50 μg/mL	
			Vibrio cholerae 785	$\Phi = 10.0$ mm MIC = 50 μg/mL	
			Vibrio cholerae 1037	$\Phi = 9.0$ mm MIC = 50 μg/mL	
Leaves	Essential oils	Agar well diffusion method Dilution method	*Staphylococcus aureus*	$\Phi = 17 \pm 0.01$ mm MIC = 0.4 ± 0.01 μg/mL	[79]
			Escherichia coli	$\Phi = 14 \pm 0.01$ mm MIC = 0.5 ± 0.02 μg/mL	
			Erwinia carotovora	$\Phi = 14 \pm 0.01$ mm MIC = 0.5 ± 0.02 μg/mL	
			Bacillus subtilis	$\Phi = 17 \pm 0.01$ mm MIC = 0.6 ± 0.01 μg/mL	
			Xanthomonas campestris	$\Phi = 22 \pm 0.01$ mm MIC = 0.5 ± 0.02 μg/mL	
			Klebsiella pneumoniae	$\Phi = 20 \pm 0.01$ mm MIC = 0.4 ± 0.01 μg/mL	

Table 4: Continued.

Parts used	Extracts	Methods used	Strains tested	Key results	References
Leaves	Essential oils	Diffusion method	*Bacillus subtilis* *Staphylococcus aureus* *Staphylococcus epidermidis* *Escherichia coli* *Pseudomonas aeruginosa* *Salmonella enterica* subsp.	$\Phi = 11.5 \pm 0.61$ mm $\Phi = 13 \pm 1.52$ mm $\Phi = 11.2 \pm 1.61$ mm $\Phi = 21 \pm 0.90$ mm $\Phi = 16 \pm 1.9$ mm $\Phi = 18 \pm 1.33$ mm	[80]
Aerial parts	Essential oils	Disc diffusion method Microdilution broth assay	*Staphylococcus aureus* *Bacillus subtilis* *Pasteurella multocida* *Escherichia coli*	$\Phi = 26.0 \pm 1.1$ mm MIC = 0.07 ± 0.00 μg/mL $\Phi = 27.1 \pm 1.1$ mm MIC = 0.05 ± 0.00 μg/mL $\Phi = 24.3 \pm 0.9$ mm MIC = 0.12 ± 0.01 μg/mL $\Phi = 20.3 \pm 0.9$ mm MIC = 0.21 ± 0.01 μg/mL	[5]
Whole plant	Essential oils	Disc diffusion method	*Escherichia coli*	MIC = 1/250 (V/V) MBC = 1/250 (V/V)	[81]
Not reported	Essential oil	Disc diffusion method	*Escherichia coli* *Staphylococcus aureus* *Saccharomyces cerevisiae* *Penicillium citrinum*	MIC = 1.56 μg/mL MBC = 25 μg/mL MIC = 25 μg/mL MBC = 50 μg/mL MIC = 0.78 μg/mL MBC = 6.25 μg/mL MIC = 3.12 μg/mL MBC = 12.50 μg/mL	[11]
Leaves	Hexane	Agar well diffusion techniques	*Pseudomonas aeruginosa* *Bacillus subtilis* *Escherichia coli* *Staphylococcus aureus*	$\Phi = 15$ mm $\Phi = 10$ mm $\Phi = 25$ mm $\Phi = 26$ mm	[63]
Leaves	Petroleum ether	Agar well diffusion techniques	*Pseudomonas aeruginosa* *Bacillus subtilis* *Escherichia coli* *Staphylococcus aureus*	$\Phi = 17$ mm $\Phi = 12$ mm $\Phi = 26$ mm $\Phi = 27$ mm	[63]
Aerial parts	Essential oils	Disc diffusion method	*Staphylococcus aureus* *Enterococcus faecalis* *Pseudomonas aeruginosa* *Escherichia coli*	MIC = 15.6 μg/mL MIC = 125 μg/mL MIC = 125 μg/mL MIC < 3.19 μg/mL	[67]
Leaves	Essential oil	Broth microdilution method	*Serratia* spp. *Salmonella* spp. *Kluyvera* spp. *Klebsiella* spp. *Escherichia coli* (F5) *Escherichia coli* (F17) *Escherichia coli* (CS31 A)	MIC = 4.75 mg/mL MBC > 9.5 mg/mL MIC = 2.37 mg/mL MBC > 9.5 mg/mL MIC = 2.37 mg/mL MBC > 9.5 mg/mL MIC = 2.37 mg/mL MBC = 9.5 mg/mL MIC = 2.37 mg/mL MBC > 9.5 mg/mL MIC > 9.5 mg/mL MBC > 9.5 mg/mL MIC = 2.37 mg/mL MBC = 9.5 mg/mL	[82]
Aerial parts	Essential oil	Disc diffusion method	MRSA *Staphylococcus aureus* (ATCC 6538) *Pseudomonas aeruginosa* *Escherichia coli* Q157 : H7 *Bacillus cereus* (CCM99) *Enterococcus faecium* (DSM 13590)	$\Phi = 17.5 \pm 0.7$ mm $\Phi = 11 \pm 1.4$ mm $\Phi = 21 \pm 8.4$ mm $\Phi = 20.5 \pm 2.1$ mm $\Phi = 22.5 \pm 0.7$ mm $\Phi = 13 \pm 4.2$ mm	[31]

Table 4: Continued.

Parts used	Extracts	Methods used	Strains tested	Key results	References
Leaves	Essential oil	Broth microdilution method	*Staphylococcus aureus* (ATCC 6538)	MIC = 10 μg/mL MBC = 10 μg/mL	[83]
			Staphylococcus aureus (ATCC 29213)	MIC = 8 μg/mL MBC = 8 μg/mL	
			Bacillus subtilis (ATCC 6633)	MIC = 2.5 μg/mL MBC = 5 μg/mL	
			Bacillus cereus (ATCC 11774)	MIC = 2.5 μg/mL MBC = 5 μg/mL	
			Listeria monocytogenes (ATCC 19118)	MIC = 2.5 μg/mL MBC = 2.5 μg/mL	
			Salmonella typhimurium (ATCC 14028)	MIC = 10 μg/mL MBC = 10 μg/mL	
			Escherichia coli O157 : H7 (ATCC 10536)	MIC = 10 μg/mL MBC = 10 μg/mL	
Not reported	Essential oil	Microdilution method	*Staphylococcus aureus*	MIC = 0.005 μg/mL	[84]
			Bacillus subtilis	MIC = 0.005 μg/mL	
			Bacillus cereus	MIC = 0.005 μg/mL	
			Listeria monocytogenes	MIC = 0.005 μg/mL	
			Salmonella typhimurium	MIC = 0.005 μg/mL	
			Escherichia coli O157 : H7	MIC = 0.005 μg/mL	
Leaves	Essential oil	Agar diffusion method	*Escherichia coli* (ATCC 25922)	Φ = 17 mm	[69]
			Bacillus subtilis (NCTC 8236)	Φ = 16 mm	
Not reported	Decanted essential oil	Disc diffusion assay	*Staphylococcus epidermidis*	Φ = 2 mm	[85]
			Enterococcus faecalis	Φ = 5 mm	
			Streptococcus mutans	Φ = 5 mm	
			Escherichia coli	Φ = 6 mm	
			Pseudomonas aeruginosa	No inhibition	
Not reported	Recovered essential oil	Disc diffusion assay	*Staphylococcus epidermidis*	Φ = 2 mm	[85]
			Enterococcus faecalis	Φ = 4 mm	
			Streptococcus mutans	Φ = 5 mm	
			Escherichia coli	Φ = 6 mm	
			Pseudomonas aeruginosa	No inhibition	
Not reported	Essential oil	Agar well diffusion method	*Escherichia coli*	$\Phi = 14 \pm 0.05$ mm	[70]
			Salmonella typhi	No inhibition	
			Salmonella paratyphi	No inhibition	
			Staphylococcus aureus	$\Phi = 21 \pm 0.09$ mm	
			Klebsiella pneumoniae	$\Phi = 12.7 \pm 0.07$ mm	
			Pseudomonas aeruginosa	No inhibition	
			Acinetobacter spp.	$\Phi = 18 \pm 0.11$ mm	
Leaves	Essential oil	Agar diffusion method	*Bacillus subtilis*	Φ = 15 mm at a concentration of 100 mg/mL	[71]
			Escherichia coli	Φ = 17 mm at concentration of 100 mg/mL	
			Staphylococcus aureus	Φ = 16 mm at a concentration of 100 mg/mL	
			Pseudomonas aeruginosa	Φ = 16 mm at a concentration of 100 mg/mL	
Aerial parts	Essential oil	Agar diffusion method	*Pseudomonas aeruginosa* (ATCC 27853)	No inhibition	[7]
			Escherichia coli (ATCC 25922)	Φ = 9 mm	
			Staphylococcus aureus (ATCC 25923)	Φ = 11 mm	
			Staphylococcus epidermidis	Φ = 10 mm	
			Streptococcus pneumoniae	Φ = 13 mm	
			Streptococcus pyogenes	Φ = 16 mm	
			Klebsiella pneumoniae	Φ = 8 mm	
			Salmonella typhi	Φ = 8 mm	
			Shigella sonnei	Φ = 9 mm	

Table 4: Continued.

Parts used	Extracts	Methods used	Strains tested	Key results	References
Leaves	Essential oil	Agar-well diffusion assay Broth microdilution assay	*Staphylococcus aureus*	Φ = 32.00 ± 2.65 mm MIC = 0.25% (v/v) MBC = 0.25% (v/v)	[86]
			Pseudomonas aeruginosa	Φ = 13.33 ± 1.53 mm MIC = 0.5% (v/v) MBC = 2% (v/v)	
			Listeria monocytogenes	Φ = 26.67 ± 2.08 mm MIC = 0.25% (v/v) MBC = 0.25% (v/v)	
			Bacillus subtilis	Φ = 17.00 ± 2.00 mm MIC = 1% (v/v) MBC = 1% (v/v)	
			Proteus mirabilis	Φ = 29.33 ± 1.53 mm MIC = 0.5% (v/v) MBC = 1% (v/v)	
			Escherichia coli	Φ = 15.33 ± 1.89 mm MIC = 2% (v/v) MBC > 2% (v/v)	
Whole plant			*Staphylococcus aureus* (MBLA)	Φ = 18 ± 1.34 mm MIC = 4% (v/v) MBC > 8% (v/v)	[87]
			Staphylococcus aureus 976	Φ = 9 ± 1.9 mm	
			Listeria monocytogenes	Φ = 21 ± 3.11 mm MIC = 1% (v/v) MBC = 4% (v/v)	
			Staphylococcus aureus 994	Φ = 7 ± 0.66 mm	
			Bacillus subtilis 6633	Φ = 15 ± 0.80 mm	
			Escherichia coli K12	Φ = 9 ± 0.65 mm	
			Pseudomonas aeruginosa IH	No activity	
			Proteus mirabilis	Φ = 19 ± 0.41 mm MIC = 4% (v/v) MBC > 8% (v/v)	
Leaves	Essential oil	Agar well diffusion method	*Klebsiella pneumoniae* (CIP8291)	Φ = 25 mm	[72]
			Escherichia coli (ATCC10536)	No activity	
			Staphylococcus aureus (CIP7625)	No activity	
			Listeria monocytogenes (Scott A 724)	Φ = 29 mm	
Leaves	Essential oil	Agar well diffusion method	*Escherichia coli*	Φ = 8 mm at a concentration of 500 μL/mL	[88]
			Salmonella choleraesuis	Φ = 13 mm at a concentration of 500 μL/mL	
			Staphylococcus aureus	Φ = 11 mm at a concentration of 500 μL/mL	
			Listeria monocytogenes	Φ = 9.5 mm at a concentration of 500 μL/mL	
Aerial parts	Essential oil	Disc diffusion method	*Escherichia coli*	MIC = 2.5 μL/mL MBC = 2.5 μL/mL	[89]
			Streptococcus D	MIC = 2.5 μL/mL MBC = 2.5 μL/mL	
			E. faecalis	MIC = 2.5 μL/mL MBC = 2.5 μL/mL	
			K. pneumoniae	MIC = 2.5 μL/mL MBC = 2.5 μL/mL	

discovered the possibility of using essential oil extracted from aerial parts of *M. spicata* as a pesticide against the insect pest *Callosobruchus chinensis*. According to their findings, treatment with essential oil from *M. spicata* caused 100% mortality to *C. chinensis* after 12 h at a concentration of 0.1 μL/mL air using the fumigation toxicity test, and 100% repellency was observed at 0.025 μL/mL oil concentration in air during repellent activity assay. Using the probit model, the LC_{50} and LC_{90} values obtained were 0.003 and 0.005 μL/mL air concentrations, respectively. Furthermore, the

Table 5: Antiparasitic activity of *Mentha spicata*.

Part used	Extracts	Tested strains	Key results	Reference
Leaves	Dried plant	*Varroa destructor*	Killed 26.20% of *Varroa* Infestation rates = 13.18% Reduced the infestation rate of 2.35% Mortality rate = 30.65%	[90]
Leaves	Essential oils	*Tetranychus turkestani*	LC_{50} = 15.3 mL/L LC_{95} = 23.4 mL/L Mortality = 100% at concentration of 20 μL/L	[91]

Table 6: Insecticidal activity of *Mentha spicata*.

Part used	Extracts	Tested strains	Key results	Reference
Leaves	Essential oil	*Rhyzopertha dominica*	LD_{50} = 6.1 μL/mL Mortality = 43% after 96 hours at a concentration of 2 μL/mL Repellent effect = 56.2% at a concentration of 2 μL/mL	[65]
Leaves	Essential oil	Rice weevil *Sitophilus oryzae*	LC_{50} = 100.16 μL/L air; LC_{95} = 192.197 μL/L air; mortality = 22% at a concentration of 71.43 μL/L air; LT_{50} = 45.52 h	[93]
Aerial parts	Essential oil	*Callosobruchus chinensis*	Mortality = 100% after 12 h at concentration of 0.1 μL/mL air LC_{50} = 0.003 μL/mL air LC_{90} = 0.005 μL/mL air Repellency value = 100% at 0.025 μL/mL air of oil concentration 98% oviposition deterrence at 0.1 μL/mL concentration	[66]
	Essential oil	*Sitophilus granarius*	Mortality = 43% at the 24 h exposure test Mortality = 80% at the 48 h exposure test	[94]
Whole flowering plants	Essential oil	*Acanthoscelides obtectus*	LC_{50} = 1.2 mL/L air, for males LC_{50} = 4.4 mL/L air, for females	[95]
	Essential oil	*Boophilus annulatus*	Embryonated eggs (LC_{50} = 1.20%); unfed larvae (LC_{50} = 0.90%); fed females (LC_{50} = 10.57%)	[96]
Leaves	oil	*Callosobruchus maculatus*	LC_{50} = 235 ppm	[92]
	Essential oil	*Culex quinquefasciatus* Say	LC_{50} = 92 mg/L LC_{90} = 160 mg/L	[3]
Leaves	Essential oil	*C. quinquefasciatus*	LC_{50} = 62.62 ppm; LC_{90} = 118.70 ppm Mortality = 96.8 ± 1.2% at a concentration of 125 ppm	[38]
		A. aegypti	LC_{50} = 56.08 ppm; LC_{90} = 110.28 ppm Mortality = 98.1 ± 1.2% at a concentration of 125 ppm	
		A. stephensi	LC_{50} = 49.71 ppm; LC_{90} = 100.99 ppm Mortality = 99.6 ± 1.6% at a concentration of 125 ppm	

essential oil from *M. spicata* at 0.1 μL/mL concentration has been reported as the effective fumigant with an oviposition deterrence value estimated at 98%.

In an effort to identify biopesticides for granary weevil to avoid losses of crops caused by insects, Lamiri et al. [94] screened a variety of essential oils for their pesticide effects against *Sitophilus granarius*. They discovered that essential oil of spearmint caused 80% and 43% mortality after 24 h and 48 h of exposure, respectively. These findings indicate that the rate of adult mortality rises as the concentration of oil used in the test increases. The study by Papachristos and Stamopoulos [95] assessed the repellent effects of essential oil extracted from whole flowering plants of spearmint against *Acanthoscelides obtectus*. The results showed that this oil exhibited a highly toxic effect in both males and females with LC_{50} values of 1.2 mL/L air for males and 4.4 mL/L air for females, where males are more affected than females. Also, the oil of spearmint exhibited the most repellent property against *Acanthoscelides obtectus* and appears to be more promising for potential use against this pest.

Abdel-Shafy and Soliman [96] in their research hypothesized that essential oil of spearmint (*M. viridis*) possesses the toxicity effect against embryonated eggs, larvae, and fed females of the cattle tick *Boophilus annulatus* (Acari: Ixodida: Amblyommidae) in Egypt. It was found that oil spearmint (*M. viridis*) was less toxic on embryonated eggs (LC_{50} = 1.20%) as well as on unfed larvae (LC_{50} = 0.90%) and fed females (LC_{50} = 10.57%) than other oils tested, including peppermint (*Mentha piperita*), marjoram (*Majorana hortensis*), lavender (*Lavandula officinalis*), and sweet basil (*Ocimum basilicum*). Compared to the study performed by Derbalah and Ahmed [92], spearmint oil leaf was highly effective against *Callosobruchus maculatus* with an LC_{50} value of 235 ppm. The results showed that oil spearmint could be used as a botanical product to control *C. maculatus* insect in cowpea seeds.

Pavela et al. [3] showed the effects of a variety of essential oils from the genus *Mentha* L., including *M. spicata,* against the larvae and adults of *Culex quinquefasciatus* Say (Diptera: Culicidae). Their finding indicates that the oil of *M. spicata* revealed lower larvicidal efficacy against *C. quinquefasciatus* compared to other oils tested. The lethal response of the oil towards the larvae for LC_{50} was estimated as 92 mg/L and for LC_{90} was estimated as 160 mg/L. Similarly, the study carried out by Govindarajan et al. [38] focused on the possible larvicidal properties of essential oil from *M. spicata* against three larvae species: *A. stephensi, C. quinquefasciatus*, and *A. aegypti*. After the exposure of treatment (24 h), the essential oil from *M. spicata* leaves showed a significant larvicidal effect against *A. stephensi, C. quinquefasciatus*, and *A. aegypti*, with LC_{50} and LC_{90} values of 49.71 versus 100.99 ppm, 62.62 versus 118.70 ppm, and 56.08 versus 110.28 ppm, respectively. Also, the essential oil of *M. spicata* caused 99.6 ± 1.6% mortality for *A. stephensi* and 98.1 ± 1.2% for both *C. quinquefasciatus* and *A. aegypti* at a concentration of 125 ppm.

To test the application for alone or combined, three essential oils were isolated from three medicinal plant species belonging to the *Mentha* genus to manage the rice weevil *Sitophilus oryzae* (Curculionidae). The study conducted by Haouel-Hamdi et al. [93] showed that binary combined Tunisian spearmint oils from *M. rotundifolia, M. viridis*, and *M. longifolia* leaves have exerted an important anti-insecticide activity against *Sitophilus oryzae.* However, *Mentha* essential oils alone revealed the lowest repellent activity to *S. oryzae* adults. After 24 days of exposure, LC_{50} and LC_{95} values of fumigant toxicity of *M. viridis* essential oils alone was 100.16 μL/L air and 192.197 μL/L air, respectively, against *S. oryzae* adults. In addition, the LT_{50} value was 45.52 h for *M. viridis*, and the percentage of mortality was 22% at a concentration of 71.43 μL/L air.

3.5.5. Anti-Inflammatory Activity. Table 7 provides studies focused on the anti-inflammatory propriety of the *M. spicata* in different in vivo experiments [97, 99]. Using the carrageen-induced paw edema method, Yousuf et al. [97] showed that methanol extract from the whole plant of *M. spicata* exhibited a strong anti-inflammatory activity which presenting at both doses 250 and 500 mg/kg of methanol extract a significant dose-dependent reduction of paw edema. Furthermore, the anti-inflammatory action of the extract remained significant until the 6th hour of the test. In another study, Arumugam et al. [98] evaluated in vivo anti-inflammatory effect of different solvent fractions of the ethanolic extract of the dried leaves of *M. spicata* on rats with acute and chronic inflammation by using two experimental approaches, carrageenan and cotton pellet-induced inflammation models. The finding showed that ethyl acetate extract and aqueous fraction were potent in cotton pellet (chronic) induced inflammation where the rate of inflammation was reduced by 65% and 54%, respectively. However, inflammation was reduced with less effectiveness in hexane extract (0–20%) and aqueous fraction (7–11%); only the ethyl acetate fraction was found to be effective in carrageenan (acute) induced inflammation, while chloroform fraction has not been able to decrease inflammation.

The study conducted by Jabbar and Kathem [99] evaluated the preventive effect of ethanolic extract of leaves from *M. spicata* on irinotecan-induced mucositis in mice. The results revealed that the ethanolic extract of *M. spicata* markedly reduced jejunal tissue IL-1β (3.47 ± 1.23 vs. 6.5 ± 0.36 ng/mL), and fecal β-glucuronidase activity (79.78 ± 10.7 vs. 120.6 ± 8.3 U) compared to no-treated mice. In addition, histological investigation of the jejunum section of the animal after administration of irinotecan and ethanolic extract of *M. spicata* showed enhancements in mucositis features.

3.5.6. Antidiabetic Activity. Diabetes mellitus is a metabolic disease that affects the endocrine system, often occurring when the pancreas does not secrete enough insulin or when the body cannot use this hormone effectively, resulting in chronic hyperglycemia with disruptions in protein, lipid, and carbohydrate metabolism.

In order to understand the mechanism of antidiabetic action of *M. spicata* better, several recent studies (in vivo and in vitro) performed in chronological order were discussed in this review [8, 100, 101] (Table 8).

Regarding in vivo studies, Al-Fartosi and collaborators evaluated this activity on male rats rendered diabetic by alloxan intraperitoneal injection (125 mg/kg b.w) and treated with phenolic compounds (200 mg/kg b.w) extracted from the leaves of this plant [100]. During 14 days of daily treatment, a decrease in the level of blood glucose, triglycerides, cholesterol, plasma LDL, and VLDL and a significant increase in plasma HDL levels were recorded. This work confirmed the potential of *M. spicata* in the management of diabetes and its complications. In 2017, two similar studies verified these findings on the same animal model. Indeed, the aqueous ethanolic extract (200 and 400 mg/kg b.w) [101] and the aqueous extract (300 mg/kg b.w) [13] of the leaves of this species presented the same results as the previous study. The following year, 40 streptozotocin-induced diabetic rats were treated for 4 weeks with butanol extract from *M. spicata* roots [8]. At the end of this period, the authors observed antidiabetic properties represented by a decrease in blood glucose level and an increase in bodyweight.

A very recent investigation tested this powder on two carbohydrate hydrolyzing enzymes, namely, α-amylase and α-glucosidase [86]. In fact, inhibiting these two enzymes prevents the digestion of carbohydrates, which is a promising strategy in the treatment of diabetes. The results of this study showed that the leaf essential oil of this herb at doses of 200 and 250 μL was able to inhibit α-amylase (IC_{50} = 101.72 ± 1.86 μg/mL) and α-glucosidase (IC_{50} = 86.93 ± 2.43 μg/mL), respectively.

From these studies, it can be inferred that *M. spicata* may be used as an antidiabetic agent; however, further investigations, as well as clinical trials, must be carried out to evaluate this benefit in humans.

Table 7: Anti-inflammatory activity of *Mentha spicata*.

Used part	Extracts	Experimental approach	Key results	References
Whole plant	Methanol extract	Carrageen-induced paw edema method	Significant dose-dependent reduction of paw edema	[97]
Leaves	Hexane extract	Carrageenan-induced paw edema in rats	Reduced the inflammation with less effectiveness Reduced the inflammation by 0–20%	[98]
	Ethyl acetate extract		Reduced the inflammation by 9–85%	
	Chloroform fraction		The inflammation did not decrease	
	Aqueous fraction		Enhances inflammation by about 7–11%	
	Hexane extract	Cotton pellet-induced granuloma in rats	Reduced inflammation with 20%	
	Ethyl acetate extract		Reduced inflammation with 65%	
	Chloroform fraction		Reduced inflammation with 20%	
	Aqueous fraction		Reduced inflammation with 54%	
Leaves	Methanol extract	Irinotecan-induced mucositis in mice	Significantly decreased both jejunal tissue IL-1β and fecal β-glucuronidase activity Improvements in mucositis features	[99]

Table 8: Antidiabetic effects of *Mentha spicata*.

Part used	Extracts	Dose	Model	Keys results	References
Leaves	Aqueous ethanolic extract	200 mg/kg and 400 mg/kg bodyweight	Alloxan-induced hyperglycemic rats	Reduced blood glucose level, reduced serum cholesterol, triglycerides, LDL, and VLDL and increased bodyweights and HDL levels	[101]
Leaves	Phenolic extract	200 mg/kg bodyweight	Alloxan-induced hyperglycemic rats	Significant decrease in glucose concentration of blood serum; significant decrease in cholesterol and TG; significant increase in plasma HDL; significant decrease in plasma LDL, VLDL	[100]
Leaves	Aqueous extract	300 mg/kg bodyweight	Alloxan-induced hyperglycemic rats	Decreased blood glucose level; decreased bodyweight; significant reduction of total cholesterol, triglyceride, and LDL-cholesterol levels; significant increase in plasma HDL; significant reduction in the level of MDA	[13]
Roots	Butanol extract	100 mg/kg bodyweight	Streptozotocin-induced hyperglycemic rats	Increased bodyweight; reduced blood glucose	[8]
Leaves	Essential oil	200 μL	α-Glucosidase inhibitory assay	$IC_{50} = 86.93 \pm 2.43\,\mu g/mL$	[86]
		250 μL	α-Amylase inhibitory assay	$IC_{50} = 101.72 \pm 1.86\,\mu g/mL$	

3.5.7. Antioxidant Activity. Oxidative stress corresponds to an attack on cells by free radicals, also called reactive oxygen species (ROS), produced continuously from oxygen in the cell, particularly in the mitochondrial respiratory chain. ROS are reactive and very toxic substances. Oxidative stress is caused by an imbalance between the production of prooxidant free radicals and antioxidants. Regarding *M. spicata*, many studies have evaluated its antioxidant activity either by measuring its effectiveness in scavenging free radicals or by directly assaying the products formed using photometric techniques [5, 78, 102] (Table 9). Indeed, Getahun et al. [78] obtained essential oils by hydrodistillation from *M. spicata* leaves to determine their radical scavenging potentials in vitro in DPPH and deoxyribose degradation assays. These oils exhibited potent radical scavenging activities, with IC_{50} values of 5.96 and 0.57 μL/mL in the DPPH and deoxyribose degradation assays, respectively. In the same year, Nickavar et al. [102] found that the ethanolic extract of *M. spicata* aerial parts showed IC_{50} values of 87.89 and 173.80 μg/mL by the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays, respectively. The following year, using the same methods, Mkaddem et al. [72] showed that the essential oil from the leaves of this plant has significant anti-free radical potential.

By respecting the chronology of the studies carried out over time, Ebrahimzadeh et al. [9] examined the antioxidant capacity of *M. spicata* aerial parts in vitro using eight assay systems. They recorded the best activity with the DPPH test ($IC_{50} = 105.8 \pm 3.98\,\mu g/mL$), followed by the assay of nitric oxide-scavenging activity ($IC_{50} = 210.6 \pm 7.7\,\mu g/mL$) and scavenging of H_2O_2 ($IC_{50} = 631.1 \pm 26.0\,\mu g/mL$). In addition, good antioxidant activity has been demonstrated by Hussain

Table 9: Antioxidant activity of *Mentha spicata*.

Parts used	Extracts	Methods used	Key results	References
Whole plant	Methanol extract	DPPH	$IC_{50} = 65.13 \pm 1.29\ \mu g/mL$	[103]
		ABTS	$IC_{50} = 52.31 \pm 0.81\ \mu g/mL$	
Aerial parts	Ethanol extract	DPPH	$IC_{50} = 87.89\ \mu g/mL$	[102]
		ABTS	$IC_{50} = 173.80\ \mu g/mL$	
Aerial parts	Essential oil	DPPH	$IC_{50} = 3450 \pm 172.5\ \mu g/mL$	[48]
		ABTS	$IC_{50} = 40.2 \pm 0.2\ \mu g/mL$	
		FRAP	$IC_{50} = 215 \pm 4.50\ \mu g/mL$	
Leaves	Essential oil	DPPH	$IC_{50} = 9544.6 \pm 196.2\ \mu g/mL$	[65]
		ABTS	$IC_{50} = 36.2 \pm 3.2\ \mu g/mL$	
		Reducing power	$RP_{50} = 452.3 \pm 0.4\ \mu g/mL$	
		Phosphomolybdate	$RP_{50} = 53.3 \pm 2.8\ \mu g/mL$	
Leaves	Essential oil	DPPH	$IC_{50} = 10 \pm 0.24\ \mu g/mL$	[104]
		Superoxide anion	$IC_{50} = 1.33 \pm 0.10\ \mu g/mL$	
Aerial parts	Ethanol-water extract	DPPH	$IC_{50} = 105.8 \pm 3.98\ \mu g/mL$	[9]
		Nitric oxide radical scavenger	$IC_{50} = 210.6 \pm 7.7\ \mu g/mL$	
		Metal chelating	$IC_{50} = 757.4 \pm 29.5\ \mu g/mL$	
		Scavenging of H_2O_2	$IC_{50} = 631.1 \pm 26.0\ \mu g/mL$	
Seeds	Methanol extract	DPPH	Inhibition = 89.91 ± 2.12%	[33]
Leaves	Ethanol extract	DPPH	$IC_{50} = 16.2 \pm 0.2\ \mu g/mL$	[105]
		ABTS	$IC_{50} = 10.3 \pm 0.9\ \mu g/mL$	
		TEAC	TEAC = 0.90 ± 0.07 mM	
Leaves	Essential oils	DPPH	$IC_{50} = 8.81\ \mu L/mL$	[78]
Leaves	Essential oils	DPPH	$IC_{50} = 41{,}23\ \mu L/mL$	[80]
Aerial parts	Essential oils	DPPH	$IC_{50} = 13.3 \pm 0.6\ \mu L/mL$	[5]
	Essential oil	DPPH	$IC_{50} = 72.07 \pm 0.34$ mg/mL	[11]
Aerial parts	Water extract	DPPH	Inhibition = 74.2 ± 0.2%	[106]
		β-Carotene	Inhibition = 79.1 ± 2.4%	
Leaves	Water extract	DPPH	$EC_{50} = 336 \pm 3\ \mu g/mL$	[107]
		Reducing power	$EC_{50} = 198 \pm 2\ \mu g/mL$	
		TBARS	$EC_{50} = 152 \pm 5\ \mu g/mL$	
Leaves	Essential oil	DPPH	$IC_{50} = 21.19 \pm 7.17\ \mu g/mL$	[82]
		Reducing power	$IC_{50} = 2.28 \pm 0.68\ \mu g/mL$	
		DPPH	Inhibition = 30.52 ± 0.09% at a concentration of 10 μg/mL	
Aerial parts	Essential oil	DPPH	$IC_{50} = 3.08 \pm 0.07\ \mu g/mL$	[108]
		Reducing power	$EC_{50} = 2.49 \pm 0.07\ \mu g/mL$	
		Chelating power	$IC_{50} = 6.33 \pm 0.12\ \mu g/mL$	
		β-Carotene	$IC_{50} = 6.4 \pm 0.07\ \mu g/mL$	
Leaves	Essential oil	β-Carotene-linoleic acid	Antioxidant activity = 25.37% at 500 μg/mL	[44]
		DPPH	Antioxidant activity = 13.81% at 500 μg/mL	
Aerial parts	Ethanol extract	DPPH	Radical scavenging effect = 18.34 ± 2.2% at a concentration of 0.4 mg/mL	[50]
	Essential oil	DPPH	$IC_{50} > 2.5\ \mu g/mL$	[87]
Leaves	Essential oil	DPPH	$IC_{50} = 80.45 \pm 1.86\ \mu g/mL$	[86]
		FRAP	$IC_{50} = 101.78 \pm 3.14\ \mu g/mL$	
		ABTS	$IC_{50} = 139.59 \pm 3.12\ \mu g/mL$	
Leaves	Essential oil	ABTS	$IC_{50} = 195.1 \pm 4.2$ mg/L	[72]
		DPPH	$IC_{50} = 3476.3 \pm 133$ mg/L	

et al. [5] ($IC_{50} = 13.3 \pm 0.6\ \mu L/mL$) and by Liu et al. [11] ($IC_{50} = 72.07 \pm 0.34$ mg/mL), using DPPH free radical-scavenging ability. Moreover, the antioxidant power of *M. spicata* aerial parts has been tested by Benedec et al. [50] using only the DPPH radical scavenging assay, which showed a value of 18.34 ± 2.2% at the concentration of 0.4 mg/mL. A Tunisian research team also confirmed this when they recorded an important antiradical ($IC_{50} = 10 \pm 0.24\ \mu g/mL$) and superoxide anion ($IC_{50} = 1.33 \pm 0.10\ \mu g/mL$) scavenging ability [104]. Furthermore, according to Teixeira and collaborators, the essential oil of this plant was shown to be a potent antioxidant by exhibiting a dose-dependent antioxidant effect at the concentrations tested (25, 50, 100, 150, 200, 250, 300, and 500 μg/mL), determined by the sequestration of the DPPH radical and by the β-carotene-linoleic acid method [44].

Using the same methods as previous studies, other more recent investigations have confirmed the important antioxidant activity of *M. spicata*, regardless of its harvest region or parts used (Table 9).

The antioxidant activity of different parts of *M. spicata* is certainly attributed to its major compounds. Indeed,

L-menthone (32.74%) and pulegone (26.67%) were the main volatiles of its essential oil, while apigenin (38.4 mg/100 g dry weight) was the main flavonoid in methanolic extracts [104]. These molecules are renowned for their antioxidant potential [109].

3.5.8. Diuretic Activity. The in vivo study performed by Aziz et al. [110] assessed the diuretic property of the aqueous methanol extract from aerial parts of spearmint in rat models. The treatment administered to experimental rats at dose 100 mg/kg revealed significant diuresis (3.74 ± 0.41 mL). The values obtained are more or less close to the reference standard (furosemide, 4.05 ± 0.34 mL) ($p < 0.05$). Also, the extract of spearmint significantly increased the excretion of potassium and sodium ($p < 0.05$), while a significant change in the pH has not been observed after administration of *M. viridis* extract.

3.5.9. Analgesic and Antipyretic Activities. For testing the analgesic and antipyretic effects of methanol extract from *M. spicata*, Yousuf et al. [97] in their study demonstrated that the methanol extract from the whole plant of *M. spicata* had markedly increased the reaction time of mice in a dose-dependent manner by the hot-plate test ($p < 0.001$) proving its marked analgesic effect. In addition, using the acetic acid-induced writhing method, the methanol extract of *M. spicata* also exhibited a significant analgesic action. The inhibition at the dose of 500 mg/kg was estimated at 60.30%. On the other hand, using Brewer's yeast-induced pyrexia in rats, the methanol extract of *M. spicata* was revealed to exert a strong marked ($p < 0.01$) antipyretic activity at the dose of 500 mg/kg at 3 h than at a dose of 100 mg/kg at 2 h.

3.5.10. Antihemolytic Activity. In order to investigate the biological functions of *M. spicata*, Ebrahimzadeh et al. [9] decided to study the antihemolytic effect of ethanol-water extract from aerial parts of *M. spicata*. The results showed that this extract possesses a weak inhibiting effect with an $IC_{50} = 1250.7 \pm 46.1\ \mu g/mL$ by H_2O_2-induced membrane damage and hemolysis.

3.5.11. Protective Effects. In their research, Saad et al. [111] were interested in studying the protective activity of *M. spicata* treatment against nicotine-induced oxidative damage in the liver and erythrocytes Wistar rats. The findings showed that aqueous extract from aerial parts of *M. spicata* exhibited a strong protective action. On the hematological parameters, it was found to restore to normal levels the levels of erythrocytes, haematocrit, hemoglobin, and white blood cells. However, on hepatic dysfunction parameters, the aqueous extract of spearmint significantly decreased ALT and ALP activities resulting in a decrease in liver toxicity. Furthermore, the aqueous extract of *M. spicata* to nicotine-treated rats provided a statistically significant ($p \leq 0.01$) enhancement of antioxidant enzyme capacities, including CAT, SOD, and GPX activities, suggesting an improvement in antioxidant status. According to liver histological analysis, the treatment with the aqueous extract of *M. spicata* showed considerable recovery in the form of hepatic histoarchitecture. Similarly, Saad et al. [111] aimed to screen the in vivo and in vitro antioxidative effect of *M. spicata* extract against nicotine-induced oxidative injury in the kidney and brain of rats. The in vivo results obtained reported that *Mentha* extract significantly increased the bodyweight of rats as well as exhibited a significant increase in testis, brain, and accessory sex organ weights. In addition, treatment with the aqueous extract of *M. spicata* had a significant decrease in the MDA levels, but no significant changes in brain AChE were recorded. Also, *M. spicata* extract supplementation could restore the antioxidant enzymes activities to normal levels and participate to ameliorate cerebral cortex histological pictures and histological damages.

3.6. Toxicity Investigations. In pharmacology, the efficacy of a plant or a natural constituent is not sufficient to justify its therapeutic use. Indeed, each bioactive substance is likely to have deleterious effects for human health, at least in high doses and over long periods [112]. In addition to efficacy, the active dose must be free from any toxicity and demonstrate safety. Therefore, in the therapeutic indication of any substance, it is imperative to define its risk-benefit ratio.

Despite the data paucity on its safety profile and given its wide use, the acute and subacute toxicities of *M. spicata* have been tested in four studies to optimize its use [66, 113, 114] (Table 10).

Initially, Yousuf et al. [97] orally administered single doses of 500, 1000, and 2000 mg/kg of whole plant methanolic extract to mice of both sexes. After 24 hours of observation, no mortality or signs of toxicity were noticed. One year later, aerial parts of the same extract at a dose of 5000 mg/kg of extract (the limit test dose according to OECD guidelines 425) showed similar results in female rats [113]. Indeed, during the 14 days of oral gavage, no mortality was recorded, considering the LD_{50} to be greater than 5000 mg/kg. In addition, no changes in the behavior and the bodyweight of the animals were observed. At the end of the experiment and after sacrificing animals, there were no toxicologically significant biochemical and hematological changes compared to the control group. The histological evaluation did not reveal any morphological changes or gross lesions in the lung, kidney, liver, and heart tissues. These results corroborate those obtained by Kedia et al. [66]. They recorded low toxicity ($LD_{50} = 8342.33\ \mu L/kg$) of the essential oil of *M. spicata* aerial parts following oral administration of different doses (0.05–0.5 mL) to mice (*Mus musculus* L.).

In the same year, Mugisha and colleagues tested the acute and subacute toxicities of the leaves of this plant in Swiss mice and Wistar albino rats, respectively [114]. For acute toxicity, animals received intragastrically over 72 hours, doses of 10000, 12000, 14000, 16000, and 18000 g/kg b.w of the 70% ethanolic extract. Therefore, a death rate of 100% was obtained at the highest dose with some signs of toxicity (convulsions, abdominal muscle contractions, and hyperurination) above 12000 mg/kg b.w. The LD_{50} value was

Table 10: Toxicity study of *Mentha spicata*.

Parts used	Extracts	Experimental approaches	Key results	References
Aerial parts	Methanol extract	The animals treated with a single dose of 5000 mg/kg of *M. spicata* extract by oral gavage	No mortality during the observation period No toxicologically significant hematological and biochemical changes Any morphological changes in the heart, liver, kidney, and lung tissues of the rats	[113]
Leaves	Ethanolic extract	The animals treated with a single dose of 10000, 12000, 14000, 16000, and 18000 mg/kg BW of *M. spicata* extract by oral gavage	LD_{50} is considered to be >5000 mg/kg NOEL dose for *M. spicata* was 10000 mg/kg 100% mortality at 18000 mg/kg BW LD_{50} = 13606 mg/kg BW	[114]
Leaves	Ethanolic extract	The animals treated with 500, 1000, and 1500 mg/kg BW daily for 28 days	No signs of toxicity; no mortalities; no significant change in bodyweight; significant increase in WBC, Lym, and MCHC levels; significant reduction in HCT level; significant increase in AST levels; unaffected serum creatinine and urea; no significant histopathological change	[114]
Whole plant	Methanol extract	Mice treated with a single dose of 500, 1000, and 2000 mg/kg of *M. spicata* extract by oral gavage	No mortality during the observation period	[97]
Aerial parts	Essential oil	EO (0.05–0.5 mL) orally administered to mice (*Mus musculus* L., average weight 30.0 g, age 3 months)	LD_{50} = 8342.33 μL/kg	[66]
Leaves	Ethanolic extract	The animals treated with 500, 1000, and 1500 mg/kg BW daily for 28 days	No signs of toxicity; no mortalities; no significant change in bodyweight; significant increase in WBC, Lym, and MCHC levels; significant reduction in HCT level; significant increase in AST levels; unaffected serum creatinine and urea; no significant histopathological change	[114]
Whole plant	Methanol extract	Mice treated with a single dose of 500, 1000, and 2000 mg/kg of *M. spicata* extract by oral gavage	No mortality during the observation period	[97]
Aerial parts	Essential oil	EO (0.05–0.5 mL) orally administered to mice (*Mus musculus* L., average weight 30.0 g, age 3 months)	LD_{50} = 8342.33 μL/kg	[66]

13606 mg/kg b.w. Regarding subacute toxicity (28 days), ethanol leaf extract (500, 1000, and 1500 mg/kg b.w) caused no mortality or signs of toxicity. However, it significantly increased the levels of mean corpuscular hemoglobin concentration, lymphocytes, blood cells count, and aspartate transferase and significantly reduced haematocrit. At the same time, serum urea and creatinine levels were not affected, confirmed by histopathological data.

From these toxicological investigations, it can be declared that *M. spicata* is an experimentally safe plant, thus justifying its use in treating numerous abnormalities. However, prolonged treatment in high doses can lead to specific problems. For this, other studies on this plant's chronic toxicity are necessary to complete its toxicological profile.

4. Conclusion and Perspectives

In this work, we reported the ethnobotanical, phytochemical, and pharmacological aspects of *M. spicata* (*M. viridis*). This medicinal plant is frequently used in traditional practices to treat certain diseases and showed interesting biological properties in various scientific investigations. Phytochemical studies of this species showed its richness in numerous bioactive compounds in particularly terpenoid components, exhibiting important biological effects. Pharmacological biology explorations demonstrated that extracts and essential oils of *M. spicata* showed different pharmacological properties such as antibacterial, antiparasitic activity, insecticidal, anti-inflammatory, antidiabetic, antioxidant, diuretic, analgesic, antipyretic, antihemolytic, and protective activities. However, these effects were evaluated often using in vitro and in vivo approaches, and therefore, further investigations to validate these activities with determining mechanisms of their actions are needed. Toxicological investigation of *M. spicata* extracts was examined by some studies and showed a safety of this plant. However, clinical trials were not conducted, and there is an urgent need to perform such trials to promote the use of the plant especially after proving its excellent safety profile in the toxicological investigation. Indeed, bioactive compounds of *M. spicata* need further investigations concerning the pharmacodynamic and pharmacokinetic aspects to determine their bioavailability and their mechanisms of action of different targets.

Data Availability

The data used to support this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors wish to thank the Research Center at College of Pharmacy and Deanship of Scientific Research at King Saud University, Riyadh, Saudi Arabia, for providing financial support and access to online database for literature survey.

Supplementary Materials

Graphical abstract of this study is attached in supplementary file. (*Supplementary Materials*)

References

[1] O. Benkhnigue, B. A. Fatiha, S. Salhi, F. Mohamed, A. Douira, and L. Zidane, "Catalogue des plantes médicinales utilisées dans le traitement du diabète dans la région d'Al Haouz-Rhamna (Maroc)," *The Journal of Animal and Plant Sciences*, vol. 23, no. 1, pp. 3539–3568, 2014.

[2] A. Bouyahya, J. Abrini, A. Et-Touys, Y. Bakri, and N. Dakka, "Indigenous knowledge of the use of medicinal plants in the North-West of Morocco and their biological activities," *European Journal of Integrative Medicine*, vol. 13, pp. 9–25, 2017.

[3] R. Pavela, K. Kaffková, and M. Kumšta, "Chemical composition and larvicidal activity of essential oils from different Mentha L. and Pulegium species against Culex quinquefasciatus say (Diptera: Culicidae)," *Plant Protection Science*, vol. 50, no. 1, pp. 36–42, 2014.

[4] I. Print, A. R. Golparvar, A. Hadipanah, and A. M. Mehrabi, "Diversity in chemical composition from two ecotypes of (Mentha longifolia L.) and (Mentha spicata L. in Iran climatic conditions)," *Journal of Biodiversity and Environmental Sciences JBES.*, vol. 6, no. 4, pp. 26–33, 2015.

[5] A. I. Hussain, F. Anwar, and M. Shahid, "Chemical composition, and antioxidant and antimicrobial activities of essential oil of spearmint (mentha spicata L.) from Pakistan," *Journal of Essential Oil Research*, vol. 22, no. 1, pp. 78–84, 2010.

[6] L. M. S. S. Franciscato, M. R. Silva, F. Andrich et al., "Chemical characterization and antimicrobial activity of essential oils of mint (mentha spicata L.) and surinam cherry (eugenia uniflora L.)," *Orbital - The Electronic Journal of Chemistry*, vol. 10, no. 6, pp. 475–481, 2018.

[7] H. Boukhebti, A. N. Chaker, H. Belhadj, F. Sahli, H. Laouer, and D. Harzallah, "Chemical composition and antibacterial activity of Mentha pulegium L. and Mentha spicata L. essential oils," *Der Pharmacia Lettre*, vol. 3, no. 4, p. 10, 2011.

[8] M. Rakebizadeh, M. Zahedizadeh, and Y. E. Panah, "Supplemental effect of zinc oxide nanoparticles and mentha spicata butanol extract on blood glucose of diabetic wistar rats," *Journal of Science, Engineering & Technology*, vol. 3, no. 5, 2018.

[9] M. A. Ebrahimzadeh, S. M. Nabavi, and S. F. Nabavi, *Biological Activities of Me Tha Spicata L.,* Pharmacology Online, vol. 1, pp. 841–848, 2010.

[10] G. Mahendran and L. U. Rahman, "Ethnomedicinal, phytochemical and pharmacological updates on Peppermint (Mentha × piperita L.)-A review," *Phytotherapy Research*, vol. 34, no. 9, pp. 2088–2139, 2020.

[11] K. H. Liu, Q. Zhu, J. J. Zhang, J. F. Xu, and X. C. Wang, "Chemical composition and biological activities of the essential oil of Mentha spicata Lamiaceae," *Advanced Materials Research*, vol. 524-527, pp. 2269–2272, 2012.

[12] G. Kunwar, C. Pande, and G. Tewari, "Essential oil composition of the aerial parts of mentha spicata L." *Journal of essential oil-bearing plants JEOP*, vol. 13, no. 3, pp. 353–356, 2017.

[13] M. Bayani, M. Ahmadi-hamedani, and A. Jebelli Javan, "Study of hypoglycemic, hypocholesterolemic and antioxidant activities of Iranian mentha spicata leaves aqueous extract in diabetic rats," *Iranian Journal of Pharmaceutical Research: IJPR*, vol. 16, no. 8, pp. 75–82, 2017.

[14] B. M. Kassahun, D. F. Egata, T. Lulseged, W. B. Yosef, and S. Tadesse, "Variability in agronomic and chemical characteristics of spearmint (mentha spicata L.) genotypes in Ethiopia," *International Journal of Advanced Biological and Biomedical Research*, vol. 2, no. 10, pp. 2704–2711, 2014.

[15] L. A. Kee, A. B. Shori, and A. S. Baba, "Bioactivity and health effects of Mentha spicata," *Integrative Food, Nutrition and Metabolism*, vol. 5, no. 1, pp. 1-2, 2017.

[16] J. El-hilaly, M. Hmammouchi, and B. Lyoussi, "Ethnobotanical studies and economic evaluation of medicinal plants in Taounate province (Northern Morocco)," *Journal of Ethnopharmacology*, vol. 86, no. 2-3, pp. 149–158, 2003.

[17] E. Idm'hand, F. Msanda, and K. Cherifi, "Ethnopharmacological review of medicinal plants used to manage diabetes in Morocco," *Clin. Phytoscience.*, vol. 6, no. 1, 2020.

[18] J. Fakchich and M. Elachouri, "Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments," *Journal of Ethnopharmacology*, vol. 154, no. 1, pp. 76–87, 2014.

[19] H. Labiad, A. Et-Tahir, M. Ghanmi et al., "Ethnopharmacological survey of aromatic and medicinal plants of the pharmacopoeia of northern Morocco," *Ethnobotany Research and Applications*, vol. 19, pp. 1–16, 2020.

[20] H. Orch, L. Zidane, and A. Douira, "Ethnobotanical study of plants used in the treatment of respiratory diseases in a population bordering the forest of Izarène," *Journal of Pharmacy & Pharmacognosy Research*, vol. 8, no. 5, pp. 392–409, 2020.

[21] N. Salhi, A. Bouyahya, S. Fettach, A. Zellou, and Y. Cherrah, "Ethnopharmacological study of medicinal plants used in the treatment of skin burns in Occidental Morocco (area of Rabat)," *South African Journal of Botany*, vol. 121, pp. 128–142, 2019.

[22] P. A. El Abbouyi, N. Filali-Ansari, P. S. Khyari, and H. Loukili, "Inventory of medicinal plants prescribed by traditional healers in El Jadida city and suburbs (Morocco)," *International Journal of Green Pharmacy*, vol. 8, no. 4, pp. 242–251, 2014.

[23] M. El Hafian, N. Benlandini, H. Elyacoubi, L. Zidane, and A. Rochdi, "Étude floristique et ethnobotanique des plantes médicinales utilisées au niveau de la préfecture d'Agadir-Ida-Outanane (Maroc)," *Journal of Applied Biosciences*, vol. 81, no. 1, pp. 7198–7213, 2014.

[24] I. Telci, N. Sahbaz, G. Yilmaz, and M. E. Tugay, "Agronomical and chemical characterization of spearmint (Mentha spicata L.) originating in Turkey," *Economic Botany*, vol. 58, no. 4, pp. 721–728, 2004.

[25] A. Ainane, "Chemical study by GC-MS of the essential oils of certain mints grown in the region of settat (Morocco):

mentha piperita, mentha pulegium and mentha spicata," *Drug Designing & Intellectual Properties International Journal*, vol. 1, no. 4, 2018.

[26] N. Zekri, H. Elazzouzi, F. El Makhoukhi, M. Alaoui El Belghiti, and T. Zair, "Drying effect on yields and chemical composition of essential oils extracted from the Moroccan mentha spicata (L.) aerial parts," *Journal of Essential Oil Bearing Plants*, vol. 22, no. 3, pp. 789–798, 2019.

[27] F. Bensabah, S. Houbairi, M. Essahli, A. Lamiri, and J. Naja, "Chemical composition and inhibitory effect of the essential oil from Mentha Spicata irrigated by wastewater on the corrosion of aluminum in 1 molar hydrochloric acid," *Portugaliae Electrochimica Acta*, vol. 31, no. 4, pp. 195–206, 2013.

[28] M. Znini, M. Bouklah, L. Majidi et al., "Chemical composition and inhibitory effect of Mentha spicata essential oil on the corrosion of steel in molar hydrochloric acid," *International Journal of Electrochemical Science*, vol. 6, no. 3, pp. 691–704, 2011.

[29] M. Maffei, A. Codignola, and M. Fieschi, "Essential oil from Mentha spicata L. (spearmint) cultivated in Italy," *Flavour and Fragrance Journal*, vol. 1, no. 3, pp. 105–109, 1986.

[30] M. Cirlini, P. Mena, M. Tassotti et al., "Phenolic and volatile composition of a dry spearmint (Mentha spicata L.) extract," *Molecules*, vol. 21, no. 8, pp. 1–15, 2016.

[31] E. Sevinďik, Ç. Yamaner, C. Kurtoğlu, and B. Tin, "Chemical composition of mentha spicata L. Subsp. tomentosa and M. Pulegium L., and their antimicrobial activity on strong pathogen microorganisms," *Notulae Scientia Biologicae*, vol. 9, 2017.

[32] S. Kizil and Ö. Tonçer, "Influence of different harvest times on the yield and oil composition of spearmint (Mentha spicata L. var. spicata)," *Journal of Food Agriculture and Environment*, vol. 4, no. 3–4, pp. 135–137, 2006.

[33] İ. Emre, Y. Kurşat, M. Kurşat, Ö. Yilmaz, and P. Erecevit, "Chemical compositions, radical scavenging capacities and antimicrobial activities in seeds of satureja hortensis l. And mentha spicata l. subsp. spicata from Turkey," *Brazilian Journal of Biology*, vol. 81, no. 1, pp. 144–153, 2021.

[34] R. S. Chauhan, M. K. Kaul, A. K. Shahi, A. Kumar, G. Ram, and A. Tawa, "Chemical composition of essential oils in Mentha spicata L. accession [IIIM(J)26] from North-West Himalayan region, India," *Industrial Crops and Products*, vol. 29, no. 2–3, pp. 654–656, 2009.

[35] P. K. Jain, A. Soni, P. Jain, and J. Bhawsar, "Phytochemical analysis of Mentha spicata plant extract using UV-VIS, FTIR and GC/MS technique," *Journal of Chemical and Pharmaceutical Research*, vol. 8, no. 2, pp. 1–6, 2016.

[36] G. Tewari, "Essential oil composition of the aerial parts of mentha spicata L," *World Journal of Pharmaceutical Research*, vol. 6, pp. 853–859, 2017.

[37] M. A. Aziz, M. Adnan, A. H. Khan, A. A. Shahat, M. S. Al-Said, and R. Ullah, "Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan," *Journal of Ethnobiology and Ethnomedicine*, vol. 14, no. 1, pp. 1–16, 2018.

[38] M. Govindarajan, R. Sivakumar, M. Rajeswari, and K. Yogalakshmi, "Chemical composition and larvicidal activity of essential oil from Mentha spicata (Linn.) against three mosquito species," *Parasitology Research*, vol. 110, no. 5, pp. 2023–2032, 2012.

[39] M. Mahmoodi and M. M. Sourestani, *Oil Content, Yield and Composition of Spearmint (Mentha Spicata L. Var. Kashan) at Different Cuts the First Iranian Congress of Essential Oil, Oil Content, Yield and Composition of Spearmint (Mentha Spicata L. Var. Kashan) at Different Cuts*, Researchgate.Net, Berlin, Germany, 2016.

[40] J. Farahbakhsh, S. Najafian, M. Hosseinifarahi, and S. Gholipour, "Essential oil storage conditions affect the chemical composition in cultivated Mentha spicata," *Iranian Journal of Plant Physiology*, vol. 11, 2020.

[41] A. R. Golparvar, A. Hadipanah, and M. M. Gheisari, "Comparative analysis of chemical composition of three ecotypes of spearmint (Mentha spicata L. (in Isfahan province," no. 2011, pp. 2011–2013, 2013.

[42] M. Bimakr, R. A. Rahman, F. S. Taip et al., "Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (Mentha spicata L.) leaves," *Food and Bioproducts Processing*, vol. 89, no. 1, pp. 67–72, 2011.

[43] P. P. Almeida, N. Mezzomo, and S. R. S. Ferreira, "Extraction of mentha spicata L. Volatile compounds: evaluation of process parameters and extract composition," *Food and Bioprocess Technology*, vol. 5, no. 2, pp. 548–559, 2012.

[44] M. L. Teixeira, M. d. G. Cardoso, A. C. S. Figueiredo et al., "Essential oils from lippia origanoides kunth. And mentha spicata L.: chemical composition, insecticidal and antioxidant activities," *American Journal of Plant Sciences*, vol. 05, no. 09, pp. 1181–1190, 2014.

[45] A. de Sousa Barros, S. M. de Morais, P. A. T. Ferreira et al., "Chemical composition and functional properties of essential oils from Mentha species," *Industrial Crops and Products*, vol. 76, pp. 557–564, 2015.

[46] D. Zhao, Y. W. Xu, G. L. Yang, A. M. Husaini, and W. Wu, "Variation of essential oil of Mentha haplocalyx Briq. and Mentha spicata L. from China," *Industrial Crops and Products*, vol. 42, no. 1, pp. 251–260, 2013.

[47] T. Antal, A. Figiel, B. Kerekes, and L. Sikolya, "Effect of drying methods on the quality of the essential oil of spearmint leaves (mentha spicataL.)," *Drying Technology*, vol. 29, no. 15, pp. 1836–1844, 2011.

[48] S. K. Bardaweel, B. Bakchiche, H. A. ALSalamat, M. Rezzoug, A. Gherib, and G. Flamini, "Chemical composition, antioxidant, antimicrobial and Antiproliferative activities of essential oil of Mentha spicata L. (Lamiaceae) from Algerian Saharan atlas," *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, pp. 201–207, 2018.

[49] A. H. Al-Marzouqi, M. V. Rao, and B. Jobe, "Comparative evaluation of SFE and steam distillation methods on the yield and composition of essential oil extracted from spearmint (Mentha spicata)," *Journal of Liquid Chromatography & Related Technologies*, vol. 30, no. 4, pp. 463–475, 2007.

[50] D. Benedec, L. Vlase, I. Oniga et al., "LC-MS analysis and antioxidant activity of phenolic compounds from two indigenous species of Mentha," *Farmacia*, vol. 61, no. 2, pp. 262–267, 2013.

[51] Z. Soilhi, A. Rhimi, S. Heuskin, M. Fauconnier, and M. Mekki, "Essential oil chemical diversity of Tunisian mentha spp. collection," *Industrial Crops and Products*, vol. 131, 2018.

[52] J. U. Chowdhury, N. C. Nandi, M. Uddin, and M. Rahman, "Chemical constituents of essential oils from two types of spearmint (Mentha spicata L. And M. cardiaca L.) introduced in Bangladesh," *Bangladesh Journal of Scientific & Industrial Research*, vol. 42, no. 1, pp. 79–82, 1970.

[53] M. D. Soković, J. Vukojević, P. D. Marin, D. D. Brkić, V. Vajs, and L. J. Van Griensven, "Chemical composition of essential oils of Thymus and mentha species and their antifungal activities," *Molecules*, vol. 14, no. 1, pp. 238–249, 2009.

[54] M. S. Ali-Shtayeh, R. M. Jamous, S. Y. Abu-Zaitoun, A. I. Khasati, and S. R. Kalbouneh, "Biological properties and bioactive components of mentha spicata L. Essential oil: focus on potential benefits in the treatment of obesity, alzheimer's disease, dermatophytosis, and drug-resistant infections," *Evidence-Based Complementary and Alternative Medicine*, vol. 2019, 2019.

[55] A. Chrysargyris, P. Xylia, G. Botsaris, and N. Tzortzakis, "Antioxidant and antibacterial activities, mineral and essential oil composition of spearmint (Mentha spicata L.) affected by the potassium levels," *Industrial Crops and Products*, vol. 103, pp. 202–212, 2016.

[56] L. C. A. Barbosa, C. A. Filomeno, and R. R. Teixeira, "Chemical variability and biological activities of Eucalyptus spp. essential oils," *Molecules*, vol. 21, no. 12, 2016.

[57] S. Laggoune, M. Öztürk, E. Erol et al., "Chemical composition, antioxidant and antibacterial activities of the essential oil of Mentha spicata L. from Algeria," *BMC Complementary and Alternative Medicine*, vol. 18, 2016.

[58] H. EL-Haoud, M. Boufellous, A. Berrani, H. Tazougart, and R. Bengueddour, "Screening phytochimique D' une plante medicinale: mentha spicata L," *American Journal of Innovative Research & Applied Sciences*, vol. 7, no. 4, pp. 226–233, 2018.

[59] R. Subramanian, S. Gayathri, C. Rathnavel, and V. Raj, "Analysis of mineral and heavy metals in some medicinal plants collected from local market," *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 1, pp. S74–S78, 2012.

[60] R. P. Choudhury, A. Kumar, and A. N. Garg, "Analysis of Indian mint (Mentha spicata) for essential, trace and toxic elements and its antioxidant behaviour," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 41, no. 3, pp. 825–832, 2006.

[61] M. A. Ebrahimzadeh, S. Eslami, S. M. Nabavi, S. F. Nabavi, A. H. Moghaddam, and A. R. Bekhradnia, "Estimation of essential and toxic mineral elements in mentha species," *Asian Journal of Chemistry*, vol. 23, no. 4, p. 3, 2011.

[62] A. Alaklabi, I. A. Arif, A. Ahamed, A. Manilal, R. Surendrakumar, and A. Idhayadhulla, "Larvicidal, nematicidal, antifeedant and antifungal, antioxidant activities of Mentha spicata (Lamiaceae) root extracts," *Tropical Journal of Pharmaceutical Research*, vol. 15, no. 11, p. 2383, 2016.

[63] M. E. Ojewumi, S. O. Adedokun, O. J. Omodara, E. A. Oyeniyi, O. S. Taiwo, and E. O. Ojewumi, "Phytochemical and antimicrobial activities of the leaf oil extract of mentha spicata and its efficacy in repelling mosquito," *International Journal of Pharmaceutical Research and Allied Sciences*, vol. 6, no. 4, p. 12, 2017.

[64] Y. Bayan, M. Küsek, and M. Küsek, "Chemical composition and antifungal and antibacterial activity of mentha spicata L. Volatile oil," *Ciencia e investigación agraria*, vol. 45, no. 1, pp. 64–69, 2018.

[65] F. Brahmi, A. Adjaoud, B. Marongiu et al., "Chemical and biological profiles of essential oils fromMentha spicataL. leaf from Bejaia in Algeria," *Journal of Essential Oil Research*, vol. 28, no. 3, pp. 211–220, 2016.

[66] A. Kedia, B. Prakash, P. K. Mishra, C. S. Chanotiya, and N. K. Dubey, "Antifungal, antiaflatoxigenic, and insecticidal efficacy of spearmint (Mentha spicata L.) essential oil," *International Biodeterioration & Biodegradation*, vol. 89, pp. 29–36, 2014.

[67] E. Şarer, S. Y. Toprak, B. Otlu, and R. Durmaz, "Composition and antimicrobial activity of the essential oil from mentha spicata L. subsp. spicata," *Journal of Essential Oil Research*, vol. 23, 2014.

[68] J. Singh, A. K. Dubeyd, and N. N. Tripathi, "Antifungal activity of mentha spicata," *International Journal of Pharmacognosy*, vol. 32, no. 4, pp. 314–319, 1994.

[69] A. M. Sulieman, S. E. Abdelrahman, and A. M. Abdel Rahim, "Phytochemical analysis of local spearmint (mentha spicata) leaves and detection of the antimicrobial activity of its oil," *Journal of Microbiology Research*, vol. 1, no. 1, pp. 1–4, 2012.

[70] S. Zaidi and P. Dahiya, "In vitro antimicrobial activity, phytochemical analysis and total phenolic content of essential oil from Mentha spicata and Mentha piperita," *International Food Research Journal*, vol. 22, 2015.

[71] O. Yahia Balla, "Chemical composition and antimicrobial activity of essential oil of mentha viridis," *Biochemistry and Molecular Biology*, vol. 2, no. 5, p. 60, 2017.

[72] M. Mkaddem, J. Bouajila, M. Ennajar, A. Lebrihi, F. Mathieu, and M. Romdhane, "Chemical composition and antimicrobial and antioxidant activities of mentha (longifolia L. And viridis) essential oils," *Journal of Food Science*, vol. 74, 2009.

[73] S. Schwarz and E. Chaslus-Dancla, "Use of antimicrobials in veterinary medicine and mechanisms of resistance," *Veterinary Research*, vol. 32, no. 3–4, pp. 201–225, 2001.

[74] P. Arumugam, R. Murugan, M. Subathra, and A. Ramesh, "Superoxide radical scavenging and antibacterial activities of different fractions of ethanol extract of Mentha spicata (L.)," *Medicinal Chemistry Research*, vol. 19, no. 7, pp. 664–673, 2010.

[75] F. Bensabah, H. Sbayou, S. Amghar, A. Lamiri, and J. Naja, "Chemical composition and antibacterial activity of essential oils of two aromatic plants: mentha spicata and lippia citriodora irrigated by urban wastewater," *International Journal of Engineering Research*, vol. 2, no. 12, p. 11, 2013.

[76] S. S. Chauhan and R. Agarwal, "Evaluation of antibacterial activity of volatile oil from mentha spicata l," *Journal of Drug Delivery and Therapeutics*, vol. 3, no. 4, pp. 120-121, 2013.

[77] M. Ekhtelat, Z. Bahrani, A. Siahpoosh, and A. Ameri, "Evaluation of antibacterial effects of mentha spicata L., cuminum cyminum L. And mentha longifolia L. Essential oils individually and in combination with sodium benzoate against Escherichia coli O157:H7 and Listeria monocytogenes," *Jundishapur Journal of Natural Pharmaceutical Products*, vol. 14, no. 3, 2019.

[78] Z. Getahun, K. Asres, and A. Mazumder, "Essential oil composition, antibacterial and antioxidant activities of four mentha species growing in Ethiopia," *Ethiopian Pharmaceutical Journal*, vol. 25, no. 2, 2008.

[79] B. M. Githaiga, E. M. Gathuru, E. M. Gathuru, P. N. Waithaka, and L. W. Kiarie, "Determination of antibacterial activity of essential oils from mint (Mentha spicata) leaves on selected pathogenic bacteria," *Journal of Drugs and Pharmaceutical Science*, vol. 2, no. 2, pp. 8–14, 2018.

[80] N. Ha, K. Durić, E. meragić, N. He, S. Muratović, and F. Bečić, "Chemical characterization, antimicrobial and antioxidant properties of Mentha spicata L. (Lamiaceae) essential oil," *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, vol. 50, 2018.

[81] H. Jaber, A. Oubihi, I. Ouryemchi et al., "Chemical composition and antibacterial activities of eight plant essential oils from Morocco against Escherichia coli strains isolated from different Turkey organs," *Biochemistry Research International*, vol. 2021, 2021.

[82] S. M. A. Selles, M. Kouidri, Y. Bellik et al., "Chemical composition, antioxidant and in vitro antibacterial activities of essential oils of mentha spicata leaf from tiaret area (Algeria)," *Dhaka University Journal of Pharmaceutical Sciences*, vol. 17, no. 1, pp. 87–96, 2018.

[83] Y. Shahbazi, "Chemical composition andIn VitroAnti-bacterial activity ofMentha spicataEssential oil against common food-borne pathogenic bacteria," *Journal of Pathogens*, vol. 2015, pp. 1–5, 2015.

[84] Y. Shahbazi, "Antibacterial effects of ziziphora clinopodioides and mentha spicata essential oils against common food-borne pathogen biofilms on stainless steel surface," *Bulgarian Journal of Veterinary Medicine*, vol. 23, 2020.

[85] R. S. Verma, V. Pandey, R. C. Padalia, D. Saikia, and B. Krishna, "Chemical composition and antimicrobial potential of aqueous distillate volatiles of Indian peppermint (mentha piperita) and spearmint (mentha spicata)," *Journal of Herbs Spices & Medicinal Plants Spices & Medicinal Plants*, vol. 17, 2011.

[86] A. Bouyahya, F. Lagrouh, N. El Omari et al., "Essential oils of Mentha viridis rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, anti-dermatophyte and antibacterial properties," *Biocatalysis and Agricultural Biotechnology*, vol. 23, p. 101471, 2020.

[87] A. Bouyahya, Y. Bakri, A. Et-Touys et al., "In vitro screening of antibacterial and antioxidant activities of essential oils from four Moroccan medicinal plants," *Microbiology Research Journal International*, vol. 18, no. 4, pp. 1–10, 2017.

[88] L. F. Silva, M. d. G. Cardoso, L. R. Batista et al., "Chemical characterization, antibacterial and antioxidant activities of essential oils of mentha viridis L. And mentha pulegium L. (L)," *American Journal of Plant Sciences*, vol. 06, no. 05, pp. 666–675, 2015.

[89] A. Talbaoui, "Chemical composition and antibacterial activity of essential oils from six Moroccan plants," *Journal of Medicinal Plants Research*, vol. 6, no. 31, 2012.

[90] N. Zandi-Sohani and L. Ramezani, "Evaluation of five essential oils as botanical acaricides against the strawberry spider mite Tetranychus turkestani Ugarov and Nikolskii," *International Biodeterioration & Biodegradation*, vol. 98, pp. 101–106, 2015.

[91] S. Koumad and M. L. Berkani, "Assessment of the efficacy of four medicinal plants as fumigants against Varroa destrutor in Algeria," *Animal Husbandry Archives*, vol. 68, 2019.

[92] A. Derbalah and S. Ahmed, "Efficacy of spearmint oil and powder as alternative of chemical control against C. maculatus in Cowpea Seeds," *Egyptian Academic Journal of Biological Sciences, F. Toxicology & Pest Control*, vol. 2, no. 1, pp. 53–61, 2010.

[93] S. Haouel-Hamdi, A. Soltani, R. Jmal et al., "Use of binary mixtures of three Mentha essential oils for the control of rice weevil Sitophilus oryzae (Curculionidae)," *International Journal of Tropical Insect Science*, vol. 41, no. 2, pp. 1333–1342, 2021.

[94] A. Lamiri, S. Lhaloui, B. Benjilali, and M. Berrada, "Fumigant toxic activity of essential oils on Sitophilus granarius (linné)," *Physical and Chemical News*, vol. 1, 2001.

[95] D. P. Papachristos and D. C. Stamopoulos, "Repellent, toxic and reproduction inhibitoryeffects of essential oil vapours on Acanthoscelides obtectus (Say) (Coleoptera: bruchidae)," *Journal of Stored Products Research*, vol. 38, 2002.

[96] S. Abdel-Shafy and M. M. M. Soliman, "Toxicity of some essential oils on eggs, larvae and females of Boophilus annulatus (Acari: Ixodida: Amblyommidae) infesting cattle in Egypt," *Open Science in Acarology*, vol. 44, 2004.

[97] P. H. Yousuf, N. Y. Noba, M. Shohel, R. Bhattacherjee, and B. K. Das, "Analgesic, anti-inflammatory and antipyretic effect of mentha spicata (spearmint)," *British Journal of Pharmaceutical Research*, vol. 11, 2013.

[98] P. Arumugam, N. G. Priya, M. Subathra, and A. Ramesh, "Anti-inflammatory activity of four solvent fractions of ethanol extract of Mentha spicata L. investigated on acute and chronic inflammation induced rats," *Environmental Toxicology and Pharmacology*, vol. 26, no. 1, pp. 92–95, 2008.

[99] A. A. S. A. Jabbar and S. H. Kathem, "The protective effect of mentha spicata ethanolic extract on irinotecan-induced mucositis in mice," *Iraqi Journal of Pharmaceutical Sciences*, vol. 28, 2019.

[100] K. G. Al-Fartosi, H. Radi, and E. A. Al-Rekabi, "Lipid profile of diabetic male rats treated with phenolic compounds of leaves extracts from mentha longifolia and mentha spicata," *ISSN*, vol. 3, no. 2, p. 6, 2014.

[101] A. Mushtaq, N. Iqbal, M. Jamil, N. Khawaja, U. Gohar, and M. Mehmood, "Anti-diabetic and anti-hyperlipidemic action of aqueous ethanolic extracts of mentha spicata (leaves), plumeria alba (leaves) and nymphaea alba (flowers and rhizomes)," *IJBPAS*, vol. 6, 2017.

[102] B. Nickavar, A. Alinaghi, and M. Kamalinejad, "Evaluation of the antioxidant properties of five mentha species," *Iranian Journal of Pharmaceutical Research*, vol. 7, no. 3, p. 7, 2008.

[103] H. Abdel-Hady, E. A. El-Wakil, and M. Abdel-Gawad, "GC-MS analysis, antioxidant and cytotoxic activities of mentha spicata," *European Journal of Medicinal Plants*, vol. 26, no. 1, pp. 1–12, 2018.

[104] W. Dhifi, N. Jelali, W. Mnif, M. Litaiem, and N. Hamdi, "Chemical composition of the essential oil ofmentha spicatal. From Tunisia and its biological activities," *Journal of Food Biochemistry*, vol. 37, no. 3, pp. 362–368, 2013.

[105] B. Fatiha, H. Didier, G. Naima et al., "Phenolic composition, in vitro antioxidant effects and tyrosinase inhibitory activity of three Algerian Mentha species: M. spicata (L.), M. pulegium (L.) and M. rotundifolia (L.) Huds (Lamiaceae)," *Industrial Crops and Products*, vol. 74, pp. 722–730, 2015.

[106] Z. Özer, "Investigation of phenolic compounds and antioxidant activity of mentha spicata L. subsp. spicata and M. longifolia (L.) L. subsp. typhoides (briq.) harley decoction and infusion," *Journal of the Turkish Chemical Society Section A: Chemistry*, vol. 5, pp. 445–456, 2018.

[107] I. Rita, C. Pereira, L. Barros, C. Santos-Buelga, and I. C. F. R. Ferreira, "Mentha spicata L. infusions as sources of antioxidant phenolic compounds: emerging reserve lots with special harvest requirements," *Food & Function*, vol. 7, no. 10, pp. 4188–4192, 2016.

[108] M. Snoussi, E. Noumi, N. Trabelsi, G. Flamini, A. Papetti, and V. De Feo, "Mentha spicata essential oil: chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of Vibrio spp. strains," *Molecules*, vol. 20, no. 8, pp. 14402–14424, 2015.

[109] J. Prince Vijeya Singh, K. Selvendiran, S. Mumtaz Banu, R. Padmavathi, and D. Sakthisekaran, "Protective role of Apigenin on the status of lipid peroxidation and antioxidant defense against hepatocarcinogenesis in Wistar albino rats," *Phytomedicine*, vol. 11, no. 4, pp. 309–314, 2004.

[110] M. Aziz, N. Saqib, N. Akhtar et al., "Phytochemical screening and evaluation of the diuretic activity of aqueous methanol extract from aerial parts of mentha viridis linn (labiatae) in

albino rats," *Tropical Journal of Pharmaceutical Research*, vol. 13, no. 7, pp. 1121–1125, 2014.

[111] A. B. Saad, I. Rjeibi, N. Brahmi, E. Elaloui, and N. Zouari, "Nicotine-induced oxidative stress, testis injury, AChE inhibition and brain damage alleviated by Mentha spicata," *Inflammopharmacology*, vol. 28, no. 4, pp. 939–948, 2020.

[112] A. S Alqahtani, R. Ullah, and A. A. Shahat, "Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia," *Evidence-based Complementary and Alternative Medicine*, vol. 2022, p. 23, Article ID 7123521, 2022.

[113] J. Naidu, R. Ismail, and S. Sasidharan, "Acute oral toxicity and brine shrimp lethality of methanol extract of mentha spicata L (Lamiaceae)," *Tropical Journal of Pharmaceutical Research*, vol. 13, no. 1, p. 101, 2014.

[114] M. K. Mugisha, J. G. Ndukui, A. Namutembi, P. Waako, A.-K. B. Karlson, and P. Vudriko, "Acute and sub-acute toxicity of ethanolic leaf extracts of rumex abyssinica jacq. (Polygonaceae) and mentha spicata L. (Lamiaceae)," *Pharmacology & Pharmacy*, vol. 05, no. 03, pp. 309–318, 2014.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7736378, 16 pages
https://doi.org/10.1155/2022/7736378

Research Article

Antioxidant and Antiaging Properties of Agar Obtained from Brown Seaweed *Laminaria digitata* (Hudson) in D-Galactose-Induced Swiss Albino Mice

B. S. Reshma,[1] Thabitha Aavula,[1] Vignesh Narasimman,[1] Saravanan Ramachandran,[1] Musthafa Mohamed Essa,[2] and M. Walid Qoronfleh[3]

[1]*Native Medicine and Marine Pharmacology Laboratory, Department of Medical Biotechnology, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education, Kelambakkam, TN 603 103, India*
[2]*Department of Food Science and Nutrition, CAMS, Sultan Qaboos University, Muscat, Oman*
[3]*Q3CG Research Institute (QRI), Research & Policy Division, 7227 Rachel Drive, Ypsilanti, MI 48917, USA*

Correspondence should be addressed to Saravanan Ramachandran; saran_prp@yahoo.com

Received 13 October 2021; Revised 24 December 2021; Accepted 1 February 2022; Published 24 February 2022

Academic Editor: Vijaya Anand

The present paper explores the antioxidant and antiaging properties of agar extracted from *Laminaria digitata* (*L. digitata*) on a D-galactose (D-Gal)-induced mouse model. Experimental mice were divided into four groups: group I comprised of control nontreated mice, group II comprised of D-Gal-induced mice, group III mice were treated with extracted agar after D-Gal induction, and group IV mice were given ascorbic acid as a positive control. Antioxidant enzymes and aging marker proteins declined significantly in group II, whereas they were normal in group III and group IV mice. Expressions of interleukin-1β (IL-1β) in D-Gal-induced mice were significantly enhanced in the liver and brain of the experimental mice, which were otherwise normal in agar-treated mice. Also, IL-6 levels were significantly increased in the liver and reversed in the brain of D-gal mice, while it was regularly in the agar-treated mice. The histopathological analysis of D-Gal-induced mice showed spongiosis and tangles in brain cells, increased fat and decreased collagen contents in the skin, and few dilated sinuses in the hepatic cells. The changes were under control in group III and group IV mice, suggesting the protective effects of agar extracted from *L. digitata* and ascorbic acid.

1. Introduction

Aging is a multifactorial natural process of the skin. The changes in the structure and functions of the entire body system are connected to the physical decline associated with aging. Biological aging is rooted in genetics, endocrine, and immune metabolic function factors. Then, environmental influences such as gravity, ultraviolet light exposure, climate, and pollution exacerbate the natural aging process. Several research reports show a correlation between the process of aging and antioxidant functions such as amplified mitochondrial reactive oxygen species (ROS) generation and increased accumulation of oxidant products [1–3]. ROS produced by lipid peroxidation has been implicated in the progressive oxidative deterioration of polyunsaturated fatty acids (PUFAs) of the cell membranes [4]. The ROS originates unstable lipid peroxide species incorporating carbonyl group with the end products, namely, reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), ultimately causing tissue damage [5, 6]. Galactose (Gal) is a monosaccharide that has a similar structure to glucose, differing only in the position of one hydroxyl group. Chronic systemic exposure of rodents to D-Gal causes the acceleration of aging and has been used as an aging agent in experimental models. D-Gal, which is completely metabolized at normal concentrations, is a natural compound in the body. At higher concentrations, however, it converts to aldose and hydrogen peroxide (H_2O_2), a reaction catalyzed by galactose oxidase; conversely, superoxide dismutase (SOD) facilitates the transformation of superoxide anions to H_2O_2. The overall increase in ROS leads to disruptions in the activities of

macromolecules inside the cell [6]. Cosmetic products are used for human skincare by adolescents. The usage intends to maintain the skin in good condition to shield it from the environment's harsh effects and minimize the aging processes [7, 8]. Unfortunately, some of the adverse effects of commercial cosmetics are skin irritation and allergic reactions, swelling, blisters, and surface oozing. On the extreme side, they can cause reproductive toxicity, endocrine disruptions, and cancer to consumers [9, 10]. As a result, consumers are on the lookout for more natural sources of skincare products [11]. Seaweeds are rich sources of polysaccharides used as gelling and thickening agents (e.g., alginates, agar, and carrageenan) including some that have become valuable additives in food manufacturing due to their rheological properties. They are also known to have multiple biological activities, including antioxidant, anticoagulant, antiviral, and immunoinflammatory activities that could find their relevance in nutraceuticals, functional foods, and pharmaceutical applications [12–15]. Previously, the authors have reported on the antioxidant, anticoagulant, and mosquitocidal properties of water-soluble polysaccharides from green, red, and brown seaweeds [16]. However, to the authors' knowledge, there is no report on the antiaging property of agar. *Laminaria digitata* belongs to the phylum Ochrophyta, family Laminariaceae, genus *Laminaria*, and species *digitata*. Hence, the present study was designed and conducted to evaluate the texture, antioxidant, and *in vivo* antiaging properties of agar extracted from the brown seaweed *L. digitata*.

2. Methods

2.1. Seaweed Collection and Processing. Brown seaweeds (*L. digitata*) were collected from Kanyakumari (Lat 8.0866oN and Long 77.5544oE), Tamil Nadu, India. The samples were identified according to the renowned monograph of Marine Biology, Annamalai University, Tamil Nadu, India. First, the seaweeds were washed in seawater, followed by distilled water, and finally were washed with 70% alcohol to remove microflora and other contaminants on the seaweed surface. Finally, the processed seaweeds were shadow dried and stored at −20°C for further study.

2.2. Extraction of Aqueous Polysaccharide. The dried seaweed samples were blended and ground into a fine powder using a blender. A 5 g of powdered seaweeds was dissolved in 100 ml of Milli-Q water and then autoclaved at 121°C for 1 hour. The water-soluble polysaccharides were filtered through a Whatman no. 1 filter paper to remove debris in the extract and further centrifuged at 5000xg for 15 min at room temperature to eliminate any particulate matter. The solution was kept at room temperature until gelation. The gelated agar was frozen at room temperature overnight. After bleaching, the samples were dried at 60°C overnight [17].

2.3. Bleaching of Agar. The gelated agar seaweed samples were washed with sodium hypochlorite (NaClO). The washing was repeated up to 5 times. After NaClO treatment, it was diluted with H_2O_2. The color change has been noted as dark brown to pale yellow color [18].

2.4. Food Texture Analyses. The texture parameters of the extracted agar from *L. digitata* were measured using the TA.XTplus Texture Analyzer (Stable Microsystems, UK) in back extrusion mode and constituted with a 36 mm cylinder probe. The parameters were set as follows: data acquisition rate: 200 PPS, test speed: 1.0 mm/s, load cell: 5 mm, and distance: 40 mm. The agar from *L. digitata* was loaded onto the analyzer platform, and the probe was allowed to be flush with the surface. The extract holders were supported by a heavy-duty rig plate attached to the crosshead of the instrument. Upon data acquisition, it was plotted as a graph. The peaks on the graph represent the force at which the probe reaches a maximum penetration depth of 75% of the original agar height. A relaxation of the sample over the two-second holding period resulted in subsequent decay in force, followed by the withdrawal of the probe to a tracking force of 5 g, thus allowing the sample to "spring back". The maximum force (g) required to penetrate the product was recorded as the hardness, adhesiveness, cohesiveness, springiness, and gumminess of agar from *L. digitata*. The experiments were run in triplicate, and an average value was statistically reported [19].

2.5. In Vitro Antioxidant Activity

2.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Activity. The DPPH radical scavenging assay was performed using a 96-well microtiter plate. A 50 µl of 100 µg/ml methanolic DPPH solution was added to 200 µl of different concentrations of agar sample (12.5–100 µg/ml) and mixed with a vortex mixer. Ascorbic acid was used as a standard [20].

2.5.2. H_2O_2 Scavenging Activity. The scavenging activity of the agar was determined. A 0.3 ml agar solution was reacted with 600 µl H_2O_2 40 mM solution. Then, it was allowed to stand for 10 min at room temperature. The absorbance of the reaction mixture was measured at 230 nm by a UV spectrophotometer [21].

2.5.3. Total Antioxidant Capacity. The total antioxidant activities of the agar from the seaweeds were determined. A 0.3 ml agar sample was reacted with 3 ml total antioxidant capacity reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated in a water bath at 95°C for 90 min. The absorbance of the reaction mixture was measured at 695 nm by a UV-visible spectrophotometer [21].

2.5.4. Phosphomolybdate Activity. The antioxidant activity of agar was evaluated by the green phosphomolybdenum complex formation protocol. A reaction mixture solution was prepared by adding 0.588 ml of sulfuric acid (H_2SO_4), 0.049 g ammonium molybdate, and 0.036 g sodium

phosphate. The volume of the final solution was made up to 10 ml with distilled H_2O. Afterwards, 10 mg of agar was dissolved in 1 ml of dimethyl sulfoxide (DMSO). A 100 μl from the prepared agar sample was taken, and 1 ml of the reagent mixture solution was added to it and then incubated in a boiling water bath at 95°C for 90 min. After 90 min, the absorbance of the solution was read at 695 nm. Ascorbic acid (10 mg/ml DMSO) was used as a standard [22].

2.5.5. Ferric Reducing Antioxidant Power Assay (FRAP Assay). The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution, and 20 mM $FeCl_3.6H_2O$ in a 10 : 1: 1 ratio before use and was heated to 37°C in a water bath for complete dissolution. The blank reading was measured by adding 200 μl of this working solution to the microtiter plate and read at 593 nm using a UV spectrophotometer. The sample reading was calculated by adding 3 ml of the freshly prepared working solution with 100 μl of agar extracts and 300 μl of distilled water and then left for 4 min at room temperature before reading absorbance at 593 nm using a UV spectrophotometer. Here, ferrous sulfate ($FeSO_4$) was used as standard and for calibration in the concentrations of 0 to 1000 μM [23].

2.6. Behavioral Studies. Antiaging was experimentally induced in female Swiss albino mice following the ethical clearance obtained from Institutional Animal Ethics Committee (IAEC4/Alr.No. 23/dated 12.12.17). Extracted agar (100 mg/kg) was suspended in 0.1 M phosphate-buffered saline (PBS) and administered orally using an 18 mm gauge for 42 days. Control mice received regular food and water *ad libitum*. Ascorbic acid (100 mg/kg)-treated mice served as the positive control. The animals were divided into four groups (Table 1). Food and water intake of all mice groups were measured up to 42 days. The behavioral parameters such as locomotor function, light-dark, hot plate, and rotarod test of experimental mice groups were determined on 0, 2nd, 4th, and 6th weeks [24]. At the end of the experimental period, blood was collected using a 1 ml syringe from the tail vein of the mice. The mice were then sacrificed by halothane inhalation. The liver, brain, kidney, and portions of the skin were dissected, fixed in formalin, and embedded in paraffin, and the morphological changes were detected under the microscope. The rest of the dissected organs were stored in PBS and stored at −20°C for further use.

2.6.1. Locomotor Activity. Locomotor activity was evaluated by placing a mouse into the center of a clear Plexiglas (40 × 40 × 30 cm) open-field arena and allowing the mouse to explore for 5 min. Bright overhead lighting was approximately 500 lux inside the arenas, while noise was present at about 60 dB (INCI Photoactometer, India). The total distance (locomotor activity), movement time (sec), movement speed (cm/sec), and center distance (the distance traveled in the center of the arena) were recorded manually. The center distance was divided by the total distance to obtain a center distance-to-total distance ratio. The center distance-to-total distance ratio can be used as an index of anxiety-related responses. Data were collected at 2, 3, 4 and 5 min intervals over the session [25].

2.6.2. Light-Dark Exploration Test. Experimental mice were put through the light-dark exploration test, which consists of a polypropylene chamber (44 × 21 × 21 cm) unequally divided into two chambers by a black partition containing a small opening. The large chamber was open and brightly illuminated (800 lx), while the small chamber was closed and dark. White noise was present in the room at approximately 55 dB in the test chamber. Mice were placed on the illuminated side and allowed to move freely between the two chambers for 5 min [26].

2.6.3. Hotplate Test. The hotplate test was used to evaluate sensitivity to a painful stimulus. Mice were placed on a 55.0°C (±0.3) hotplate, and the latency to the first hind-paw response was recorded. The hind-paw response was either a foot shake or a paw lick [27].

2.6.4. Rotarod Test. Mice were placed on a rotating rod (INCI Rotarod, India) that accelerated from 4 to 40 rpm. For two consecutive days, four trials were performed per day with 45–60 min intervals between trials. The maximum duration of each trial was 5 min. The time at which the mice fell off the rod was recorded [28].

2.7. In Vivo Antiaging Marker Studies

2.7.1. Collagen Measurement. The amount of collagen in the dermis was measured using a Sircol Collagen Assay Kit based on the fact that the Sirius red dye binds to the side chains of the amino acids in collagen [29].

2.7.2. Elastin Measurement. The amount of elastin in the dermis was measured using a Fastin Elastin Assay Kit based on the fact that the Fastin dye binds to the side chains of the amino acids in elastin [29].

2.7.3. Protein Extraction. The frozen skin samples were minced and homogenized in 10 volumes of extraction buffer Tris-HCl (pH 7.8). The homogenate was centrifuged at 9,000xg at 4°C for 15 min, and the supernatant was collected as an extracted protein. The protein concentration of the supernatant was measured by Lowry's method using a DC Protein Assay Kit [29].

2.7.4. Skin Aging Assay. The levels of elastin, collagen, hyaluronidase, and tyrosinase in skin tissues were examined using the kits procured from Cayman Chemicals, USA [30].

Table 1: Experimental groups of mice.

Group 1	Normal mice (normal diet and water)
Group 2	Aging-induced mice (100 mg/kg D-Gal; s.c. injection)
Group 3	Aging-induced (100 mg/kg; s.c.) mice + 100 mg/kg of agar (orally)
Group 4	Positive control mice (100 mg/kg D-Gal; s.c.) + ascorbic acid 100 mg/kg (orally)

2.8. Biochemical Analysis and Histological Analysis. The blood and tissue levels of reduced glutathione (GSH), hydroxyproline, malondialdehyde (MDA), total protein, and the activities of total SOD, catalase (CAT), and total antioxidant capacity (T-AOC) in the blood and tissues were determined using commercial kits (Sigma, USA). The brain, liver, kidney, and skin of each group of mice were fixed in 10% formalin for 24 h; tissues and dorsal skin samples were dehydrated in various ways before being implanted in paraffin, sectioned thinly, dewaxed, and stained with hematoxylin-eosin [31]. Cross sections were selected from three plates per sample, and the morphological changes were examined under the microscope.

2.9. RNA Extraction and Quantitative PCR. A 100 mg of the brain tissue sample was taken, and 1 ml of TRIzol (Sigma, USA) was added and homogenized. The solution was incubated at room temperature for 30 min and then centrifuged at 12,000 rpm for 10 min. The supernatant was transferred, and after 5 min, about 200 μl of chloroform was added, mixed, and incubated at room temperature for 15 min. Then, it was followed by centrifugation at 12,000 rpm for 10 min at 4°C. The upper aqueous layer was transferred, and a 500 μl of isopropanol was added and then incubated at room temperature for 10 min and finally centrifuged at 12,000 rpm for 10 min at 4°C. To the pellet, a 500 μl of 70% ethanol was added, washed (5000 rpm for 5 min at 4°C), and then air-dried. The pellet was then dissolved in 20 μl of diethyl pyrocarbonate (DEPC)-treated water. The RNA was quantified using a UV spectrophotometer by measuring the ratio of absorbance at wavelengths 260 nm and 280 nm (NanoDrop, Thermo Scientific, USA).

2.10. Statistical Analysis. Each test was performed on six mice, and all results were expressed as the mean ± SD. A P value of <0.05 was considered significant. Statistical analysis was performed using the IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA).

3. Results and Discussion

Aging is an intricate progression that disturbs the tasks of all organs and tissues and most often has an irreversible impact on their mechanical behavior. The most significant noticeable effects of aging are perceived in the skin and have been broadly investigated for medical and cosmetic resolutions. From a biological appearance, aging is a predictable natural process [24].

About 5 kg of the selected brown seaweed, *L. digitata* was collected from the Kanyakumari coast, Tamil Nadu, India, and processed. After being shadow dried, the dry weight and the yield of agar from *L. digitata* were found to be 266.66 g and 20%, respectively. This yield of agar from *L. digitata* was high when compared to prior results [32]. It has been documented that the difference in agar yield varies from species to species; also, it is dependent on the harvested season. The study results were in accordance with the report of Armisen et al. [33] who pointed out that the yield of agar varied between species and geographical areas of collections.

The proximate biochemical composition and food texture analysis of *L. digitata* are shown in Table 2. The moisture content of the *L. digitata* was reported to be 22.18%, and among the selected macroelements, sodium (Na) was found to be maximum (1.74%) while phosphorous (P) was found to be minimum (0.03%). Further, the carbon and phosphorous ratio (C : P) was found to be 0.72% of the sample of harvested brown seaweed *L. digitata*. Moreover, among the selected microelements, the content of copper was observed at maximum (73.41 ppm), and cobalt was recorded at minimum (2 ppm). The elemental content of agar from *L. digitata* was higher when compared to the report of Fleuerence et al. [34]. The difference in the mineral content could be attributed to the species difference and the method selection for quantification. The overall biochemical analyses findings were in agreement with Fleuerence et al. [34] who had mentioned that the biochemical concentration of seaweed varies according to the factors stated above. The texture properties such as hardness (234.43 ± 0.60 g), adhesiveness (50.61 ± 0.40 N/mm), cohesiveness (0.825 ± 0.11), springiness (3.250 ± 0.12), and gumminess (2.969 ± 0.10) of agar from *L. digitata* were maximum when compared to the texture profile analysis of the starch gel prepared from corn [35]. The difference in the texture analysis was due to the monosaccharide composition differences between the species [36], and the results support the present finding that aqueous extract can be easily degraded due to the presence of monomeric chains [37].

Antioxidant activity was determined from the brown seaweed *L. digitata*. The methods differ in terms of their assay and experimental conditions. The *in vitro* antioxidant activity of agar from the brown seaweed *L. digitata* showed higher activity at higher concentrations [35]. The DPPH scavenging activity in agar from brown seaweed was assayed and is presented in Table 3. The 200 µl concentration of agar showed significantly elevated levels of scavenging activity (83.70 ± 3.83%) ($P < 0.05$) when compared to the concentration of 50 µl (32.68 ± 1.21%). The standard 100 µl of ascorbic acid was at (62.42 ± 1.11%) DPPH scavenging activity. Hence, the significant variations in the DPPH scavenging abilities of the bioactive compounds isolated from seaweed might be due to variations in the type of extraction and/or differences in the zone of collection of the seaweeds [35].

Table 2: Proximate composition and food texture analysis of agar from *L. digitata*.

Proximate analysis											
Brown seaweed	Moisture (%)	C : P (%)	Ca (%)	P (%)	Na (%)	K (%)	Mn (%)	Zn (%)	Fe (%)	Cu (%)	Co (%)
L. digitata	22.18	0.72	0.1	0.03	1.74	0.99	14.50	40.50	29.00	73.41	2.00

Food texture				
Hardness (g)	Adhesiveness (N/mm)	Cohesiveness	Springiness	Gumminess
243.43 ± 0.6	50.61 ± 0.40	0.825 ± 0.11	3.250 ± 0.12	2.969 ± 0.10

Table 3: *In vitro* antioxidant activity in agar from brown seaweed.

S. no.	Parameters	Agar (50 µl)	Agar (100 µl)	Agar (200 µl)	Ascorbic acid (100 µl)
1.	DPPH scavenging activity (%)	32.68 ± 1.21	65.33 ± 2.41	*83.70 ± 3.83	*62.42 ± 1.11
2.	H_2O_2 scavenging assay (%)	47.47 ± 0.97	64.99 ± 1.93	*89.85 ± 3.86	*80.82 ± 3.53
3.	T-AOC (%)	16.77 ± 1.08	33.49 ± 2.14	*67.03 ± 3.30	*72.28 ± 3.73
4.	Phosphomolybdate assay (%)	16.63 ± 2.92	33.21 ± 2.05	*66.48 ± 3.03	*75.18 ± 3.54
5.	FRAP assay (%)	20.51 ± 1.30	41.05 ± 2.61	*72.03 ± 3.21	*82.31 ± 3.78

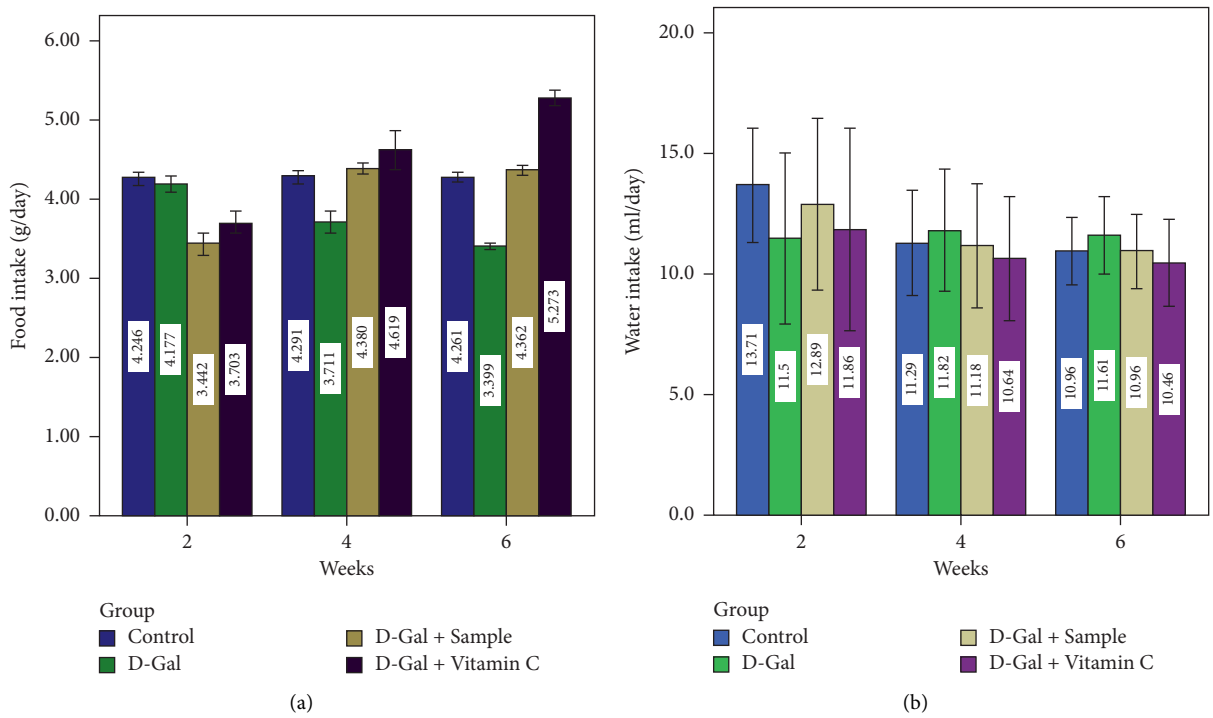


Figure 1: Food and water intake of experimental mice.

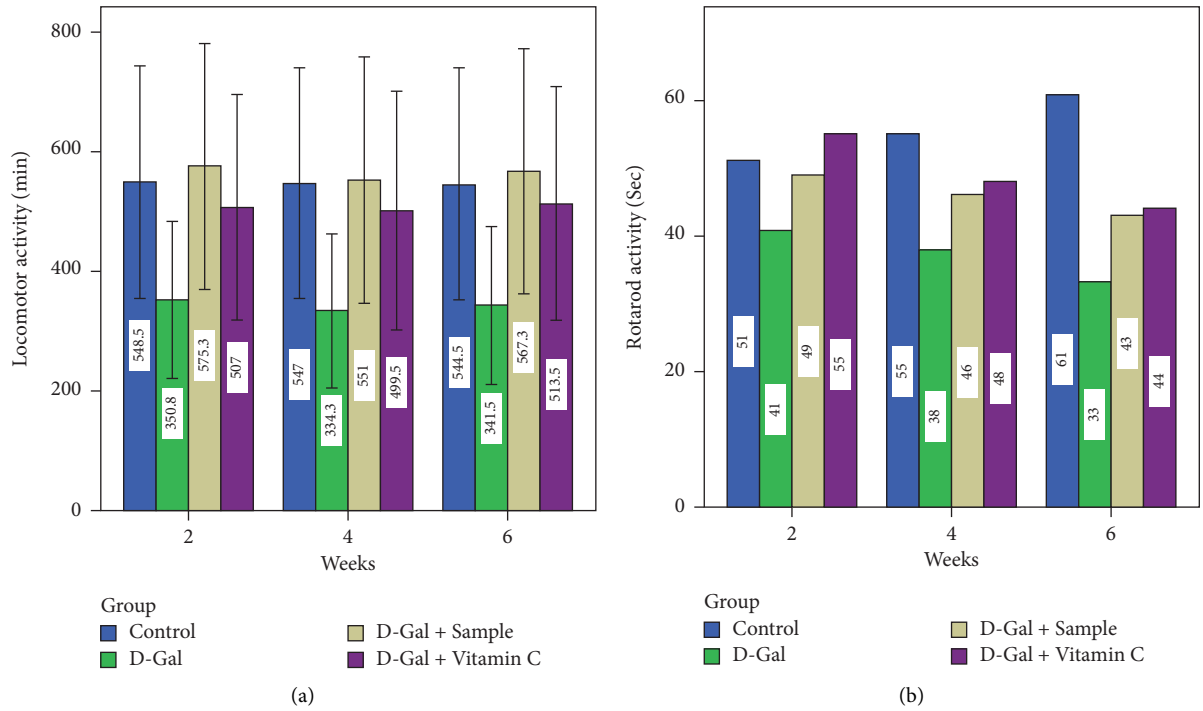


Figure 2: Locomotor and rotarod activities of experimental mice.

Table 4: Body weight of experimental mice.

S. no.	Weeks	Group I (g)	Group II (g)	Group III (g)	Group IV (g)
1.	0	*26.17 ± 2.36	*29.50 ± 2.29	27.33 ± 1.42	28.67 ± 1.05
2.	2	*28.67 ± 2.91	*29.50 ± 2.50	26.33 ± 1.49	28.00 ± 1.29
3.	4	*34.33 ± 1.50	*36.83 ± 2.01	31.00 ± 1.53	31.67 ± 1.67
4.	6	*27.33 ± 1.31	*39.83 ± 1.35	31.00 ± 1.53	31.33 ± 1.33

Statistical significance: $P < 0.05$ (DMRT). *Comparisons were made between groups I and II.

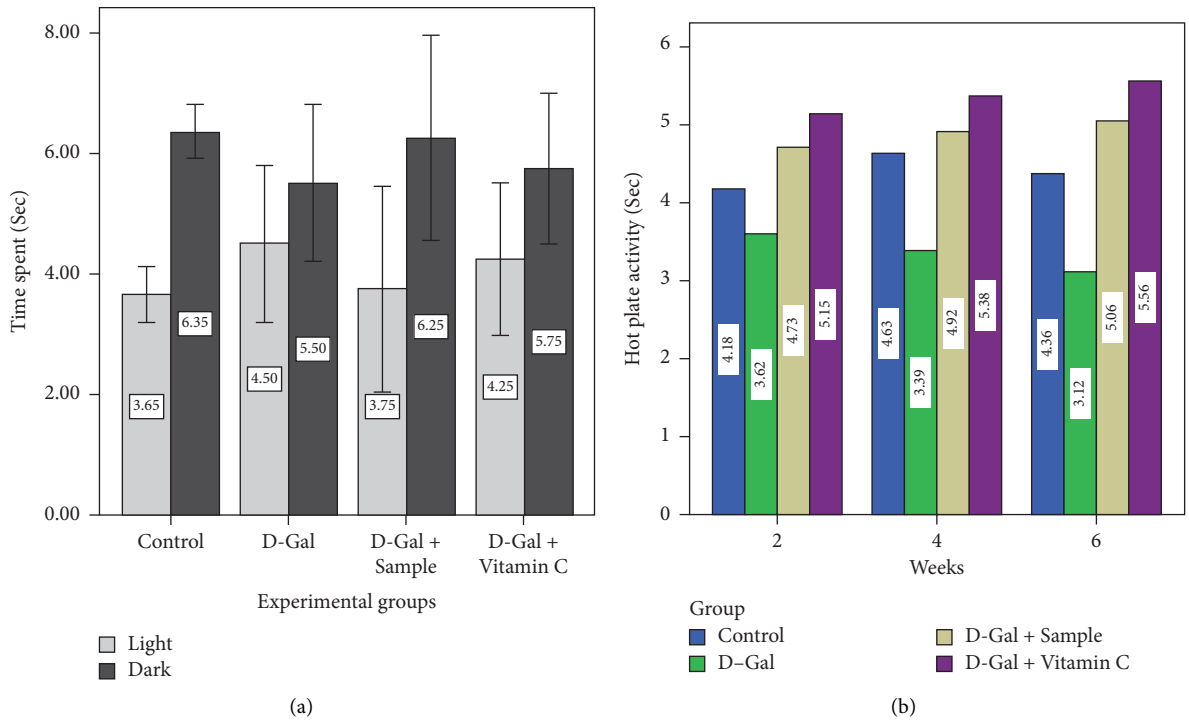


Figure 3: Light and dark and hotplate activities of experimental mice.

The H_2O_2 radical scavenging activity of agar extracted from brown seaweed is shown in Table 3. The agar exhibited increased H_2O_2 scavenging activity (89.85 ± 3.86%) at a concentration of 200 µl, closely followed by the concentrations of 100 µl (64.99 ± 1.93%) and 50 µl (47.47 ± 0.97%), and the difference was observed to be significant ($P < 0.05$). Ascorbic acid at 100 µl displayed H_2O_2 radical scavenging activity of 80.82 ± 3.53%, resulting in complete buffering of the free radicals that might be generated. The results of the present investigation seemed higher when compared to the H_2O_2 radical scavenging activity of the aqueous extract of Indian seaweed [38]. The observed differences in the percentage of H_2O_2 scavenging activity might be attributed to the variations in the geographical areas of collection from the sea or season of harvesting. It might also be due to the differences in extraction procedures, like using different solvents and filtration procedures.

The total antioxidant activity of agar extracted from brown seaweed is shown in Table 3. The agar significantly showed increased total antioxidant activity (67.03 ± 3.30%) ($P < 0.05$) at a concentration of 200 µl, followed by concentrations of 100 µl (33.49 ± 2.14%) and 50 µl (16.77 ± 1.08%), respectively. The phosphomolybdate activity of agar extracted from brown seaweed is given in Table 3, which is caused by the reduction of Mo^{6+} ions to form a green phosphate/molybdenum V complex. The agar from *L. digitata* was significantly increased ($P < 0.05$) by the scavenging phosphomolybdate assay (66.48 ± 3.03%) at a concentration of 200 µl and followed by concentrations of 100 µl (33.21 ± 2.05%) and 50 µl (16.63 ± 2.92%), correspondingly. The standard ascorbic acid exhibited (75.18 ± 3.54%) scavenging phosphomolybdate activity, resulting in complete buffering of the free radicals that might be generated.

The potency reduction activity of agar extracted from the selected brown algae was calculated, and the result was expressed as a percentage. The potency reduction activity of FRAP is estimated by measuring the Fe_3^{+}-Fe_2^{+} transformation in the presence of agar extracted from *L. digitata*. The selected brown seaweed exhibited increased FRAP reducing power activity (72.03 ± 3.21%) at 200 µl followed by (82.31 ± 3.78%) at 100 µl ($P < 0.05$). The antioxidant activity is brought about by the ability to scavenge free radicals, and thus, in the current study, the agar of brown seaweed showed good antioxidant activity by scavenging ROS.

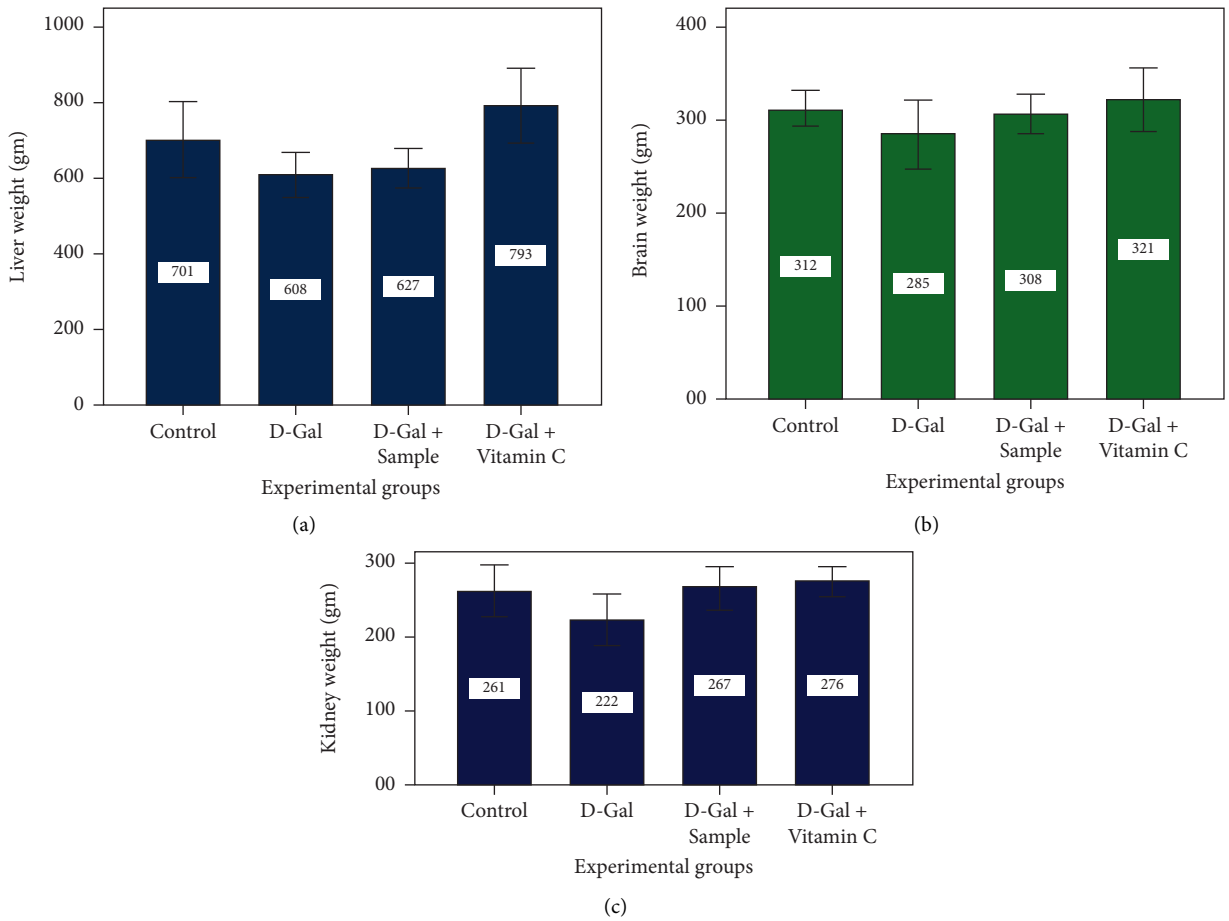


Figure 4: Brain, liver, and kidney weight of experimental mice.

Animal experiments revealed significant variations in food and water intake (Figures 1(a) and 2(b)) and body weight change (Table 4), in the D-Gal (group II)-injected mice when compared with the control group (group I) ($P < 0.05$). The water intake was significantly lower in D-Gal-injected mice when compared to group I mice in the second week ($P < 0.05$). However, it significantly increased in the subsequent weeks ($P < 0.05$). Prior to the animals being sacrificed, no mice died during the entire experiment. However, at the beginning of the second week, mice demonstrated noticeable effects of D-Gal aging, such as apathy, delayed response, and slow motion, which continued for up to six weeks. Furthermore, group II mice had a significantly higher body weight than group I mice ($P < 0.05$).

Current outcomes were endorsed by Swallow et al. [37] who claimed that food consumption and lack of physical activity played a major role in body composition. For the locomotor and rotarod (Figure 2) and light and dark and hot plate activities (Figure 3), group II mice experienced a significant decline when compared to group I mice in early (0 weeks) and late (6 weeks) ($P < 0.05$). The results of the present study suggested that all the abovementioned activities clearly proved that aging was induced in the D-Gal (group II) mice. On the other hand, the characteristics in the control sample (group III) and in the ascorbic acid orally administered mice (group IV) were found to be normal.

Figures 3(a) and 3(b) present the light and dark exposure and hot plate activity of experimental mice. According to the data, the time spent in light and dark in group II mice was significant ($P < 0.05$) compared to group I mice during the 6th week. The response of hot plate activity of group II mice was lower than that of group I mice during the second week onwards, and it was further decreased at the end of the experiment period (6th week) ($P < 0.05$). The results strongly suggested that all the abovementioned activities clearly proved that aging was induced in the D-Gal (group II) mice, while these behaviors were stabilized in the control sample (group III) and in the ascorbic acid coadministered mice (group IV) [38]. It was also suggested that behavioral activities were slow in the aging mice. It slows down the rate of aging by influencing the basic cellular metabolism of motor activity at the organism level [24].

Mice injected with D-Gal showed noticeable symptoms of aging. The organ indices in the brain, liver, and kidney of the model group were lower than those of the control group as depicted in Figures 4(a) and 4(c) ($P < 0.05$). With age comes functional weakening and tissue/organ degeneration. Mostly, changes in the organs such as the brain, liver, and kidney weight were evident. So, the change in organ index is

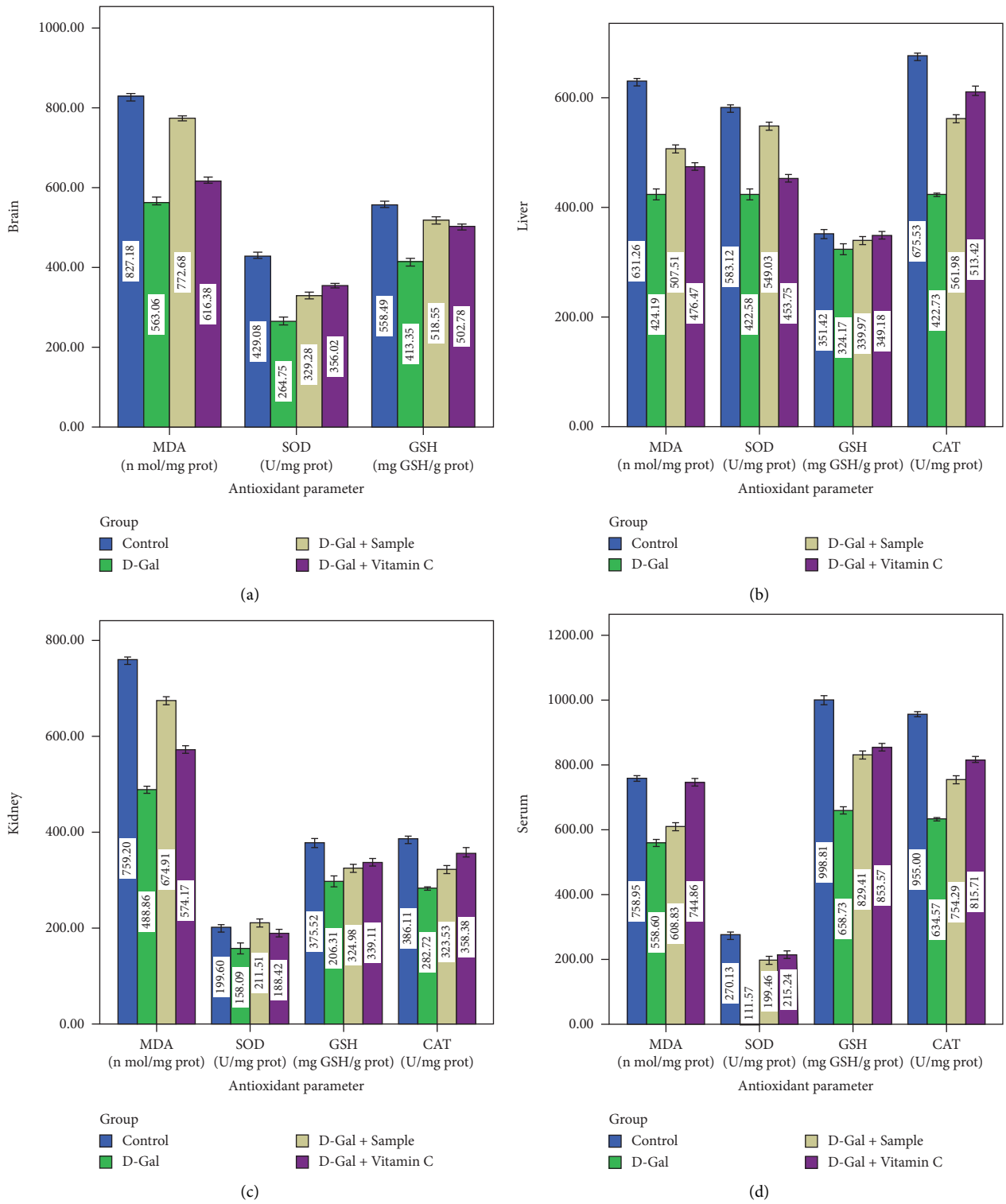


Figure 5: *In vivo* antioxidant activity of experimental mice.

a significant sign of organism senescence. D-Gal could induce physical aging and diminish organ indices of the brain, liver, and kidney in group II, according to the present experimental data. In group II, agar from brown seaweed could efficiently recover all organ indices. It demonstrated that seaweed agar could play a role in preserving the weight of all these organs from declining in the D-Gal experimental mouse model. With increased lifespan comes functional decline and atrophy of all organs and tissues. Differences in brain and kidney mass were noticeable [39]. Consequently, an organ index change was an essential sign of creature senescence, proving that the extracted agar and ascorbic acid

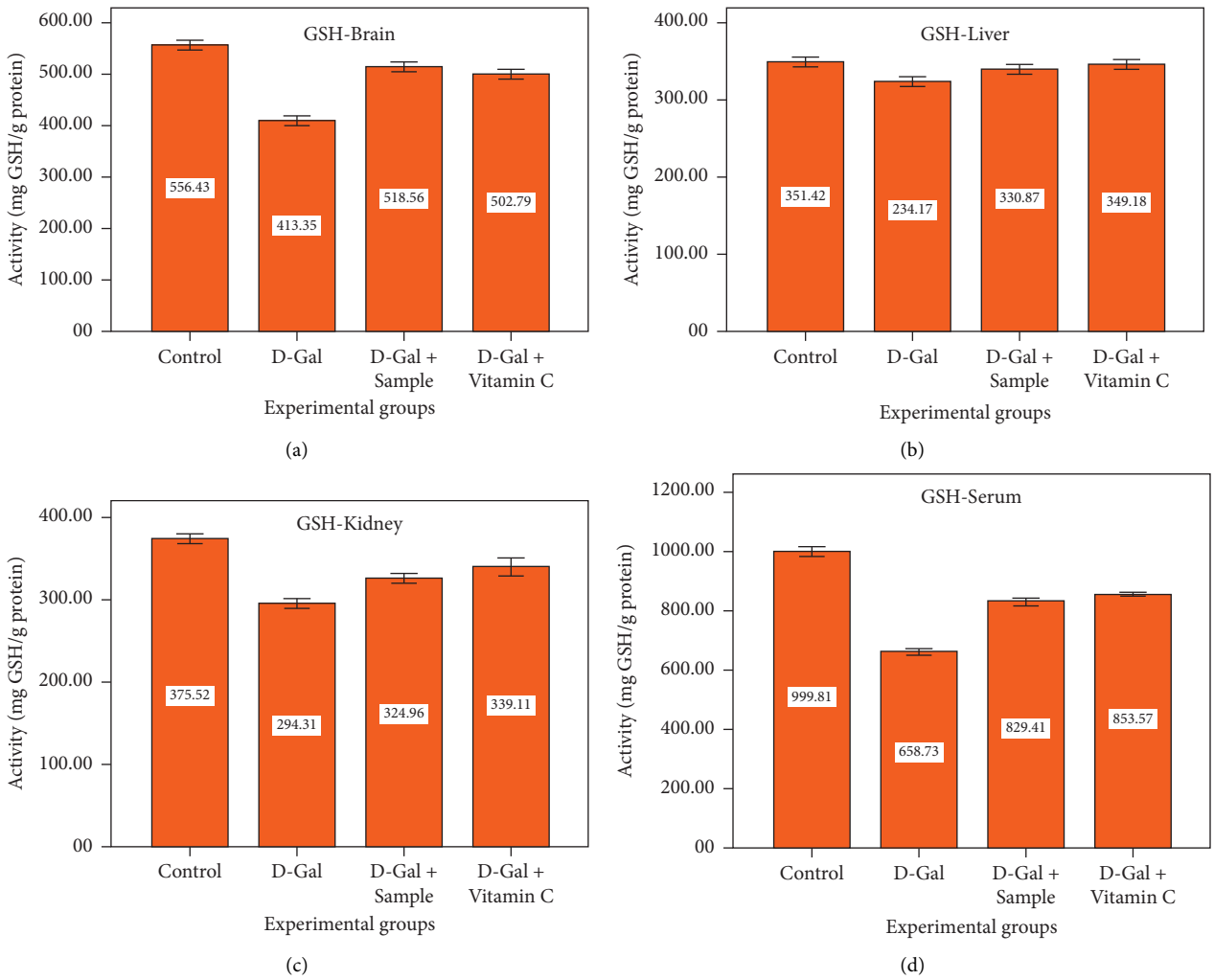


Figure 6: GSH level of experimental groups.

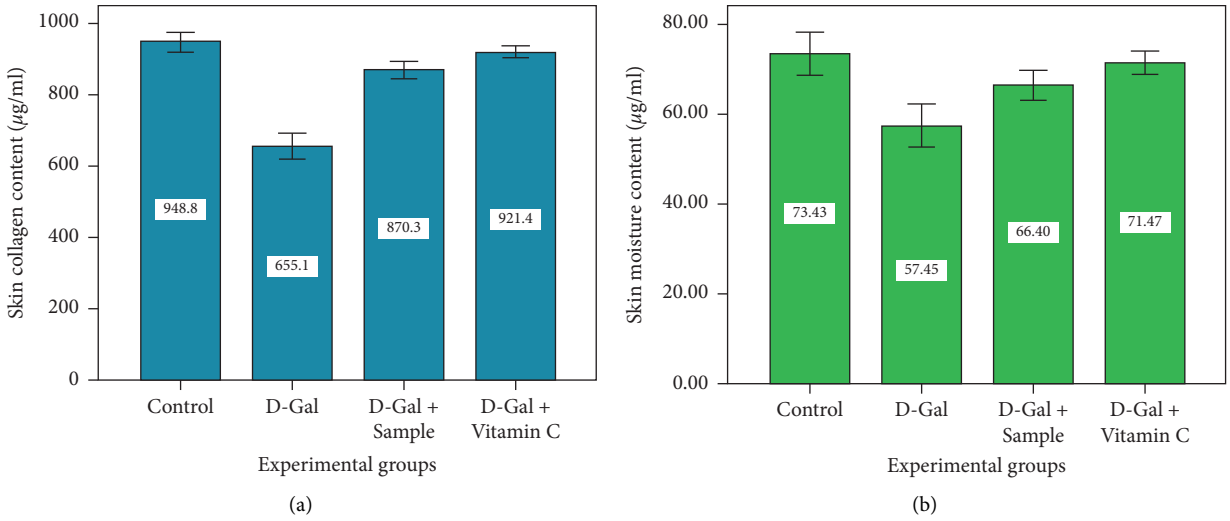


Figure 7: Elastin and moisture contents of experimental mice.

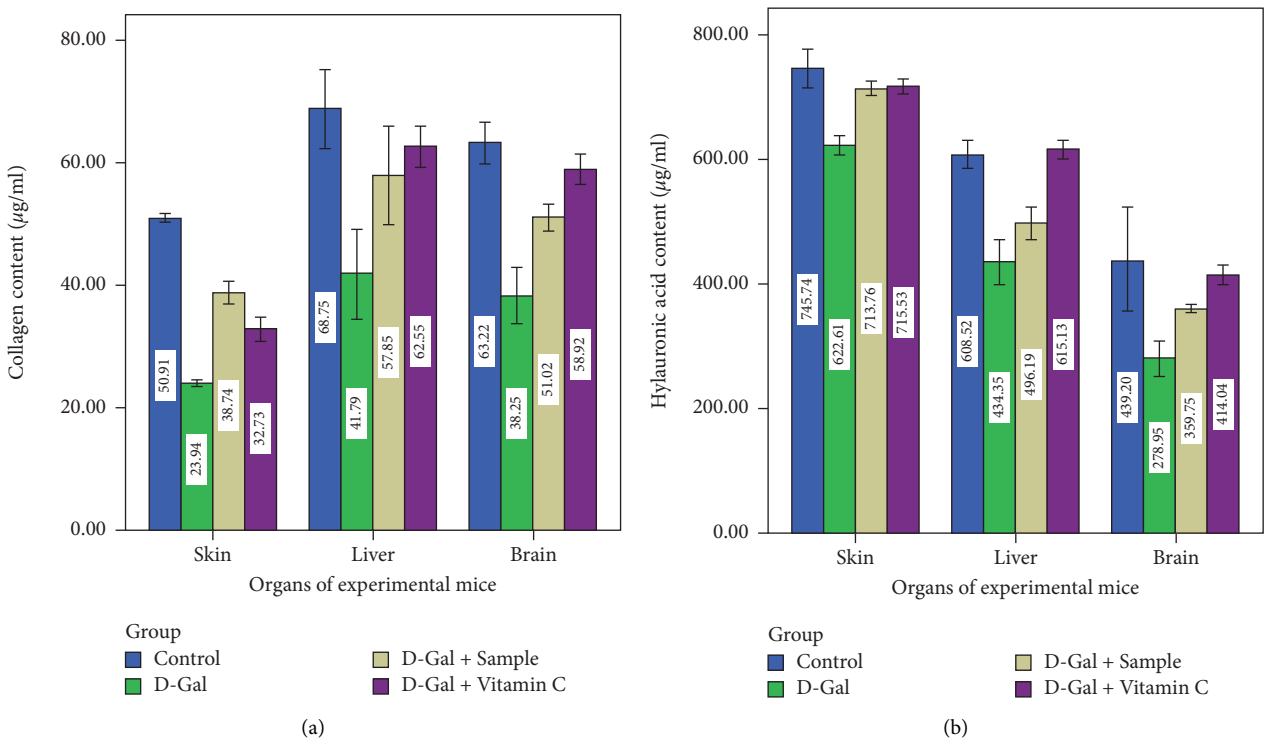


Figure 8: Collagen and hyaluronic acid contents of experimental mice.

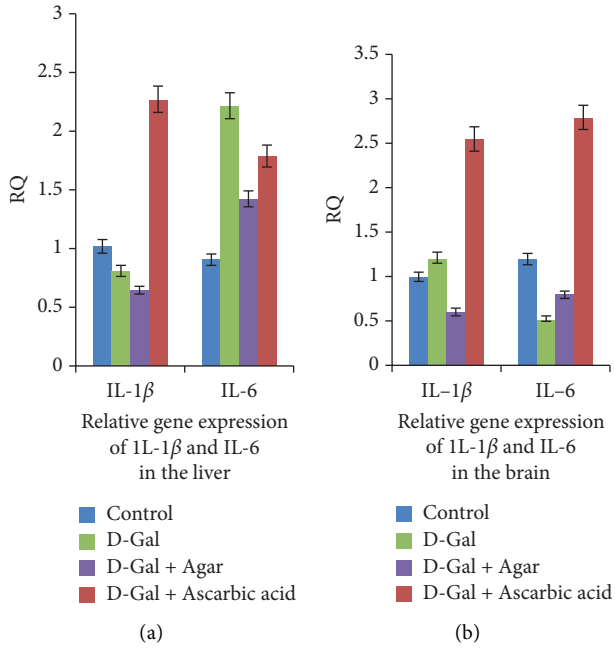


Figure 9: Relative quantification (RQ) of gene expression of IL-1β and IL-6 in the liver and brain of experimental groups of mice.

could play a vital role in protecting the weight of those organs from decreasing [40].

The *in vivo* antioxidant defense systems such as MDA, SOD, CAT, and GSH in mice administered with D-Gal (group II) exhibited a significant decrease in the tissues of the liver, brain, and kidney when compared to the control group I mice ($P < 0.05$). Group III mice showed a significant increase in the activities of all of these parameters ($P < 0.05$, Figures 5(a)–5(d)). Cell life in an oxygenated background has necessitated the progression of effective cellular tactics to identify and detoxify metabolites of ROS. The effects mentioned above could significantly disturb a host of physiological practices and metabolic pathways contributing to the aging of the animal and skin. For example, excess ROS

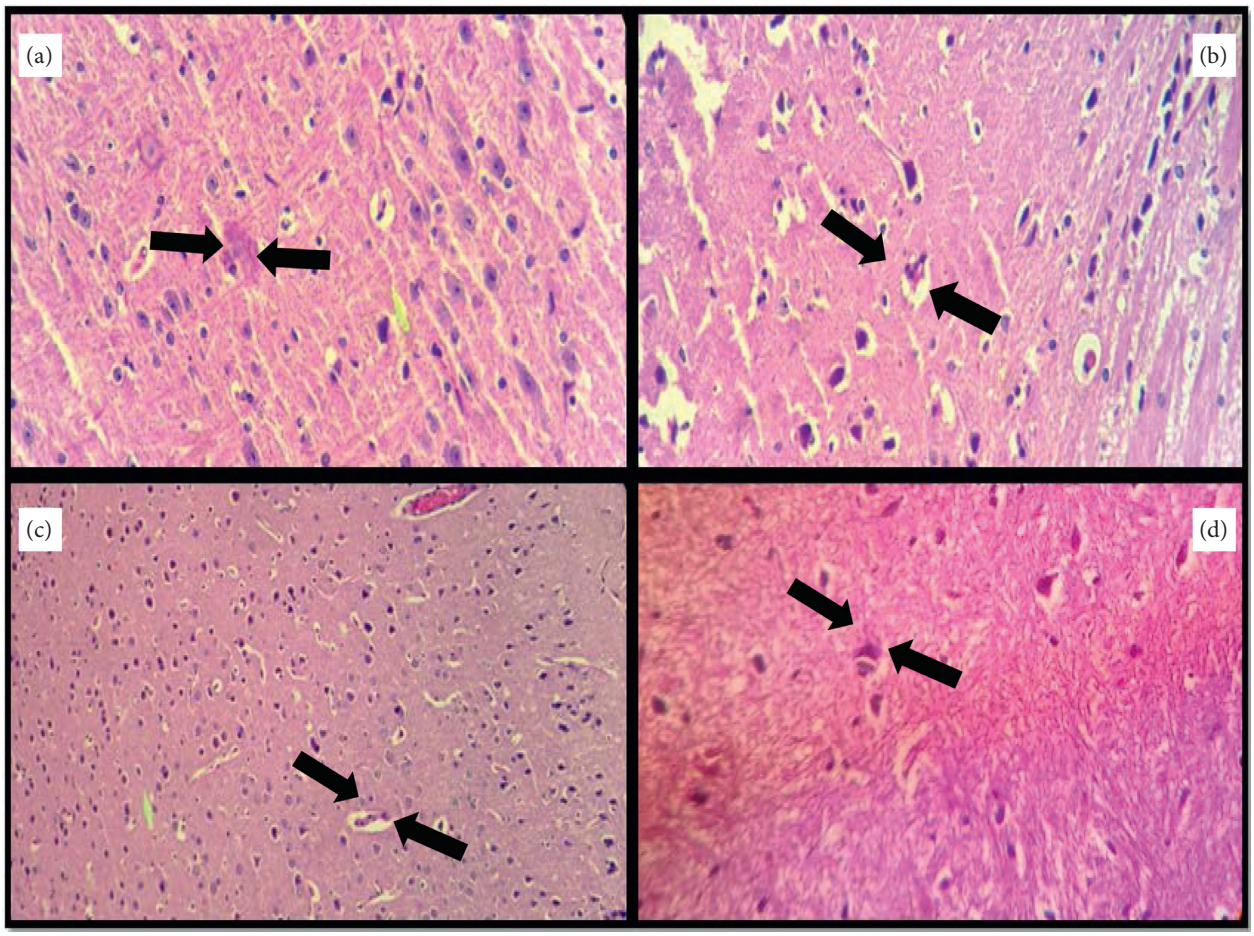


Figure 10: Histological evaluation of the brain of experimental group mice.

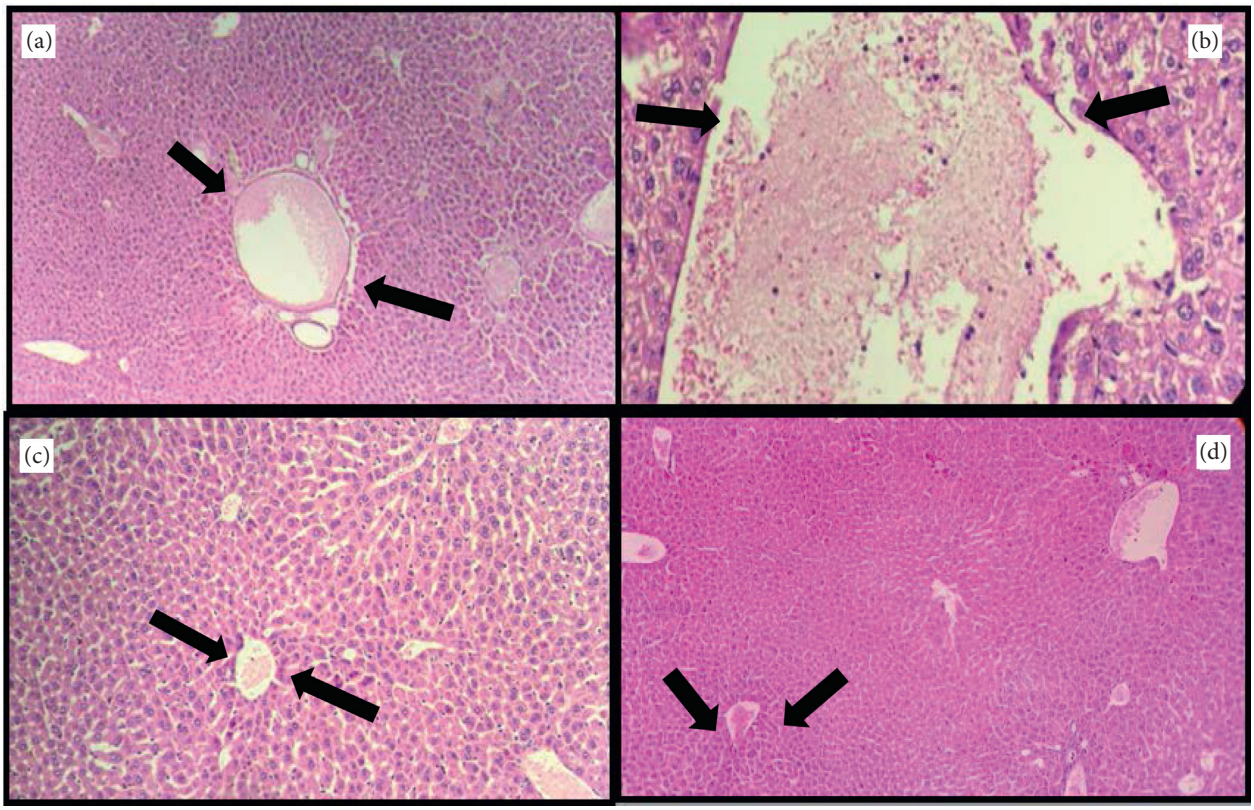


Figure 11: Histological evaluation of the liver of experimental group mice.

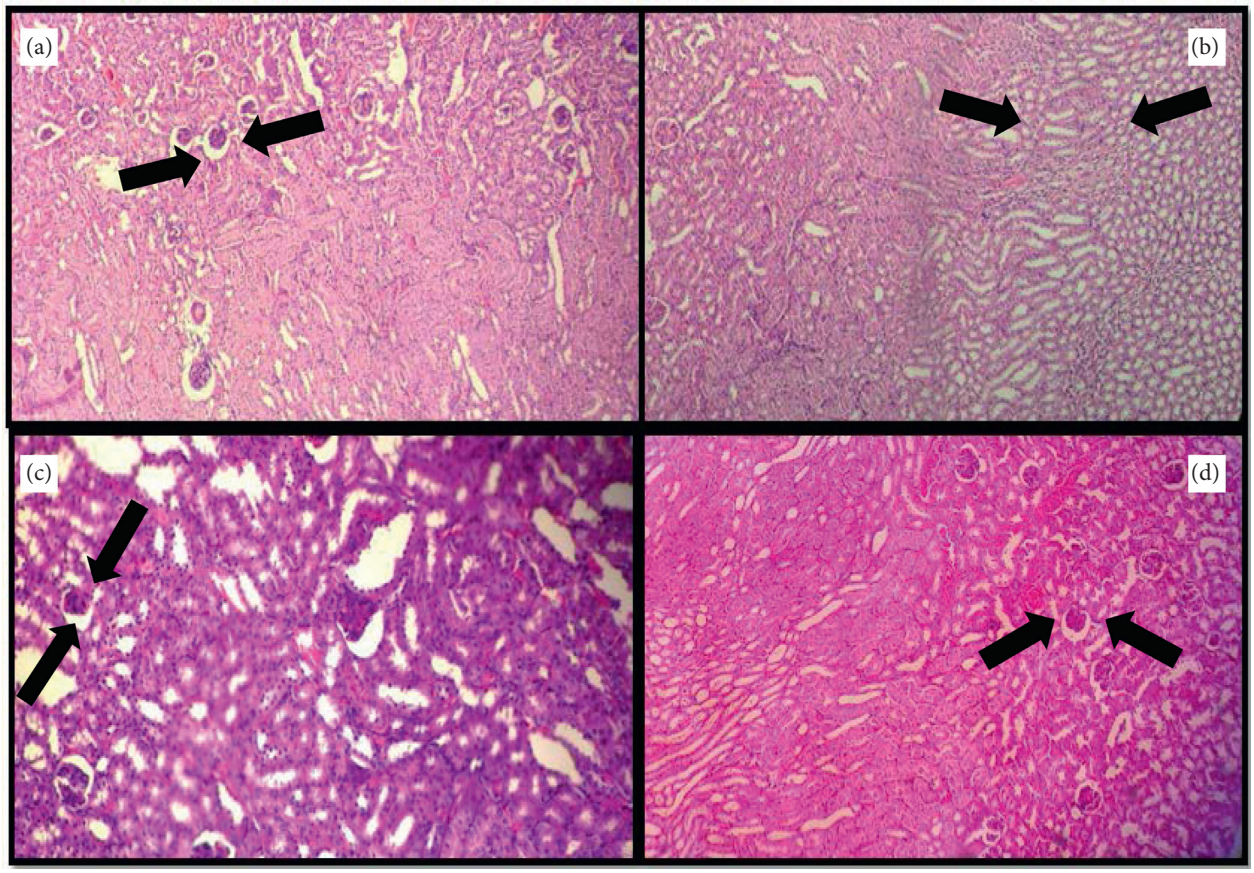


Figure 12: Histological evaluation of the kidney of experimental group mice.

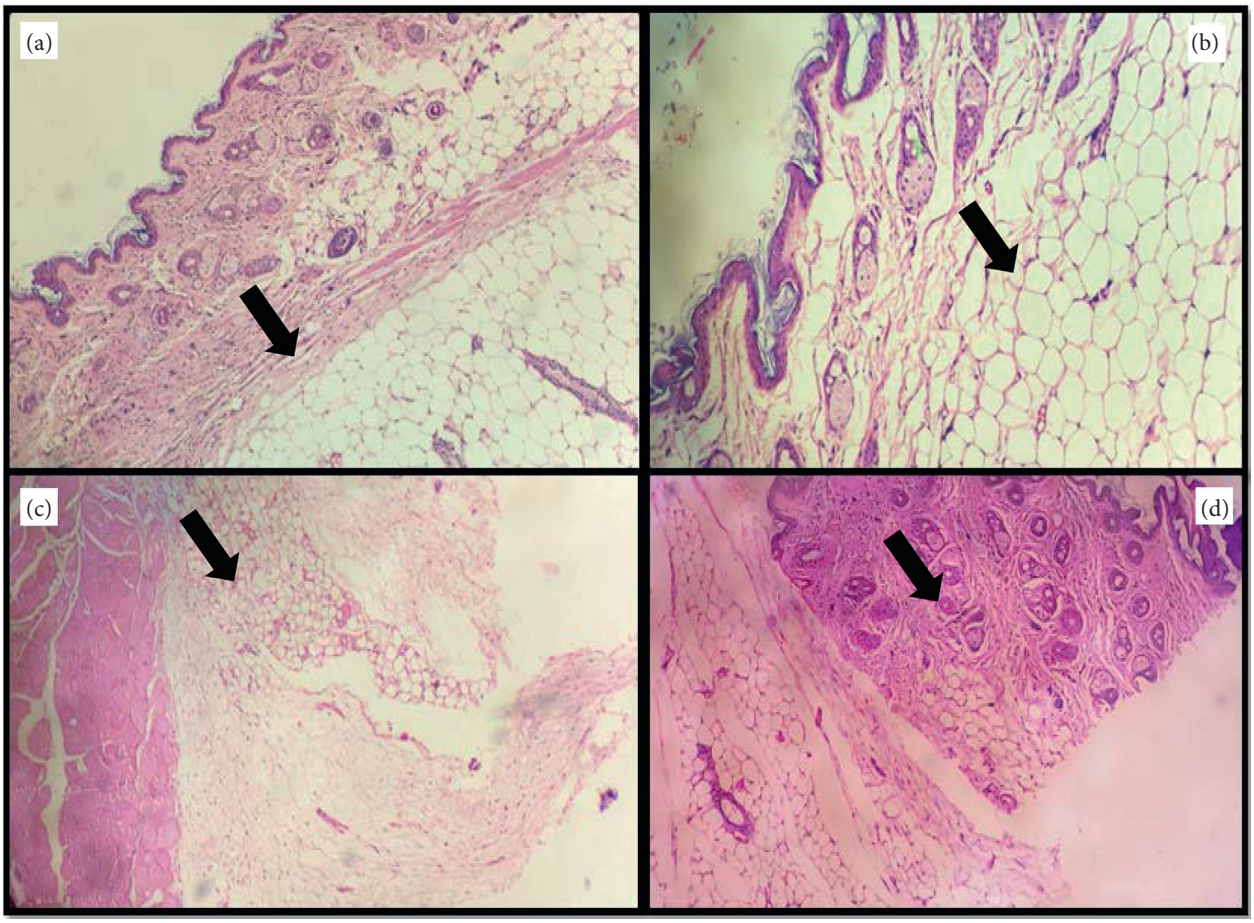


Figure 13: Histological evaluation of the skin of experimental group mice.

can cause lipid peroxidation *in vivo*. One of the final oxidation products is MDA, which induces cytotoxicity. Therefore, the content of MDA might indicate the body's level of lipid peroxidation [19] and reveal the extent of cell injury carried out by ROS. The rise in the activities of the primary enzymatic antioxidant defenses—MDA, SOD, CAT, and GSH—in the sample coadministered group (group III) and vitamin C (group IV) mice was significant ($P < 0.05$). Presumably small amounts of agar may enhance the antioxidant activity of SOD. The GSH activity of the brain, liver, kidney, and serum (Figures 6(a)–6(d)) of D-Gal-induced (group II) mice was significantly lower than that of the control, the sample, and vitamin C administered groups of mice ($P < 0.05$). The present findings were supported by the report of Ye et al. [24] who suggested that ethyl acetate extract from the plant (*I. polycarpa* defatted fruit) increased the antioxidant levels in experimental mice. The GSH level is a significant factor for quantifying the level of antioxidative activity *in vivo* and has tremendous antioxidant and detoxifying activities. It can protect almost every cell in the body. GSH not only eradicates free radicals *in vivo* but also boosts the organism's immunity level [41].

All the parameters in behavioral studies and the organ indices of D-Gal-induced aging mice were reduced. The antioxidant enzymes, elastin, collagen, and hyaluronic acid levels were low in D-Gal-induced mice, which endorsed the role of D-Gal on metabolic age changes in the skin. At the same time, all these levels were high in agar-treated mice. Further, the skin's moisture content affects the skin's physiological function. These expressions (IL-1β and IL-6) were altered in D-Gal-induced mice which were brought to a normal level by mice administered with agar. The loss of elastin and moisture caused by the D-Gal-induced mice group II was significantly lower than that of control mice (group I) ($P < 0.05$), indicating that elastin and moisture would progressively alter with the age of the skin. Agar-administered group III mice had significantly increased elastin content when compared to group II mice ($P < 0.05$, Figures 7(a) and 7(b)).

The present results were concurrent with the report of Ye et al. [24] who indicated that D-Gal reduced these cytokine factors, which were normalized by the ethyl acetate extract of *I. polycarpa* defatted fruit. Many scientific experts pointed out that cosmetic usage makes women lose elastin quicker than men [42]. Collagen fiber and hyaluronic acid are critical components of the skin. Collagen and hyaluronic acid content in group II mice decreased significantly relative to group I mice ($P < 0.05$). The variation in the amount of collagen can therefore accelerate skin aging (Figures 8(a) and 8(b)). Fittingly, brown algae agar improved the condition of mouse skin due to its high moisture and element content. Brown algae agar can effectively enhance the antioxidant activity, maintain collagen, elastin, and hydration, and reduce the MDA content of aging mouse skin. The morphological modifications of mouse skin have demonstrated the antiaging activity of agar. Collagen fibers decrease significantly with age and therefore make the skin inflexible and saggy. Thus, modifying the collagen content can accelerate the aging process of the skin [42].

The effect on IL-1β and IL-6 in the experimental groups is shown in Figure 9. The D-Gal-treated group exhibited increased IL-1β and IL-6 gene expression in the brain ($P < 0.001$ and $P < 0.01$, respectively); meanwhile, they only increased IL-1β gene expression in the liver ($P < 0.01$). In mice treated with D-Gal + agar 100 mg/kg, the IL-1β and IL-6 gene expression in the brain were inhibited, when compared with the D-gal-treated group alone. Further, IL-1β gene expression in the liver decreased conspicuously in the D-Gal + agar-treated group of mice. The present study reinforced the finding that D-Gal induced noticeable aging and oxidative stress in the brain and liver of experimental animals [24]. The paraffin sections of the brain, the liver, the kidney, and the skin of experimental mice are presented in Figures 10–13. It was noticed that the brain cell number of group II was less than that of groups I, III, and IV. Typically, cells in the brain degenerate with age, much like the reductions seen in group II mice. However, this number was regenerated in the agar-treated (group III) and vitamin C (group IV)-treated mice. In D-Gal-injected mice, hepatocytes were considerably damaged, displaying few appearances of ballooning degeneration and becoming shallow in comparison with group I mice. Further, all these changes were normalized in the sample-administered and ascorbic acid-treated mice. Similar histopathological findings were also reported by Ye et al. [24] who indicated that morphological alterations were seen in organs such as the brain, liver, and skin of the aging mice that administered D-Gal.

4. Conclusions

The in vitro antioxidant activity of agar from the brown algae *L. digitata* showed higher potency at higher concentrations. All parameters in the behavioral studies and organ indices for aging were reduced in the D-Gal-induced mice. Antioxidant enzymes, elastin, collagen, and hyaluronic acid levels were weak in D-Gal-induced mice, suggesting that D-Gal modified the metabolic age of the skin. All the parameters were elevated in mice exposed to agar. The expression of IL-1β and IL-6 was altered in D-Gal-induced mice, which was in turn brought back to normal by mice administered with agar. Finally, the anti-skin aging properties of agar were also confirmed by histopathology. All the outcomes suggested that agar from *L. digitata* holds promise as an antiaging natural phytomedicine and skincare product in the future.

Data Availability

All data generated or analyzed during this study are included in this published article.

Ethical Approval

This research does not involve human participants, human material, or human data. This research involves experimental animals approved by the Institutional Animal Ethics Committee, Chettinad Academy of Research and Education (Deemed to be University), Kelambakkam 603 103, Tamil Nadu, India, and adhered to the Committee for the Purpose of

Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India guidelines. Animal use complied with institutional, national, and international guidelines, including the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India guidelines. Guidelines of "*Guide for the Care and Use of Laboratory Animals*" (Institute of Laboratory Animal Resources, National Academic Press 1996; *NIH publication* number #85-23, revised 1996) were strictly followed throughout the study. Institutional Animal Ethical Committee (IAEC) approved this study. Research ethical clearance was obtained (IAEC4/Proposal: 13/A.Lr:31/dated: 20.12.17).

Consent

Not applicable.

Conflicts of Interest

All authors declare that there are no conflicts of interest.

Authors' Contributions

RBS and RS conducted general experiments and assay and in vivo experiments. AT and NV supported data analysis and wide-ranging aspects of the manuscript preparation and development process. MME and MWQ provided research insight, examined the content, and supported wide-ranging aspects of the manuscript development process. RS critically reviewed the final manuscript draft and provided feedback on data and analyses. RS carried out the conceptual work, framework, manuscript preparation, and critical editing/evaluation. All authors read and approved the final manuscript.

Acknowledgments

The authors want to thank their respective institutions for their continued support. All authors gratefully acknowledge the histopathology facility provided by Prof. Vijayashree, Dept. of Pathology, CHRI. The corresponding author (Saravanan Ramachandran) gratefully acknowledges the Department of Biotechnology (BT/PR15676/AAQ/03/794/2016), Ministry of Science and Technology, Govt. of India, New Delhi, for their financial support.

Supplementary Materials

In the early (0 weeks) and late (6 weeks), group II mice showed a significant decrease in light and dark activity (Figure S1) as compared to group I mice ($P < 0.05$). All of the abovementioned activities clearly indicated that ageing was induced in the D-Gal (group II) mice, according to the findings of this study. When compared to D-Gal, there are no significant changes (shrinkage) in the skin of the experimental mice (group I), as shown in Figure S1 (group II). However, in the agar-treated (group III) mice, this change was reversed (Figures S2–S4). Statistical significance: $P < 0.05$ (DMRT). *Comparison was made between 200 μl of sample and standard. (*Supplementary Materials*)

References

[1] M. Kasapoglu and T. Özben, "Alterations of antioxidant enzymes and oxidative stress markers in aging," *Experimental Gerontology*, vol. 36, no. 2, pp. 209–220, 2001.

[2] X. Li, "Anti-aging cosmetics and its efficacy assessment methods," *IOP Conference Series: Materials Science and Engineering*, vol. 87, no. 1, p. 012043, 2015.

[3] C. Aldag, D. Leventhal, and S. L Phillip, "Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature," *Clinical, Cosmetic and Investigational Dermatology*, vol. 9, pp. 411–419, 2016.

[4] M. Mladenov, I. Gjorgoski, T. Stafilov, and D. Duridanova, "Effect of vitamin C on lipid hydroperoxides and carbonyl groups content of rat plasma depending on age and acute heat exposure," *Journal of Thermal Biology*, vol. 31, no. 8, pp. 588–593, 2006.

[5] K. B. Pandey and S. I. Rizvi, "Markers of oxidative stress in erythrocytes and plasma during aging in humans," *Oxidative Medicine and Cellular Longevity*, vol. 3, no. 1, pp. 2–12, 2010.

[6] M. Sárdy, "Role of matrix metalloproteinases in skin ageing," *Connective Tissue Research*, vol. 50, no. 2, pp. 132–138, 2009.

[7] L. J. Loretz, A. M. Api, L. M. Barraj et al., "Exposure data for cosmetic products: lipstick, body lotion, and face cream," *Food and Chemical Toxicology*, vol. 43, no. 2, pp. 279–291, 2005.

[8] W. Amasa, D. Santiago, S. Mekonen, and A. Ambelu, "Are cosmetics used in developing countries safe? Use and dermal irritation of body care products in Jimma Town, Southwestern Ethiopia," *Journal of Toxicology*, vol. 2012, pp. 1–8, 2012.

[9] P. Nigam, "Adverse reactions to cosmetics and methods of testing," *Indian Journal of Dermatology, Venereology and Leprology*, vol. 75, no. 1, pp. 10–19, 2009.

[10] C. S. Cortez-Pereira, A. R. Baby, and M. V. R. Velasco, "Review Article: fragrance technology for the dermatologist - a review and practical application," *Journal of Cosmetic Dermatology*, vol. 9, no. 3, pp. 230–241, 2010.

[11] S. Sen and R. Chakraborty, "Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: importance, challenges and future," *Journal of Traditional and Complementary Medicine*, vol. 7, no. 2, pp. 234–244, 2017.

[12] M. Lahaye and M. A. V. Axelos, "Gelling properties of water-soluble polysaccharides from proliferating marine green seaweeds (Ulva spp.)," *Carbohydrate Polymers*, vol. 22, no. 4, pp. 261–265, 1993.

[13] J. P. Douzals, P. A. Marechal, J. C. Coquille, and P. Gervais, "Microscopic study of starch gelatinization under high hydrostatic pressure," *Journal of Agricultural and Food Chemistry*, vol. 44, no. 6, pp. 1403–1408, 1996.

[14] T. Chopin, A. H. Buschmann, C. Halling et al., "Integrating seaweeds into marine aquaculture systems: a key toward sustainability," *Journal of Phycology*, vol. 37, no. 6, pp. 975–986, 2001.

[15] M. Martin, T. Barbeyron, R. Martin, D. Portetelle, G. Michel, and M. Vandenbol, "The cultivable surface microbiota of the brown alga Ascophyllum nodosum is enriched in macroalgal-polysaccharide-degrading bacteria," *Frontiers in Microbiology*, vol. 6, p. 1487, 2015.

[16] M. Venkatesan, V. Arumugam, R. Pugalendi et al., "Antioxidant, anticoagulant and mosquitocidal properties of water soluble polysaccharides (WSPs) from Indian seaweeds," *Process Biochemistry*, vol. 84, pp. 196–204, 2019.

[17] X. Wang, D. Duan, and X. Fu, "Enzymatic desulfation of the red seaweeds agar by Marinomonas arylsulfatase," *International Journal of Biological Macromolecules*, vol. 93, pp. 600–608, 2016.

[18] S.-H. Sun, S.-J. Kim, S.-J. Kwak, and K.-S. Yoon, "Efficacy of sodium hypochlorite and acidified sodium chlorite in preventing browning and microbial growth on fresh-cut produce," *Preventive Nutrition and Food Science*, vol. 17, no. 3, pp. 210–216, 2012.

[19] I. HerCeg, A. R. Jambrak, and D. ŠubArIć, "Texture and pasting properties of ultrasonically treated corn starch," *Czech Journal of Food Sciences*, vol. 28, no. 2, pp. 83–93, 2010.

[20] X.-M. Li, "Protective effect of Lycium barbarum polysaccharides on streptozotocin-induced oxidative stress in rats," *International Journal of Biological Macromolecules*, vol. 40, no. 5, pp. 461–465, 2007.

[21] R. Karthik, V. Manigandan, R. Sheeba, R. Saravanan, and P. R. Rajesh, "Structural characterization and comparative biomedical properties of phloroglucinol from Indian brown seaweeds," *Journal of Applied Phycology*, vol. 28, no. 6, pp. 3561–3573, 2016.

[22] P. Prieto, M. Pineda, and M. Aguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E," *Analytical Biochemistry*, vol. 269, no. 2, pp. 337–341, 1999.

[23] A. L. M. Ling, "Antioxidant activity, total phenolic and flavonoid contents of selected commercial seaweeds of Sabah, Malaysia," *International Journal of Pharmaceutical & Phytopharmacological Research*, vol. 3, no. 3, pp. 234–238, 2014.

[24] Y. Ye, R. R. Jia, L. Tang, and F. Chen, "*In Vivo* antioxidant and anti-skin-aging activities of ethyl acetate extraction from Idesia polycarpa defatted fruit residue in aging mice induced by D-galactose," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, 2014.

[25] B. Costall, B. J. Jones, M. E. Kelly, R. J. Naylor, and D. M. Tomkins, "Exploration of mice in a black and white test box: validation as a model of anxiety," *Pharmacology Biochemistry and Behavior*, vol. 32, no. 3, pp. 777–785, 1989.

[26] R. S. Bains, S. Wells, R. R. Sillito et al., "Assessing mouse behaviour throughout the light/dark cycle using automated in-cage analysis tools," *Journal of Neuroscience Methods*, vol. 300, pp. 37–47, 2018.

[27] J. R. Deuis, L. S. Dvorakova, and I. Vetter, "Methods used to evaluate pain behaviors in rodents," *Frontiers in Molecular Neuroscience*, vol. 10, p. 284, 2017.

[28] V. W. Tung, T. J. Burton, E. Dababneh, and S. L. Quail, "Camp. "Behavioral assessment of the aging mouse vestibular system"," *Journal of visualized experiment*, vol. 89, 2014.

[29] A. Sayama, T. Soushin, T. Okada, K. Doi, and H. Nakayama, "Morphological and biochemical changes during aging and photoaging of the skin of C57BL/6J mice," *Journal of Toxicologic Pathology*, vol. 23, no. 3, pp. 133–139, 2010.

[30] D. M. Palmer and J. S. Kitchin, "Oxidative damage, skin aging, antioxidants and a novel antioxidant rating system," *Journal of Drugs in Dermatology : Journal of Drugs in Dermatology*, vol. 9, no. 1, pp. 11–15, 2010.

[31] S. Novella, A. P. Dantas, G. Segarra et al., "Aging enhances contraction to thromboxane A2 in aorta from female senescence-accelerated mice," *Age*, vol. 35, no. 1, pp. 117–128, 2013.

[32] J. George, A. Thabitha, and N. Vignesh, "Antiskin cancer and antioxidant activities of formulated agar from Brown seaweed Laminaria digitata (hudson) in dimethyl benzanthracene-induced Swiss albino mice," *International Journal of Polymer Science*, vol. 2021, 2012.

[33] R. Armisen, "World-wide use and importance of Gracilaria," *Journal of Applied Phycology*, vol. 7, no. 3, pp. 231–243, 1995.

[34] J. Fleurence, "Seaweed proteins," *Trends in Food Science & Technology*, vol. 10, no. 1, pp. 25–28, 1999.

[35] J. P. Mua and D. S. Jackson, "Relationships between functional attributes and molecular structures of amylose and amylopectin fractions from corn starch," *Journal of Agricultural and Food Chemistry*, vol. 45, no. 10, pp. 3848–3854, 1997.

[36] P. Rubanya, *Nutritional Profile, Antioxidant and Anticoagulant Activities of Aqueous Extracts from Selected Indian Seaweeds"*, M.Sc. Dissertation, Chettinad Academy of Research and Education, Kelambakkam, TN, India, pp. 32–41, 2016.

[37] J. G. Swallow, P. Koteja, P. A. Carter, and T. Garland, "Food consumption and body composition in mice selected for high wheel-running activity," *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, vol. 171, no. 8, pp. 651–659, 2001.

[38] D. Wang, M. Liu, J. Cao et al., "'Effect of colla corii asini (E'jiao) on D-galactose induced aging mice," *Biological and Pharmaceutical Bulletin*, vol. 35, no. 12, pp. 2128–2132, 2012.

[39] J. H. Sun, Y. M. Liu, T. Cao, and W. Q. Ouyang, "Effect of kinetin on ovary and uterus in D-galactose-induced female mouse model of aging," *Acta Physiologica Sinica*, vol. 65, no. 4, pp. 389–394, 2013.

[40] G. Lavanya, S. P. Voravuthikunchai, and N. H. Towatana, "Acetone extract fromRhodomyrtus tomentosa: a potent natural antioxidant," *Evidence-based Complementary and Alternative Medicine*, vol. 2012, pp. 1–8, Article ID 535479, 2012.

[41] E. D. Lephart, "Protective effects of equol and their polyphenolic isomers against dermal aging: microarray/protein evidence with clinical implications and unique delivery into human skin," *Pharmaceutical Biology*, vol. 51, no. 11, pp. 1393–1400, 2013.

[42] J.-G. Zhao, Q.-Q. Yan, L.-Z. Lu, and Y.-Q. Zhang, "In vivoantioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato," *Nutrition Research and Practice*, vol. 7, no. 5, pp. 359–365, 2013.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7123521, 23 pages
https://doi.org/10.1155/2022/7123521

Review Article

Bioactive Constituents and Toxicological Evaluation of Selected Antidiabetic Medicinal Plants of Saudi Arabia

Ali S. Alqahtani,[1,2] **Riaz Ullah**,[1,2] **and Abdelaaty A. Shahat**[1,2]

[1]*Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*
[2]*Medicinal Aromatic and Poisonous Plants Research Centre, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia*

Correspondence should be addressed to Riaz Ullah; rullah@ksu.edu.sa

Received 13 December 2021; Accepted 30 December 2021; Published 17 January 2022

Academic Editor: Rajadurai Murugan

The purpose of this review is to summarize the available antidiabetic medicinal plants in the Kingdom of Saudi Arabia with its phytoconstituents and toxicological findings supporting by the latest literature. Required data about medicinal plants having antidiabetic activities and growing in the Kingdom of Saudi Arabia were searched/collected from the online databases including Wiley, Google, PubMed, Google Scholar, ScienceDirect, and Scopus. Keywords used in search are in vivo antidiabetic activities, flora of Saudi Arabia, active ingredients, toxicological evaluations, and medicinal plants. A total of 50 plant species belonging to 27 families were found in the flora of Saudi Arabia. Dominant family was found Lamiaceae with 5 species (highest) followed by Moraceae with 4 species. β-Amyrin, β-sitosterol, stigmasterol, oleanolic acid, ursolic acid, rutin, chlorogenic acid, quercetin, and kaempferol are the very common bioactive constituents of these selected plant species. This paper has presented a list of antidiabetic plants used in the treatment of diabetes mellitus. Bioactive antidiabetic phytoconstituents which showed that these plants have hypoglycemic effects and highly recommended for further pharmacological purposes and to isolate/identify anti-diabetes mellitus (anti-DM) active agents also need to investigate the side effects of active ingredients.

1. Introduction

Medicinal plants are used for the treatment of different infections [1, 2]. These plants contributed as a source of inspiration for novel therapeutic compounds [3]. The medicinal value of plants is due to the presence of a wide variety of secondary metabolites including alkaloids, glycosides, tannins, volatile oil, and terpenoids [4, 5]. Medicinal plants and their extracts represent a rich source of crude medications that possess therapeutic properties. Indeed, the World Health Organization reports that various plant fractions and their dynamic constituents are utilized as traditional medicines by 80% of the world population [6]. Plants are the primary source for different pharmaceutical, perfumery, flavor, and cosmetics industries; the use of modern drugs dramatically resulted into resistant microorganisms toward different modern drugs; the researchers are now in search for alternate source of treatment of various disorders [7, 8]. For this purpose, the medicinal herbs are the best alternate to various drugs. Most of natural products possess interesting biological activities and medicinal potential. Various herbs, fruits, and grains have been found to have different important biological activities such as antioxidant, [9] antitumor, antimutagenic, antidiabetes, antianalgesic, [10] antidementia, inflammation inhibitory effect, [9] antitumor, [11] anticancer, [12] antimicrobial, antileishmanial, and antimalarial properties [13, 14]. The consumption of natural antioxidants will reduce risk of many diseases including cancer, cardiovascular disease, diabetes, and other diseases allied with aging [15]. For natural antioxidants, a larger number of medicinal herbs have been evaluated by applying laboratories' developed procedures. Plants derived substances, collectively called phytonutrients or phytochemicals, been recognized as good source of natural antioxidants [16, 17].

The Kingdom of Saudi Arabia is a huge arid land with an area of about 2,250,000 km^2 covering the major part of the Arabian Peninsula, characterized by different ecosystems

and diversity of plant species. The climate in Saudi Arabia differs greatly between the coast and the interior. High humidity coupled with more moderate temperatures is prevalent along the coast, whereas aridity and extreme temperatures characterize the interior. The flora of Saudi Arabia is one of the richest biodiversities in the Arabian Peninsula and comprises very important genetic resources of crops and medicinal plants. Saudi Arabia contains 97 trees, 564 shrubs, and about 1620 herbs, which cover, respectively, 4.25%, 24.73%, and 71.02% of higher plant diversity of the country [18].

Diabetes mellitus is one of the most prevalent diseases in endocrine gland system with an increasing incidence in human community [19]. Type I diabetes is caused by insulin secretion deficit, while type II diabetes is accompanied with progressive rate of insulin resistance in liver and peripheral tissues, reducing β-cell mass, and deficient insulin secretion [20, 21]. This disease brings about acute metabolic side effects including ketoacidosis, hyperosmolar coma accompanied with chronic disorders, and long term, adverse side effects such as retinopathy, renal failure, neuropathy, skin complications, as well as increasing cardiovascular complication risks [22, 23]. Also, common symptoms of diabetes are frequent urine, thirsty, and overeating [24]. Diabetes inflicts 100 million people yearly and is recognized as the seventh cause of death in the world [25]. It has been estimated that the number of diabetic people will increase from 150 million individuals in 2003 to 300 million by 2025 [26]. The essential and effective drugs for diabetes mellitus are insulin injection and hypoglycemic agents, but these compounds possess several adverse effects and have no effects on diabetes complications in long term. Therefore, it is important to find effective compounds with lower side effects in treating diabetes [27]. Medicinal plants are good sources as alternative or complementary treatments for this and other diseases [28–30]. Although various plants have been traditionally used throughout history to reduce blood glucose and improve diabetes complications, there is not enough scientific information about some of them. Herbal medicines are commonly prescribed throughout the world because of low side effects, availability, roughly low cost, and also its effectiveness [31, 32].

In Saudi Arabia, the number of people who suffer from DM increased from 890,000 in 2000 to a staggering projection of 2,523,000 in 2030. In 2011, Saudi Arabia reported a prevalence of DM at 30% of the total population, with a rate of 27.6% in women and 34.1% in men [33]. According to 2010 data from several sources (WHO, World Bank, UNESCO, CIA, and individual country databases), DM is the number three disease-related cause of death in Saudi Arabia [34].

In the present situation, herbal medicines' usage has significantly increased and published studies from developed countries emphasize that a paramount proportion of medicines supplied by them have herbal origins, so growing and producing the herbal medicines could be helpful to both economic development and community's health [35]. Keeping in mind the importance of medicinal plants, in the current review various medicinal plants used for antidiabetic treatment around the world, native to or cultivated in Saudi Arabia, are documented for the purpose to provide up-to-date insight on medicinal plant used for DM, so that researcher easily selects plant for bioscreening and active constituents' identification purposes. Therefore, we invite researchers' attention to carry out detailed ethnopharmacological and toxicological studies on unexplored antidiabetic plants in order to provide reliable knowledge to the patients and develop novel antidiabetic drugs.

2. Methods

Required data about medicinal plants having antidiabetic activities and growing in the Kingdom of Saudi Arabia were searched/collected from the online databases including Wiley, Google, PubMed, Google Scholar, ScienceDirect, and Scopus. Keywords used in search are in vivo antidiabetic activities, flora of Saudi Arabia, active ingredients, toxicological evaluations, and medicinal plants. Latest published data approximately in the last ten years with the key outcome of change in blood glucose level in animal model were included. One or two articles are selected as references for each plant's species on priority basis from the journals found in web of science and latest years.

3. Results

The names, families, used parts, location, and antidiabetic properties in animal model of the native/cultivated Saudi medicinal plants are summarized in Table 1. The active ingredients and toxicological effect of these plants in animal model are given in Table 2. A total of 50 plant species belong from 27 families were found in the flora of Saudi Arabia. Dominant family was found Lamiaceae with 5 species (highest) followed by Moraceae with 4 species.

4. Discussion

The majority of the experiments confirmed the benefits of medicinal plants with hypoglycemic effects in the management of diabetes mellitus. From Table 1, it can be concluded that among the plants used for the treatment of diabetes, *H. salicornicum, T. oliverianum, A. cepa, A. herba-alba, Teucrium polium, Sesamum indicum, Z. spina-christi,* and *U. dioica* seem to be most common plants used to treat diabetes and are available everywhere in the world. The leaves were most commonly used plant part, and other parts (root, stem, bark, flower, seed, and whole plant) were also useful for curing. The most common diabetic model that was used was the streptozotocin and alloxan-induced diabetic mouse or rat as diabetic models. The most commonly involved active constituents are flavonoid, alkaloid, saponin, carbohydrate, vitamins, amino acid and its derivatives, phenol and its derivatives, and benzoic acid derivatives. The very common phytoconstituents, *targeted metabolic pathways,* and its structure are given in Table 3 [194, 195]. The native to or cultivated plant species of the kingdom given in Table 1 are selected from the published literature about ethnobotanical value and antidiabetic potential of medicinal

Table 1: Antidiabetic medicinal plants growing in Saudi Arabia.

S. no.	Names of plants	Family	Part used	location	Antidiabetes Activities
1.	*Allium cepa*	Liliaceae	Bulb	Central Saudi Arabia [36]	Ethanol extract of *A. cepa* in STZ-induced diabetic rats causes 66% decreased at 200 mg/kg after 24 h in blood glucose level [37]. 0.4 g/100gbw of A. cepa reduced 50% the fasting glucose levels of diabetic rats [38]. Similar results reported by other researchers [39].
2.	*Anthemis herba-alba*	Compositae/ Asteraceae	Aerial parts	Farasan Island of Red Sea [40]	72% plasma glucose levels decreased in albino mice by ethyl alcohol extract of *Artemisia herba-alba* [41]
3.	*Cichorium intybus*	Asteraceae	Seeds	Qassim region [42]	C. intybus leaf powder, ethanol, aqueous seed extracts, and hexane extracts led to a decrease in blood glucose levels to near normal value. Hypoglycemic effects of *C. intybus* were observed in diabetic rats, and a dose of 125 mg of plant extract/kg body weight exhibited the most potent hypoglycemic effect [43–45]
4.	*Clitoria ternatea*	Fabaceae	Aerial parts	Cultivated throughout Saudi Arabia [46]	The aqueous extract of *Clitoria ternatea* leaves and flower administered for 84 days to diabetic rats significantly decreased blood glucose [46–48]
5.	*Ficus carica*	Moraceae	Leaves	Southwest of Saudi Arabia [49]	Different extracts and fractions of *F. carica* showed a clear hypoglycemic effect in diabetic rats. *F. carica* leaves exerted significant effect on carbohydrate metabolism enzymes with promising hypoglycemic and hypolipidemic activities in type 2 diabetic rats [50, 51]
6.	Ficus benghalensis	Moraceae	Bark	Riyadh [52]	In streptozotocin-induced diabetic rats, bark aqueous extract, and an isolated compound, α-amyrin acetate exhibited antidiabetic activity by decreasing the blood glucose level and increasing the HDL level [53]
7.	*Ficus religiosa*	Moraceae	Root bark, stem bark, aerial roots	Riyadh [52]	The aqueous extract of bark and ethanol extract of leaves and fruits had a promising antidiabetic effect in streptozotocin-induced diabetic rats by decreasing the blood glucose, serum triglyceride, and total cholesterol levels and increasing serum insulin, body weight, and glycogen content in the liver and skeletal muscle [53]
8.	*Ficus microcarpa*	Moraceae	Leaves	Riyadh [52]	*F. microcarpa* leaves showed protective effect against alloxan-induced diabetic rats by reducing blood glucose, cholesterol and triglyceride levels, and increased insulin level [53]
9.	*Hypericum perforatum*	Hypericaceae	Leaves	Western Saudi Arabia [54]	H. perforatum ethyl acetate extract possesses potent antihyperglycemic activity in STZ-induced diabetic rats [55].
10	*Anethum graveolens*	Apiaceae	Seeds	Makka [56]	Different extracts and tablets of *Anethum graveolens* possess potent antihyperglycemic activity in alloxan-induced diabetic mice [57]
11	*Cuminum cyminum* L.	Apiaceae or Umbelliferae	Seeds	Makka [56]	Oral administration of cumin seeds crude ethanol extract and glibenclamide to *diabetic rats* significantly and progressively restored toward normal. Cumin seeds crude ethanol extract and glibenclamide reduced *plasma glucose* levels by 38.34 and 37.73%, respectively, compared with diabetic control [58]. Other studies also reported similar results [59].
12	*Marrubium vulgare*	Lamiaceae	Whole plant	Widely distributed in Saudi Arabia [60]	M. vulgare extracts lower blood glucose level 30 to 60% in dose-dependent manner in streptozotocin-induced diabetic rats [60].

Table 1: Continued.

S. no.	Names of plants	Family	Part used	location	Antidiabetes Activities
13	*Mentha longifolia*	Lamiaceae	Whole plant	Madinah [61]	Remarkable antidiabetic, anticholinesterase, and antityrosinase effects were recorded for the mint oil [61, 62]. Still need to investigate in vivo antidiabetic potential.
14	*Origanum syriacum*	Lamiaceae	Leaves	Saudi Desert [63]	The whole plant extract of *O. syriacum* at 100 and 400 mg/kg significantly lowers glucose level in diabetic induced rats [64].
15	*Teucrium oliverianum*	Lamiaceae	Aerial parts	Throughout Saudi Arabia [65,66]	Aqueous and ethanol extract of Teucrium oliverianum were tested for antidiabetic activity in alloxan-induced diabetic mice. Both extracts significantly reduced blood sugar levels [65]
16	*Teucrium polium*	Lamiaceae	Leaves	Madinah [67]	Infusion orally (64% decrease glucose level) and intraperitoneal of different extracts of *T. polium* caused significant reductions in blood glucose concentration in STZ hyperglycemic rats [68]
17	*Achyranthes aspera*	Amaranthaceae	Whole plant	Al Hada Road Taif [69]	The methanolic and ethanolic extract of A. aspera exhibited significant hypoglycemic activity in streptozotocin-induced diabetic rats [70]
18	*Aerva lanata*	Amaranthaceae	Leaves	Southwest region of Saudi Arabia [71, 72]	Extracts of *Aerva lanata* and glibenclamide were found to significantly ($P < 0.01$ and $P < 0.05$) reduce the blood glucose level and lipid profile in streptozotocin-induced diabetic rats [73]
19	*Alternanthera sessilis*	Amaranthaceae	Whole plant	Hail region, Saudi Arabia [74]	In diabetic mice at doses of 50, 100, 200, and 400 mg per kg body weight, the extract reduced blood sugar levels by 22.9, 30.7, 45.4, and 46.1%, respectively, compared to control animals. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 48.9% [75]
20	*Carissa edulis*	Apocynaceae	Leaves	Southern region of Saudi Arabia [76]	Oral administration of *C. edulis* extracts of the leaves significantly reduced the blood glucose level in STZ diabetic rats [77].
21	*Catharanthus roseus*	Apocynaceae	Flower, leaves, stem, and root	Western Saudi Arabia [78]	C. roseus (100 mg/kg BW) lowered the glucose level more than metformin-treated group (100 mg/kg BW) in STZ-induced hyperglycemia rats. C. roseus 200 mg/kg dose was found to be more effective in reducing fasting blood glucose levels [79]
22	*Rhazya stricta*	Apocynaceae	Leaves, seeds	Middle and western region of Saudi Arabia [80]	Extracts *Rhazya stricta* lowered 37.9% blood glucose level in the streptozotocin-induced diabetic rats. Serum cholesterol and triglyceride levels were significantly ($P < 0.05$) reduced in the treated diabetic group compared to the untreated diabetic group [81]
23	*Calotropis procera*	Asclepiadaceae	Latex	Al-Kharj [82]	Different extracts of *C. procera* at dose of 250 mg/kg were orally administered as single dose per day to diabetes-induced rats for the period of 15 days significantly decreases blood glucose level to the level of standard drug glibenclamide [83]
24	*Opuntia dillenii*	Cactaceae	Fruit	Jazan Region [84]	Researcher observed the significant hypoglycemic activity of Opuntia dillenii extract in streptozotocin-induced diabetic mice and rabbits [85]

Table 1: Continued.

S. no.	Names of plants	Family	Part used	location	Antidiabetes Activities
25	*Opuntia ficus-indica*	Cactaceae	Stem	Jazan Region [84]	Powder and water extract of *O. ficus-indica* significantly (in comparison with control group) returned blood glucose level to the initial level, 180 min after administration in STZ-induced diabetic rats [86]. Many studies confirmed the hypoglycemic activities of *O. ficus-indica* [87]
26	*Capparis decidua*	Capparaceae	Fruits, seeds	Jazan Region [84]	*C. decidua* extracts at dose level of 200 and 800 mg/kg significantly reduce sugar level (in a dose-dependent manner) compared to standard drug in STZ-induced diabetic and normal rats [88].
27	*Beta vulgaris*	Chenopodiaceae	Root bark	North Hejaz and Eastern Najd region of Saudi Arabia [89]	Extract of *B. vulgaris* at does level 50, 100, and 200 mg/kg of significantly reduced sugar level and increased in insulin level (in a dose-dependent manner) in streptozotocin or alloxan-induced diabetic mice [90]. Other researchers also concluded similar finding in STZ-induced diabetic rats [91].
28	*Haloxylon salicornicum Bunge*	Chenopodiaceae	Whole plant	Wadi-Hafr-Al-Batin, Saudi Arabia [92]	Ethanol extract (100 and 200 mg/kg of bw) of *H. salicornicum* (oral administration) exhibited persistent hypoglycemic effects in STZ-induced diabetic rats [93]
29	*Evolvulus alsinoides*	Convolvulaceae	Whole plant	Jazan Region [84]	*E. alsinoides* ethanol extract at dose level (150 mg/kg bw) in normal and streptozotocin-induced diabetic rats leads to hyperglycemia in experimental diabetic rats that decreased utilization of glucose by insulin-dependent pathways [94, 95]
30	*Ipomea aquatica*	Convolvulaceae	Whole plant	Jazan Region [96]	*I. aquatica* ethanol extract at dose level (10, 100, and 1000 μg/ml in streptozotocin-induced diabetic rats significantly ($P < .05$) exhibited the ability to enhance insulin-mediated glucose uptake into 3T3F442A adipocytes cells compared to insulin alone [97]. Another study confirmed that doses (200 mg/kg and 400 mg/kg) reduced blood glucose level, and it was statistically highly significant ($P < 0.001$) in comparison with control group [98].
31	*Citrullus colocynthis*	Cucurbitaceae	Fruits	Jazan Region [84]	1 ml/kg and 2 ml/kg of *C. colocynthis* extract (orally administered) stabilized animal body weight and ameliorated hyperglycemia in a dose- and time-dependent manner in alloxan-induced diabetic rats [99]
32	*Citrullus lanatus*	Cucurbitaceae	Seed	Wadi Lajab, Saudi Arabia [100]	*C. lanatus* seed extract (2, 4 g/kg) treatment significantly lowers glucose level which suggested that *C. lanatus* had antidiabetic property in STZ-induced diabetes mice [101]. Other researcher also concluded similar finding in STZ-induced diabetic rats [102]
33	Coccinia grandis	Cucurbitaceae	Whole plant	Jazan Region [84]	The C. grandis extract (0.75 mg/kg, orally) showed remarkable glycemic effect which confirmed antidiabetic potential in streptozotocin-induced diabetic rats [103].
34	*Jatropha curcas*	Euphorbiaceae	Leaves	Jazan Region [84]	Ethanolic extract of *J. curcas* leaves at doses of (250 and 500 mg ml^{-1} bw by administered orally) reduced glucose level from 219.5 to 116.5 and 237 to 98.8, respectively, in alloxan-induced diabetic rats. The results were comparable to reduction in rats treated with the standard glibenclamide 232–94.5 at 600 μg kg^{-1} [104].

Table 1: Continued.

S. no.	Names of plants	Family	Part used	location	Antidiabetes Activities
35	*Ricinus communis*	Euphorbiaceae	Leaves	Jazan Region [84]	*R. communis* extracts at doses of 300 and 600 mg/kg/BW administered orally caused hyperglycemia in a dose-dependent manner in streptozotocin-induced diabetic rats [105].
36	*Ficus carica*	Moraceae	Leaves	Jazan Region [84]	A review article focusing on antidiabetic potential of *F. carica* confirmed that different extracts and fractions of *F. carica* and different doses significantly reducing hyperglycemia in streptozotocin-induced diabetic rats compared to standard drug [106].
37	*Ficus sycomorus*	Moraceae	Leaves	Jazan Region [84]	Alloxan-induced type 2 diabetic albino Wistar rats treated with 250, 500, and 1000 mg/kg (body weight) of the extract of *F. sycomorus* intraperitoneally reduced glucose level in diabetic rats almost to the normal as compared to diabetic control [107]
38	*Sesamum indicum*	Pedaliaceae	Seeds	Jazan Region [84]	Alloxan-induced diabetic rats treated with 5% and 10% of *Sesamum indicum* seed powder significantly decreased blood glucose and increased insulin levels as compared with the positive (diabetic) control group [108]
39	*Plantago ovata*	Plantaginaceae	Husk	Northern border region of Saudi Arabia [18]	In intravenous administration of alloxan-induced diabetic rabbits glucose level lowering effect observed (time dependent manner) with *P. ovata* husk extract of dose level (300 mg/kg, orally administered) [109]
40	*Polygala erioptera*	Polygalaceae	Aerial part	Jazan Region [84]	0.7 g/kg of *P. erioptera* extract showed significant antidiabetic effect compared to standard drug metformin and glibenclamide in normal and alloxan-induced diabetic rats [110]
41	*Polygonum aviculare* L	Polygonaceae	Aerial parts	Taif Region [111, 112]	Many ethnopharmacological investigations reported its antidiabetic potential but still need to study its in vivo and in vitro antidiabetic potential [113, 114]
42	Ziziphus *spina-christi*	Rhamnaceae	Leaves	Eastern region of Saudi Arabia [84, 112]	The strongest ($P < 0.001$) antidiabetic activity (25.59 and 39.48% after 7 and 15 days, respectively) was found following treatment with dose level of 500 mg/kg of *Z. spina-christi* extract in streptozotocin-induced diabetes mice [115].
43	*Bacopa monnieri*	Scrophulariaceae	Aerial parts	Jazan Region [84]	*B. monnieri* extract at dose level of 50, 100, 200, and 400 mg/*kg* significantly inhibited (33.3, 34.2, 42.1, and 44.2%, respectively) the increase in serum glucose concentration in a dose-dependent manner compared to standard drug [116].
44	*Lycium shawii*	Solanaceae	Aerial parts	Taif Region [112]	The strongest ($P < 0.001$) antidiabetic activity of *L. shawii* extract of 250 and 500 mg/kg bw was found in a dose-dependent manner in streptozotocin-induced diabetes rats [117].
45	*Solanum nigrum*	Solanaceae	Whole plant	Jazan Region [84]	*S. nigrum* extract was given orally in the dose level of 200 and 400 mg/kg/day (7 days) significantly lowering the blood glucose level in fasting compared to standard drug in alloxan-induced diabetic albino Wistar rats [118].

Table 1: Continued.

S. no.	Names of plants	Family	Part used	location	Antidiabetes Activities
46	*Withania somnifera*	Solanaceae	Leaves	Jazan Region [84]	*W. somnifera* extract oral administration at two doses (200 and 400 mg/kg) reduced the blood glucose level significantly ($P < 0.001$) in a dose-dependent manner in streptozotocin-induced diabetes rats. Only WS treatment did not register any significant change in the blood glucose level when compared to citrate control rats [119]. Another study also confirmed similar results in alloxan-induced diabetic rats [120]
47	*Lantana camara*	Verbenaceae	Leaves	Jazan Region [84]	Literature survey showed that *L. camara* leaf extract oral administration (200, 250, and 500 mg/kg of bw) showed antidiabetic potential in alloxan-induced diabetic rats [121]
48	*Peganum harmala*	Zygophyllaceae	Seeds	Taif Region [112]	*P. harmala* seed extract at dose level of (30, 60, and 120 mg/kg, orally administered for four weeks) significantly decreases in blood glucose (in all doses, $P < 0.001$), in comparison with diabetic group [122].
49	*Tribulus terrestris*	Zygophyllaceae	Stem, leaves	Jazan Region [84] Taif Region [112]	*T. terrestris* extract at (2 g/kg body weight) produced protective effect in streptozotocin-induced diabetic rats by inhibiting oxidative stress [123]. T. terrestris L. extract (250 mg/kg of bw orally administered) significantly lowers glucose level to normal compared to standard drug in glucose-loaded normal rabbits [124]
50	*Urtica dioica*	Urticaceae	Leaves	Wild plant, Tanhat, Saudi Arabia [125]	Urtica dioica extract at 100 mg/kg ($P < 0.01$) and 200 mg/kg ($P < 0.001$) significantly decreased serum glucose fructose-induced insulin resistance rats [126]. The aqueous extract of *U. dioica* significantly ($P < 0.001$; 67.92%) reduced the blood glucose level at dose of 300 mg/kg, IP) in streptozotocin-induced diabetes rats [127]

plants around the world. The ethnobotanical information reports about 800 plants that may possess antidiabetic potential [196, 197]. Jeeva and Anlin also reported 177 plants belonging to 156 genera and 76 families used traditionally for antidiabetic treatment [198]. In the Middle East countries, there are 129 plant species still in use in traditional Arabic medicine. This indicates that the medicinal plant species require preservation as well as the ethnobotanical and ethnopharmacological knowledge. The preservation of the herbs is an essential requirement for maintaining traditional Arabic medicine as a medicinal and cultural resource [199]. The selected plant species *H. salicornicum, T. oliverianum, A. cepa, A. herba-alba, Teucrium polium, Sesamum indicum, Z. spina-christi,* and F. religiosa are the native Saudi medicinal plants traditionally used for the treatment of DM [200]. Similarly published data showed that 20 medicinal plants are traditionally used in Tabuk region of Saudi Arabia [201]. *Anisotes trisulcus, Artemisia judaica,* and *Moringa peregrine* are used in Al Khobah village, Saudi Arabia, for DM treatment [202]. *O. europaea* is used in Al Bahah region of KSA for DM treatment [203]. *C. roseus, A. cepa, U. dioica, A. aspera, C. intybus, C. cyminum, F. bengalensis, C. colocynthis,* and *T. polium* are the highly investigated medicinal plants for antidiabetic potential [204–206].

Desiring to contribute to the conservation priorities of traditional medicine knowledge of various medicinal plants native to or cultivated in Saudi Arabia and to make it easy and familiarized with disease treatment, the present compilation was conducted. According to the International Union for Conservation of Nature and the World Wildlife Fund, there about 15,000 medicinal plant species are threatened with extinction from overharvesting and habitat destruction and 20% of their wild resources have already been nearly exhausted with the increasing human population and plant consumption [207]. Each plant species lost due to extinction phenomena could represent not only the loss of healthcare saving cures for special diseases but also the loss of probable primary metabolite liker protein- or vitamin-rich foods [208]. Medicinal plants have been cited as a potential source of heavy metal toxicity to both man and animals. The most common heavy metals implicated in human toxicity include lead, mercury, arsenic, and cadmium, although aluminum and cobalt may also cause

Table 2: Active ingredients and toxicological evaluation of the medicinal plants given in Table 1.

S. No	Names	Active ingredients	Toxicological evaluation
1	*Allium cepa*	Quercetin, N-acetylcysteine, alliuocide, cycloalliin, S-methyl-L-cysteine, S-propyl-L-cysteine, sulfoxide, dimethyl trisulfide, S-methyl-L-cysteine sulfoxide [128]	The animals tested were found healthy with no sign of toxicity up to the dose of 2 500 mg/kg. However, at 5 000 mg/kg, animals were weak and had intense extreme tachycardia and disorientation but no death was recorded. Thus, LD_{50} was more than 5 000 mg/kg [129].
2	*Anthemis herba alba*	Guainalides, eudesmanolide, pseudogua inolides, xanthonolides, flavone, flavonol glycosides, hispidulin, cirsilineol, vicenin-2, schaftoside, isoschaftoside, 5′,4-dihydroxy-6,7,3-trimethoxyflavone, quercetin-3-rutinoside, patuletin 3-rutinoside, patuletin 3-glucoside [130]	The available toxicological investigations have shown generally that *Anthemis herba-alba* is free from toxic effects at the different doses used in the studies [130]
3	*Cichorium intybus*	Chicoric acid, inulin, cichoralexin, cichoriin, esculetin, isochlorogenic acid, chlorogenic acid, caffeic acid, dicaffeoylquinic acid, aesculin, arginine, histidine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, serine, glutamic acid, glycine, alanine, aspartic acid, and proline [44, 45]	There were no treatment-related toxic effects from chicory extract administered orally at 70, 350, or 1000 mg/kg/day. There were no observed adverse effects of chicory extract in these studies [45]
4	*Clitoria ternatea*	Kaempferol, quercetin, myricetin, taxaxerol, tannic acid, 3-monoglucoside, β-sitosterol, delphinidin-3,5-diglucoside, anthoxanthin glucoside, p-hydroxycinnamic acid, kaempferol 3-neohesperidoside, myricetin 3-rutinoside, hexacosanol [48]	Ethanolic extract of aerial parts and root of CT led to lethargy in mice at the doses of 1500 mg/kg and above, orally [31]. Ptosis was seen above 2000 mg/kg dose in mice. Through intraperitoneal route, 2900 mg/kg dose was lethal within 6 hr due to severe CNS depression [47].
5	*Ficus carica*	Over 100 bioactive compounds have been identified in fig such as rutin, arabinose, chlorogenic acid, β-amyrins, syringic acid, β-carotenes, glycosides, β-sitosterols, and xanthotoxol [131]	The rats tested were found healthy with no sign of toxicity up to the dose of 5000, 5500, and 6000 mg/kg. However, at 5 000 mg/kg, animals were weak and had intense extreme tachycardia and disorientation but no death was recorded. Thus, LD_{50} was more than 6000 mg/kg [132]
6	Ficus benghalensis	Leucopelargonidin-3-0-α-L rhamnoside, eucodelphinidin, leucoanthocyanidia, leucoanthocyanin, α-amyrin acetate [53]	In acute toxicity studies, no mortality and signs of toxicity were observed at the dose of 2000 and 5000 mg/kg body weight for aqueous and ethanol extracts, respectively [53]
7	*Ficus religiosa*	Lupeol, β-sitosterol, β-sitosterol-d-glucoside, stigmasterol, lanosterol, campesterol, octacosanol, methyl oleonate, lupen-3-one, bergapten, and bergaptol [53]	Acute toxicity reported up to dose level 2000 mg/kg showed no mortality [53]
8	*Ficus microcarpa*	Polyphenols, organic acids, alkaloids, polysaccharides, megastigmanes, pheophytins, catechin, epicatechin, isovitexin, phenolic acids [53]	The oral administration of a single dose of 2000 mg/kg ethanol or methanol extract of leaves showed no mortality or behavioral alterations in the tested animals [53]
9	*Hypericum perforatum*	Quercitrin, rutin, hypericin, kaempferol, biapigenin, hyperforin [133]	Acute toxicity studies revealed the nontoxic nature of the H. perforatum [55]
10	*Anethum graveolens*	Carvone, α-phellandrene, limonene, dill ether, myristicin coumarins, flavonoids, phenolic acids, steroids [134]	The mice treated with AG of different doses of 1000, 2000, 3000, 4000, and 5000 mg/kg of body showed no toxicity [135]
11	*Cuminum cyminum*	Cuminaldehyde, limonene, α- and β-pinene, 1, 8-cineole, o- and p-cymene, α- and γ-terpinene, safranal, and linalool [58, 59]	The acute lethal toxicity test revealed that cumin crude extract was very safe [58]
12	*Marrubium vulgare*	Furanic labdane diterpenes, marrubenol, marrubiin, ladanein [60]	An acute toxicity study of *M. vulgare* (1 g/kg) extract orally administered at a dose of 1 g/kg body weight to the mice and treated mice showed tachycardia 1 h after intake of the infusion and loss of appetite 3 h after intake of the infusion. In another experiment, a single dose of 2000 mg/kg extract of M. vulgare for an acute toxicity study showed no toxicity [60].

Table 2: Continued.

S. No	Names	Active ingredients	Toxicological evaluation
13	*Mentha longifolia*	Lucenin-1, lucenin-2, camphelinone, camphene, carveol, carvone, carvone oxide, limonene, linalool, menthatriene, menthofuran, menthol, menthone, myrcene, p-cymene, piperitenone, piperitone, sabinene, α-pinene, α-terpinene, α-terpineol, longifone, pulegone, longifoamide-A, longifoamide-B, longiside-A, longiside-B, eugenol, salvianolic acid, eriodictyol-7-rutinoside, apigenin-7-O-glucoside, hypolaetin, longitin, luteolin, etc. [136]	*M. longifolia* extract was safe, and no toxicity or mortality was observed in both the oral (3200 mg/kg) and intraperitoneal (1730 mg/kg) administration in rats. Fourteen days of oral administration of the essential oil (125, 250, 375, and 500 μL/kg) resulted in the reduction of red blood cells and lymphocytes and elevation of neutrophils and monocytes compared with normal animals [136].
14	*Origanum syriacum*	Carvacrol, thymol, thymoquinone [137]	Not available
15	*Teucrium oliverianum*	8-O-acetylharpagide, 12-O-methylteucrolin A, teucrolivin A, eupatorin, teucrolivin B, μ24(S)-stigmasta-5,22,25-trin-3β-ol [66]	Not available
16	*Teucrium polium*	Apigenin, luteolin, rutin, cirsiliol, cirsimaritin, salvigenin, and eupatorin in the roots, aerial parts, and inflorescences, teucardoside, b-sitosterol, stigmasterol, campesterol, brassicasterol, and clerosterol [68]	All rats treated with different concentrations of the total extract of TP were alive during the 14 days of observation. The animals did not show visible signs of acute toxicity. It suggested that the LD50 of the total extract was higher than 8 g/kg [138]
17	*Achyranthes aspera*	Aliphatic acid, betaine, achyranthine, β-ecdysterone, achyranthes saponins A, B, C, D, oleonolic acid, glycosides, triacontanol, E-sitosterol and spinasterol, triacontanol, hydroquinone, eugenol [70]	In acute oral toxicity studies, there was no increase or decrease in any of the parameters studied, in comparison with control animals [139]
18	*Aerva lanata*	Quercetin, betulin, aervine, ervoside, methylervine, aervine, lupeol, kaempferol, aervolanine, aervolanine, ervoside, methylaervine, persinosides A and B, tannic acid, lupeol acetate, benzoic acid, methyl grevillate [140]	The LD_{50} of the extract of AL for oral and IP acute toxicity tests were 22.62 g/kg and 0.432 g/kg, respectively. The extract produced apparent changes in body weights of both male and female rats and increased the weights of lung, brain, and pancreas of female rats while reducing the weight of testes in male rats. Hematological parameters were also altered [72]
19	*Alternanthera sessilis*	Stigmasterol, β-sitosterol, β-carotene, ricinoleic acid, myristic, palmitic, stearic, oleic, and linoleic acids, α-spiraterol, uronic acid, cyclo eucalenol, choline, oleanolic acid, lupeol [141]	The crude extract did not show any toxicity in mice even at the highest dose tested [75]
20	*Carissa edulis*	B-Amyrin, (+)-carissone, 2α-carissanol, 6α-carissanol, dehydrocarissone, pinene, myrcene, limonene, sabanene, rutin, epicatechin gallate, carinol, lariciresinol, β-sitosterol, sitosterol glucoside, stigmasterol glucoside, scopoletin, isofraxidin, pinitol [77]	Lethal effects were not observed after the oral administration of the standardized ethanol extract at doses of 1600, 2900, and 5000 mg/kg. No behavioral changes were observed during the observation period. The oral LD50 of the extract was estimated to be greater than 5000 mg/kg. [142].
21	*Catharanthus roseus*	Vinblastine, vincristine, vindesine, vindeline tabersonine, ajmalicine, vinceine, vineamine, raubasin, reserpine, catharanthine, rosindin [143]	No mortality, but dose level higher than 300 mg of *C. roseus* extract can produce signs of biochemical and histopathological toxicity in liver, kidney, and heart. It is recommended that lower doses than the studied ones should be used for treatment [144]
22	*Rhazya stricta*	Polyneuridine, stemmadenine, strictanol, rhazimine, rhazinilam, rhazimanine, sewarine, vallesiachotamine, tetrahydrosecamine [145]	Daily oral dosing of *R. stricta* extract (0.25 g/kg) for 42 days was not fatal to sheep [145].

Table 2: Continued.

S. No	Names	Active ingredients	Toxicological evaluation
23	*Calotropis procera*	Calotropin, calotoxin, calactin, uscharin, voruscharin, uzarigenin, syriogenin, proceroside, calotropagenin, calotropain enzymes, α-amyrin, β-amyrin, lupeol, β-sitosterol, ursolic acid, calotropin, gigantin, giganteol [146]	2000 mg/kg body weight in single oral administration of aqueous and hydroalcoholic extract did not cause any death after 72 h post-treatment in male and female mice. Daily administration of aqueous extract to male and female Wistar rats during 3 and 6 weeks at the dose of 20 mg/kg/day induced no mortality in either sex [147]. Whoever C. procera is a toxic plant that is avoided by grazing animals. Its latex is used by tribes to poison arrows used for hunting. If in contact with human eye, it could cause ocular toxicity, causing loss of vision and photophobia [146]
24	*Opuntia dillenii*	Betanin, betanidin, kaempferol, kaempferide, quercetin, isorhamnetin, β-sitosterol, C29-5β-sterols, taraxerol, friedelin, methyl linoleate, 7-oxositosterol, 6β-hydroxystigmast-4-ene-3-one, daucosterol, methyl eucomate, eucomic acid [85]	During the oral toxicity study of the crude drug in rats, given doses up to 50 ml/kg exhibited no symptoms of toxicity [85]
25	*Opuntia ficus indica*	Quercetin, isorhamnetin, kaempferol, luteolin, isorhamnetin, isorhamnetin glycosides, gallic acid, coumaric, narcissin, rutin, nicotiflorin, isoquercetin, ferulic acid [87].	In vivo toxicity study suggests that the oral administration of *Opuntia ficus indica* extract at levels up to 2000 mg/kg/day does not cause adverse effects in male and female rats [148].
26	*Capparis decidua*	n-Triacontane, n-pentacosane, β-carotene, n-triacontanol, kaempferol, quercetin, isodulcite, nanocosane, capric acid, glucocapparin, capparine, capparinine, cappariline, codonocarpin, β-sitosterol [88]	The oral administration of *C. decidua* extract (500, 1000, 2000, and 4000 mg/kg) did not provoke any gross behavioral changes or manifestations of toxic symptoms in male rats [149].
27	*Beta vulgaris*	Betaine, betacyanins, betaxanthins, oxalic acid, and ascorbic acid [89]	In acute oral toxicity studies, the BVBF did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg dose, considered relatively safe [150]
28	*Haloxylon salicorlicum Bunge*	Kaempferol, quercetin, betaine chloride, piperidine, anabasine, aldotripiperideine, haloxine, halosaline, oxedrine, tyramine, N-methyltyramine, scopoletin, scopolin, umbelliferone, xanthotoxol, isooxyimperatorin, esculetin, β-sitosterol, ursolic acid, β-amyrin [93].	*H. salicorlicum* extract at doses 0.1, 0.2, 0.3, 0.4, and 0.5 mL/kg orally administered in rats was safe and showed no mortality or adverse effect [92].
29	*Evolvulus alsinoides*	β-Sitosterol, betaine, shankpushpin, evolvine, caffeic acid, 6-methoxy-7-O-β-glucopyranoside coumarin, 2-C-methyl erythritol, kaempferol-7-O-β-glucopyranoside, kaempferol-3-O-β-glucopyranoside, quercetin-3-O-β-glucopyranoside, scopoletin, scopolin [151]	The Evolvulus alsinoides extract did not cause any mortality up to a dose of 1500 mg/kg body weight and no behavioral, neurological, and autonomic profiles and was found to be safe [151].
30	*Ipomea aquatica*	Caffeic acid, chlorogenic acid, quercetin glucoside, quercetin malonyl glucoside, quercetin diglucoside, catechin, isochlorogenic acid A, C, aspartic acid, glycine, alanine and leucine, 7-O-β-D-glucopyranosyldihydroquercetin-3-O-α-D-glucopyranoside [97, 98]	In acute toxicity studies, *I. aquatica* extract was found to be safe up to 2g to 5 g/kg in mice. No mortality or toxic symptoms were observed during the entire duration of the study [152, 153].
31	*Citrullus colocynthis*	Cucurbitane, gallic acid, kaempferol, cucurbitacin A-E, I-L, chlorogenic acid, caffeic acid, colocynthoside A,B,C; choline, almitic acid, stearic acid, linoleic acid, oleic acids, catechin, myricetin, α-tocopherol, γ-tocopherol, β-carotene [153]	*C. colocynthis* plant is safe to use. Studies showed that lethal dose (LD_{50}) to be 200 mg/kg, which indicate that the studied plant is not toxic when comparing the LD_{50} values of most bioactive pharmaceuticals currently used in therapeutics [154]
32	*Citrullus lanatus*	Lycopene, vitamin A, cucurbitacin E, citrulline arginine, glutamine and aspartic acid, pectin, vitamin b-complex and minerals [155, 156]	In acute toxicity study, there was no mortality observed up to the maximum dose level of 2000 mg/kg body weight of the extract after administered orally [102]

Table 2: Continued.

S. No	Names	Active ingredients	Toxicological evaluation
33	Coccinia grandis	Cephalandrol, β-sitosterol, cephalandrins A and B, β-amyrin acetate, lupeol, cucurbitacin B, taraxerone, taraxerol, β-carotene, lycopene, cryptoxanthin, xyloglucan, carotenoids, β-sitosterol, stigma-7-en-3-one, lupeol, β-amyrin, β-sitosterol, taraxerol [157]	The acute toxicity study indicated that treatment of *C. grandis* is safe up to 2 g/kg tested on animal [158].
34	*Jatropha curcas*	Jatrophol, jatropha factor C1, C2, C3,C4, C5, C6, jatropholones A, B, palmarumycin CP1, JC1, JCV2, curcin, curcacycline A, curcain, β-amyrin, β-sitosterol, stigmasterol, friedelin, taraxasterol, diamide, pyrimidine-2,4-dione, nobiletin, tomentin [103]	Many researchers have confirmed that *J. curcas* is highly toxic for animal as well as human. All parts of *J. curcas* are toxic and toxic compound reported from this plant like lectins, curcin, phorbol esters, phytate, protease inhibitors [103]
35	*Ricinus communis*	Rutin, quercetin, gallic acid, ricin, ricin A, kaempferol-3-O-β rutinoside, gentistic acid, linolenic acid, α-pinene, α-thujone, stigmasterol, ricinine, β-sitosterol, lupeol, castor oil [159, 160]	*R. communis* extracts given by oral route were safe up to a dose of 2,000 mg/kg/BW and did not show any mortality and toxic effects in the behavior of the treated animals [104].
36	*Ficus carica*	Ferulic acid, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, psoralen, bergapten, coumarin, oleanolic acid, eugenol, angelicin, germacrene D, menthol, α-pinene, β-pinene [161]	Toxicity of 70% methanolic extract of Ficus carica leaves showed LD_{50} value of brine shrimp assay was 0.158 mg/ml [162]
37	*Ficus sycomorus*	Tannins, saponins, flavones, aglycones, anthraquinone glycosides, and flavonoid glycosides [53]	*F. sycomorus* methanol extract of stem bark is nontoxic up to the dose of 5000 mg/kg [53]
38	*Sesamum indicum*	Thelignans, sesamolin, sesamin, pinoresinol, lariciresinol, α-globulin, β-globulin, triacylglycerols, oleic, linoleic acids, sesamol, γ-tocopherol, 2-furfurylthiol, 2-phenylethylthiol, 2-methoxyphenol, 2-pentylpyridine, vitamin E, quinone, sesangolin [163, 164]	*S. indicum* ethanol extract is safe, up to dose level of 2000 mg/kg in acute toxicity studies in tested animals [165]
39	*Plantago ovata*	Hemicellulose,D-xylose, L-arabinose, D-glucose, D-galactose, and L-rhamnose, 5, 6,8-epiloganic acid, gardoside, plantamajoside [166]	4,5 Gram seed husk one to four times a day soaked in 150 ml of warm water recommend by WHO. Studies confirmed its side effect like bloating, gas, and allergy. No mortality reported [167]
40	*Polygala erioptera*	Helioxanthin [168]. No literature available. Recommended for natural products, isolation, biological and toxicological evaluation.	No literature available. Recommended for pharmacological and toxicological evaluation.
41	*Polygonum aviculare* L	Protocatechuic acid, catechin, myricitrin, epicatechin-3-O-gallate, avicularin, quercetin, juglanin, kaempferol, myricetin 3-0-(3′-0-galloyl-rhamnopyranoside, cinaroside, liquiritin, rutin [169, 170]	No data available
42	Ziziphus *spina-christi*	Jujuboside B1, christinin A, christinin A1 and A2, lotoside II, catechin, epicatechin, kaempferol 3-O-(6-O-rhamnosyl-galactoside), quercetin 7-O-(6-O-rhamnosyl-glucoside), quercetin 3-O-glucoside, kaempferol 3-O-glucoside [171, 172]	Butanol and water extract of Ziziphus *spina-christi* up to 100 mg/kg and 5 g/kg, respectively, in animal model produced no functional or structural disturbances in liver and kidney and no hematological changes [173, 174]
43	*Bacopa monnieri*	Bromine, β-sitosterol, betulinic acid, stigmasterol nicotinine, herpestine, bacosides A, bacopasides (I, II, III, IV, V), pseudojujubogenin glycoside, saponins (A, B, C) [175]	B. monnieri extract at the dose of 5,000 mg/kg did not cause any side effects. Similarly doses of 30, 60, 300, and 1,500 mg/kg given for 270 days did not produce any toxicity in rats [176].
44	*Lycium shawii*	Lyciumate, cyclopentapyrrolidine, imidazole, piperidine, nortropane, tropane, pyrrole, spermine, costunolide, catechin, lyciumaside, emodin, betulinic acid, β-sitosterol glucopyranoside, quercetin, gallic acid, rutin, ρ-coumaric acid, ferulic acid [177, 178]	Reported data revealed that LD_{50} of the *L. shawii* extract was greater than 2000 mg/kg b.w in animal model [179].
45	*Solanum nigrum*	Chlorogenic acid, quercetin, naringenin, solasodine, solamargine, solasonine, α-solanigrine, β-solanigrine, ascorbic acid, nigrumnins I and II [180]	*S. nigrum* extract at a dose of 2000 mg/kg p.o. was safe and showed no changes/alteration in normal behavior in animal model. No mortality was observed [181]

Table 2: Continued.

S. No	Names	Active ingredients	Toxicological evaluation
46	*Withania somnifera*	Withanolides, withaferin, withaferin A, withanone, withanolide A, withanolide IV, withanolide V, withanolide D [182].	LD_{50} *value of W. somnifera* extract in rates was greater than 2000 mg/kg body weight. Compared to the control group in subacute toxicity study, administration of extract did not show any toxicologically significant treatment-related changes in clinical observations, and the toxicological studies revealed that the reasonable doses of W. somnifera are nontoxic and safe [182, 183]
47	*Lantana camara*	Eicosane, squalene, β-ionone, caryophyllene oxide, β-caryophyllene, hexanoic acid, tiglic acid, lantanilic acid, camaric acid, lantadene B, oleanolic acid, lantadene A, lantaninilic acid, lantoic acid, ursolic acid, betulinic acid [184]	*L. camara* extracts at dose level of 2000 mg/kg and 5000 mg.kg body weight in mice and rats, respectively, showed no significant toxic signs or mortality [185, 186]
48	*Peganum harmala*	Harmine, harmaline, harmalol, harman, vasicine and vasicinone, pegamine, acacetin 7-O-rhamnoside, 7-O-6″-O-glucosyl-2 ″-O-(3‴-acetylrhamnosyl) glucoside, 7-0-(2‴-0-rhamnosyl-2″-O-glucosylglucoside), peganone 1 and 2, *p*-cymene, limonene, eugenol, thujico acid, β-cubebene [187–189]	*P. harmala* different doses of different extracts in animal and human clinical studies confirmed that this plant showed side effect like intoxications, abdominal writhing, body tremors, and toxic at high does level causing paralysis, liver degeneration, euphoria, convulsions, nausea, vomiting, hypothermia. However, therapeutic doses have been reported to be safe in a rodent model [189]
49	*Tribulus terrestris*	Naringin, rutin, hyperoside, quercitrin, naringenin, quercetin, hesperetin, kaempferol, apigenin, pyrogallol, gallic acid, catechin, catechol, chlorogenic acid, caffeic acid, vanillic acid, ferulic acid, salicylic acid, ellagic acid, coumaric acid, cinnamic acid [190]	*T. terrestris* extract showed no mortality/or toxicity at a dose up to 1 g kg^{-1} of bw in mice [190]
50	*Urtica dioica*	β-Amyrin, β-sitosterol, stigmasterol, oleanolic acid, ursolic acid, quercetin, rutin, chlorogenic, and 2-O-caffeoyl malic acid expressed as caffeic acid, isoquercetin, kaempferol 3-O-rutinoside [191–193].	*U. dioica* extracts up to dose level of 2000 mg/kg body weight in animal model showed no mortality or changes/alteration in normal behavior [127].

Table 3: Selected antidiabetic phytoconstituents and its targeted metabolic pathway.

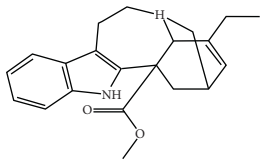
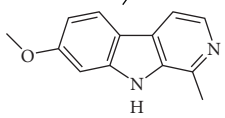
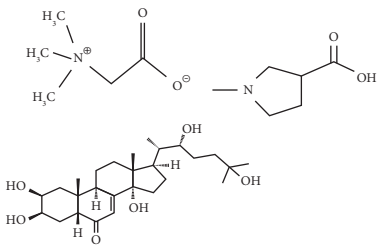
Phytoconstituents	Targeted metabolic pathways	Structures
Catharanthine	Free radical; our body has a defense system containing several enzymes, which are catalase, superoxide dismutase, and glutathione-S transferases and reduced glutathione. Catharanthine activates these free radical scavenging enzymes and prevents our body from their adverse effects.	
Harmine	Insulin secretion and β-cell regeneration	
Betaine Achyranthine β-ecdysone	Carbohydrate digestion and absorption	

Table 3: Continued.

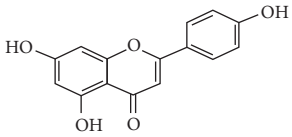
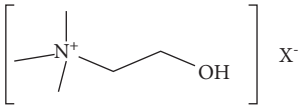
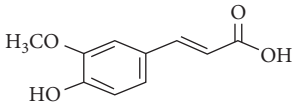
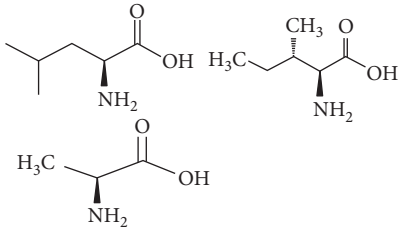
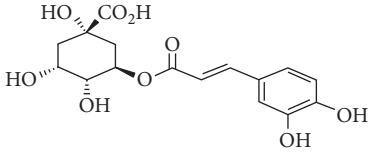
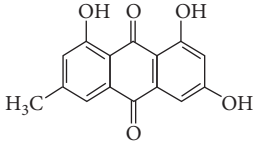
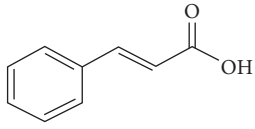
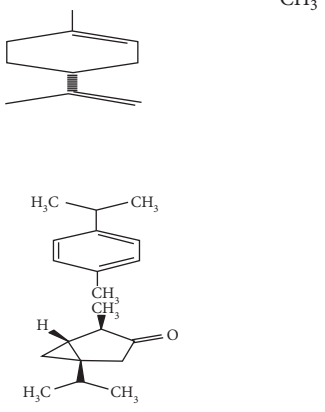
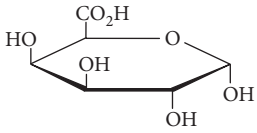
Phytoconstituents	Targeted metabolic pathways	Structures
Apigenin	Cholesterol synthesis, glycogen synthesis	
Betaine choline	Regeneration of pancreatic β cells and insulin secretion	
Ferulic acid	Free radical scavenging activity, insulin secretion	
Leucine, isoleucine, alanine	Insulin secretion	
Chlorogenic acid	Krebs cycle	
Emodin, cinnamic acid	Insulin secretion	 
Eugenol, α-pinene, limonene, p-cymene thujone	Insulin secretion, regeneration of pancreatic β cells	
Pectin	Glucose transport, carbohydrate metabolism, stabilizing agents	

Table 3: Continued.

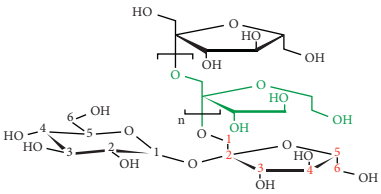
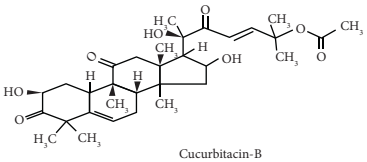
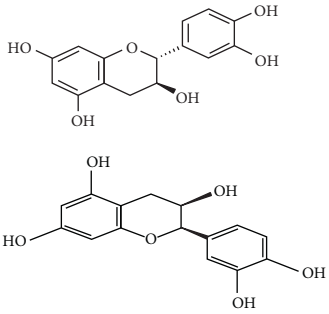
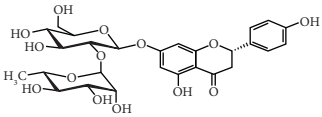
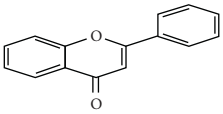
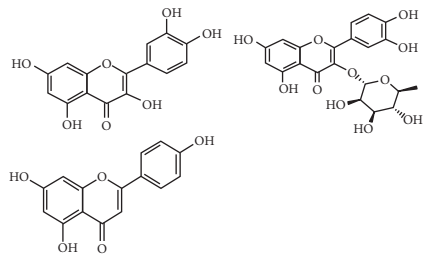
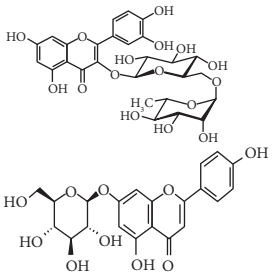
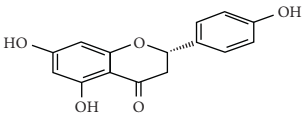
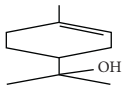
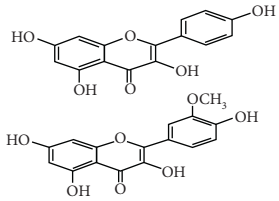
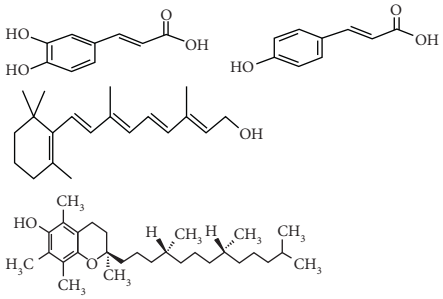
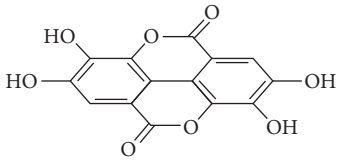
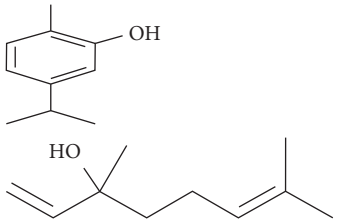
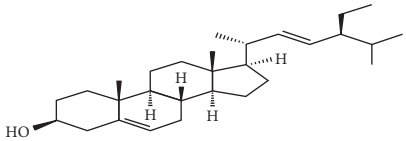
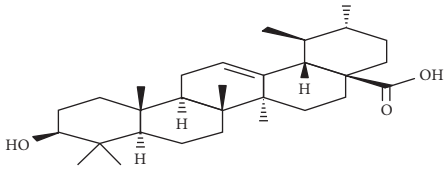
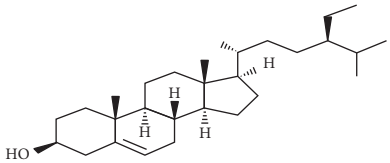
Phytoconstituents	Targeted metabolic pathways	Structures
Inulin	Glucose transport, carbohydrate digestion and absorption	
Cucurbitacin B	Insulin secretion, glycogen synthesis	 Cucurbitacin-B Cucurbitacin-B
Catechin Epicatechin	Scavenging activity Insulinomematic	
Naringin	Glycogen synthesis, glycolysis, gluconeogenesis	
Flavones	Insulin secretion	
Quercetin, quercitrin, apigenin, rutin, apigenin-7-O-glucoside	Insulin secretion	 
Naringenin	Insulin secretion	

Table 3: Continued.

Phytoconstituents	Targeted metabolic pathways	Structures
α-Terpineol	Insulin secretion	
Kaempferol, isorhamnetin, caffeic acid, p-coumaric acid	Free radical scavenging activity Carbohydrate digestion and absorption, insulin secretion	
Vitamin A, E	Free radical scavenging activity	
Ellagic acid	Carbohydrate digestion and absorption, insulin secretion	
Carvacrol, linalool	Insulin secretion, carbohydrate digestion and absorption	
Stigmasterol	Regeneration of pancreatic β cells, insulin secretion	
Ursolic acid	Regeneration of pancreatic β cells and insulin secretion	
β-Sitosterol	Insulin receptor (IR) and glucose transporter 4	

toxicity. From the study, the levels of these metals differed in the same plant collected from different geographical locations. A study conducted showed that the levels of lead in Cassia alata varied from 17.7 to 4.45 μg/g for the 5 collection sites. Similarly for Cassia occidentalis and Rauvolfia vomitoria, the level is varied between 7.85-4.35 and 9.25–1.55 μg/g, respectively. Similarly, that of aluminum varied between 105.53 and 23.3 for Rauvolfia vomitoria and 104.25–12.4 μg/

g for Paullinia pinnata. The levels of heavy metals also varied for different plants collected from the same location. Uptake of metals by plants is influenced by a number of factors including metal concentrations in soils, cation exchange capacity, soil pH, organic matter content, types and varieties of plants, and plant age. However, the prevailing factor is the concentration of the metal in the soil and thus the existing environmental conditions [209]. Another study conducted on onion bulb showed that the concentrations of Cr in onion bulb and Fe in onion leaf were above the permissible level (2.3 mg/kg, 425.5 mg/kg) set by FAO/WHO at Mojo (4.87 mg/kg, 1090.40 mg/kg), Meki (4.13 mg/kg, 1836.47 mg/kg), and Ziway (3.33 mg/kg, 764.33 mg/kg), respectively. The results generally indicate that the consumption of these onion bulbs could be the health risk respective to Cr [210]. Therefore, it is suggested that the medicinal plant source for the treatment of diabetes must not be taken from heavy metal contaminated areas to avoid their uptake by the plants because migration of these contaminants into non-contaminated areas (or leaching through the soil and spreading of heavy metal contaminated sewage sludge) are a few examples of events contributing to contamination of the ecosystem.

5. Conclusion and Recommendations

The present review provides a picture of medicinal plants that have been studied as anti-DM drugs, which can be grown either in combination with other medicinal plants or alone as treatment for diabetes and drawbacks should be properly addressed so that medicinal plants can be effectively utilized as anti-DM drugs. Diabetes is a metabolic disorder which can be considered as a major cause of high economic loss which can in turn impede the development of nations. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the development of research into new hypoglycemic and potentially antidiabetic agents is of great interest. In conclusion, this paper has presented a list of anti-DM plants used in the treatment of diabetes mellitus. Bioactive antidiabetic phytoconstituents which showed that these plants have hypoglycemic effects and highly recommended for further pharmacological purposes and to isolate/identify anti-DM active agents also need to investigate the side effects of active ingredients.

Data Availability

This is a review article. All data are taken from published research papers and available online.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

All three authors contributed equally.

Acknowledgments

The authors wish to thank Research Center College of Pharmacy at King Saud University, Riyadh, Saudi Arabia for their financial support and for providing free access to digital library and laboratory.

References

[1] B. Mahesh and S. Satish, "Antimicrobial activity of some important medicinal plant against plant and human pathogens," *World Journal of Agricultural Sciences*, vol. 4, pp. 839–843, 2008.

[2] B.-E. van Wyk and J. van Staden, "A review of ethnobotanical research in southern Africa," *South African Journal of Botany*, vol. 68, no. 1, pp. 1–13, 2002.

[3] P. Bhojane, S. Damle, A. Thite, and V. Dabholkar, "Antimicrobial effects of some leafy vegetables—a comparative analysis," *International Research Journal of Biological Sciences*, vol. 3, pp. 26–32, 2014.

[4] W. Talib and A. Mahasneh, "Antimicrobial, cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine," *Molecules*, vol. 15, no. 3, pp. 1811–1824, 2010.

[5] H. Rahman, I. Khan, A. Hussain et al., "Glycyrrhiza glabra HPLC fractions: identification of Aldehydo Iso-ophiopogonone and Liquirtigenin having activity against multidrug resistant bacteria," *BMC Complementary and Alternative Medicine*, vol. 18, no. 1, p. 140, 2018.

[6] A. A. Shahat, R. Ullah, A. S. Alqahtani, M. S. Alsaid, H. A. Husseiny, and O. T. R. Meanazel, "Hepatoprotective effect of *Eriobotrya japonica* leaf extract and its various fractions against carbon tetra chloride induced hepatotoxicity in rats," *Evidence-based Complementary and Alternative Medicine*, vol. 2018, Article ID 3782768, 8 pages, 2018.

[7] N. A. A. Ali, M. Wurster, N. Arnold, U. Lindequist, and L. Wessjohan, "Chemical composition of the essential oil of *Teucrium yemense Deflers*," *Records of Natural Products*, vol. 2, no. 2, p. 25, 2012.

[8] D. M. Livermore, "Has the era of untreatable infections arrived?" *Journal of Antimicrobial Chemotherapy*, vol. 64, no. 1, pp. 29–36, 2009.

[9] N. Mohd Azman, M. Gallego, F. Segovia, S. Abdullah, S. Shaarani, and M. Almajano Pablos, "Study of the properties of bearberry leaf extract as a natural antioxidant in model foods," *Antioxidants*, vol. 5, no. 2, p. 11, 2016.

[10] R. Radhakrishnan, M. N. M. Zakaria, M. W. Islam et al., "Analgesic and anti-inflammatory activities of *Teucrium stocksianum*," *Pharmaceutical Biology*, vol. 39, no. 6, pp. 455–459, 2001.

[11] S. Kanwal, N. Ullah, I. U. Haq, I. Afzal, and B. Mirza, "Antioxidant, antitumor activities and phytochemical investigation of *Hedera nepalensis K. Koch*, an important medicinal plant from Pakistan," *Pakistan Journal of Botany*, vol. 43, no. 8, pp. 85–89, 2011.

[12] S. Madhuri and G. Pandey, "Some anticancer medicinal plants of foreign origin," *Current Science*, vol. 96, no. 6, pp. 779–783, 2009.

[13] P. Gauniyal and U. V. S. Teotia, "Antimicrobial activity and phytochemical medicinal plants against oral microorganism," *International Journal of Pharmaceutical and Medicinal Research*, vol. 2, no. 1, pp. 21–27, 2014.

[14] A. C. Goren, G. Bilsel, M. Bilsel, H. Demir, and E. E. Kocabaş, "Analysis of essential oil of Coridothymus

capitatus (L.) and its antibacterial and antifungal activity," *Zeitschrift fur Naturforschung. C, Journal of biosciences*, vol. 58, no. 9-10, pp. 687–690, 2003.

[15] A. J. Afolayan, "Extracts from the shoots of *Arctotis arctotoides* inhibit the growth of bacteria and fungi," *Pharmaceutical Biology*, vol. 41, no. 1, pp. 22–25, 2003.

[16] V. P. Veerapur, K. R. Prabhakar, V. K. Parihar et al., "Ficus racemosaStem bark extract: a potent antioxidant and a probable natural radioprotector," *Evidence-based Complementary and Alternative Medicine*, vol. 6, no. 3, pp. 317–324, 2009.

[17] C. Manach, A. Scalbert, C. Morand, C. Rémésy, and L. Jiménez, "Polyphenols: food sources and bioavailability," *The American journal of clinical nutrition*, vol. 79, no. 5, pp. 727–747, 2004.

[18] A. K. Osman, F. Al-Ghamdi, and A. Bawadekji, "Floristic diversity and vegetation analysis of wadi arar: a typical desert wadi of the northern border region of Saudi Arabia," *Saudi Journal of Biological Sciences*, vol. 21, no. 6, pp. 554–565, 2014.

[19] S. C. Morey, "ADA recommends a lower fasting glucose value in the diagnosis of diabetes mellitus," *American Family Physician*, vol. 56, no. 8, pp. 2128–2130, 1997.

[20] K. Srinivasan and P. Ramarao, "Animal models in type 2 diabetes research: an overview," *Indian Journal of Medical Research*, vol. 125, no. 3, pp. 451–472, 2007.

[21] S. Kazemi, S. Asgary, J. Moshtaghian, M. Rafieian, A. Adelnia, and F. Shamsi, "Liver-protective effects of hydroalcoholic extract of Allium hirtifolium Boiss. In rats with alloxan-induced diabetes mellitus," *ARYA atherosclerosis*, vol. 6, no. 1, pp. 11–15, 2010.

[22] R. Gleckman and J. Morr, "Diabetes-related foot infections," *Contemporary Internal Medicine*, vol. 6, no. 8, pp. 57–64, 1994.

[23] S. Behradmanesh, F. Derees, and M. Rafieian-kopaei, "Effect of Salvia officinalis on diabetic patients," *Journal of Renal Injury Prevention*, vol. 2, no. 2, pp. 51–54, 2013.

[24] J. B. Meigs, "Epidemiology of the metabolic syndrome," *American Journal of Managed Care*, vol. 8, no. 11, pp. S283–S292, 2002.

[25] A. J. Boulton, L. Vileikyte, G. Ragnarson-Tennvall, and J. Apelqvist, "The global burden of diabetic foot disease," *The Lancet*, vol. 366, no. 9498, pp. 1719–1724, 2005.

[26] R. H. Williams and P. R. Larsen, *Textbook of Endocrinology*, Saunders, Philadelphia, PA, USA, 10th edition, 2003.

[27] S. K. Swanston-Flatt, C. Day, C. J. Bailey, and P. R. Flatt, "Evaluation of traditional plant treatments for diabetes: studies in streptozotocin diabetic mice," *Acta Diabetologica Latina*, vol. 26, no. 1, pp. 51–55, 1989.

[28] H. Nasri and M. Rafieian-Kopaei, "Herbal medicine and diabetic kidney disease," *Journal of Nephropharmacology*, vol. 2, no. 1, pp. 1-2, 2013.

[29] M. Eddouks, D. Chattopadhyay, V. De Feo, and W. C.-s. Cho, "Medicinal plants in the prevention and treatment of chronic diseases 2013," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 180981, 3 pages, 2014.

[30] F. Jamila and E. Mostafa, "Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments," *Journal of Ethnopharmacology*, vol. 154, no. 1, pp. 76–87, 2014.

[31] S. Venkatesh, G. D. Reddy, B. M. Reddy, M. Ramesh, and A. V. N. A. Rao, "Antihyperglycemic activity of Caralluma attenuata," *Fitoterapia*, vol. 74, no. 3, pp. 274–279, 2003.

[32] H. Nasri and H. Shirzad, "Toxicity and safety of medicinal plants," *Journal of Herbmed Pharmacology*, vol. 2, no. 2, pp. 21-22, 2013.

[33] M. El-Bab, N. Shawky, A. Al-Sisi, and M. Akhtar, "Retinopathy and risk factors in diabetic patients from Al-Madinah Al-Munawarah in the Kingdom of Saudi Arabia," *Clinical Ophthalmology*, vol. 6, no. 1, pp. 269–276, 2012.

[34] S. N. Alqahtani, S. Omar Alkholy, and M. Pontes Ferreira, "Antidiabetic and anticancer potential of native medicinal plants from Saudi Arabia," *Polyphenols in Human Health and Disease*, vol. 1, pp. 119–132, 2014.

[35] M. Behradmanesh, M. Ahmadi, and M. Rafieian-kopaei, "Effect of glycogol on blood glucose level of patients with type II diabetes," *Iranian Journal of Endocrinology and Metabolism*, vol. 14, no. 2, pp. 163–168, 2012.

[36] M. Abdulrahman, "Al-moshileh effects of planting date and irrigation water level on onion (Allium cepa L.) production under central Saudi arabian conditions," *Scientific Journal of King Faisal University (Basic and Applied Sciences)*, vol. 8, no. 1, pp. 75–85, 2007.

[37] A. E. Baragob, N. A. Al-Wabel, N. A. Ahmed, M. F. Babiker, A. S. Abdalkarim, and M. I. Elboshra, "Study to investigate the pancreatic regeneration and evaluation of the antidiabetic and antihyperlipidemic potential of aerial parts of Allium cepa," *Biochemistry and Biotechnology Research*, vol. 3, pp. 19–29, 2015.

[38] A. E. Ojieh, E. C. Adegor, A. C. Okolo, O. L. Ewhre, I. P. Njoku, and C. U. Onyekpe, "Hypoglycaemic and hypolipidaemic effect of Allium cepa in streptozotocin-induced diabetes," *International Journal of Scientific Engineering and Research*, vol. 6, pp. 23–29, 2015.

[39] M. Imad, T. Eldin, E. M. Ahmed, and H. M. Abd Elwahab, "Preliminary study of the clinical hypoglycemic effects of Allium cepa (red onion) in type 1 and type 2 diabetic patients," *Environmental Health Insights*, vol. 4, pp. 71–77, 2010.

[40] A. Mohammed, S. Syed, M. Y. Syed, and A. D Ali, "Use of herbal extract from Artemisia herba-alba (Shih) in pharmaceutical preparations for dental hygiene," *Saudi Pharmaceutical Journal: the Official Publication of the Saudi Pharmaceutical Society*, vol. 26, pp. 822–828, 2018.

[41] N. E. Awad, A. A. Seida, Z. El-Khayat, N. Shaffie, M. Ahmed, and Abd El-Aziz, "Hypoglycemic activity of Artemisia herba-alba (asso.) used in Egyptian traditional medicine as hypoglycemic remedy," *Journal of Applied Pharmaceutical Science*, vol. 2, no. 3, pp. 30–39, 2012.

[42] E. Pérez-López, A. F. Omar, K. M. Al-Jamhan, and T. J. Dumonceaux, "Molecular identification and characterization of the new 16SrIX-J and cpn60 UT IX-J phytoplasma subgroup associated with chicory bushy stunt disease in Saudi Arabia," *International Journal of Systematic and Evolutionary Microbiology*, vol. 68, no. 2, pp. 518–522, 2018.

[43] R. A. Street, J. Sidana, and G. Prinsloo, "Cichorium intybus: traditional uses, phytochemistry, pharmacology, and toxicology," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 579319, 13 pages, 2013.

[44] D. Kanj, K. Raafat, A. El-Lakany, S. Baydoun, and M. Aboul-Ela, "Phytochemical compounds of cichorium intybus by exploring its antioxidant and antidiabetic activities," *Pharmacognosy Journal*, vol. 11, no. 2, pp. 248–257, 2019.

[45] S. Das, N. Vasudeva, and S. Sharma, "Cichorium intybus: a concise report on its ethnomedicinal, botanical, and phytopharmacological aspects," *Drug Design, Development and Therapy*, vol. 7, pp. 1–12, 2016.

[46] A. E. Al-Snafi, "Pharmacological importance of Clitoria ternatea—a review," *IOSR Journal of Pharmacy*, vol. 6, no. 3, pp. 68–83, 2016.

[47] B. Gollen, J. Mehla, and P. Gupta, "Clitoria ternatea linn: a herb with potential pharmacological activities: future prospects as therapeutic herbal medicine," *Journal of Reports in Pharmaceutical Sciences*, vol. 3, no. 141, pp. 1–8, 2018.

[48] M. B. Lijon, N. S. Meghla, E. Jahedi, M. A. Rahman, and I. Hossain, "Phytochemistry and pharmacological activities of Clitoria ternatea," *International Journal of Natural and Social Sciences*, vol. 4, no. 1, pp. 1–10, 2017.

[49] H. Khemira and M. Mars, "Fig production in subtropical south-western Saudi Arabia," *Acta Horticulturae*, vol. 1173, no. 1173, pp. 169–172, 2017.

[50] K. Y. Khan, M. A. Khan, M. Ahmad et al., "Hypoglycemic potential of genus Ficus L.: a review of ten years of plant based medicine used to cure diabetes (2000–2010)," *Journal of Applied Pharmaceutical Science*, vol. 1, no. 6, pp. 223–227, 2011.

[51] S. Stephen Irudayaraj, S. Christudas, S. Antony, V. Duraipandiyan, A.-D. N. Abdullah, and A.-D. Ignacimuthu, "Protective effects of Ficus carica leaves on glucose and lipids levels, carbohydrate metabolism enzymes and β-cells in type 2 diabetic rats," *Pharmaceutical Biology*, vol. 55, no. 1, pp. 1074–1081, 2017.

[52] L. I. El-Juhany, A. Abdullah, and Al-Harby, "Status and diversity of ornamental plants in king Saud university campus at Riyadh, Saudi Arabia American-eurasian," *Journal of Agriculture and Environmental Sciences*, vol. 13, no. 4, pp. 471–478, 2013.

[53] P. Deepa, K. Sowndhararajan, S. Kim, and S. J. Park, "A role of Ficus species in the management of diabetes mellitus: a review," *Journal of Ethnopharmacology*, vol. 215, pp. 210–232, 2018.

[54] F. Scotti, K. Löbel, A. Booker, and M. Heinrich, "St. John's wort (Hypericum perforatum) products—how variable is the primary material?" *Frontiers of Plant Science*, vol. 9, p. 1973, 2019.

[55] S. Arokiyaraj, R. Balamurugan, and P. Augustian, "Anti-hyperglycemic effect of Hypericum perforatum ethyl acetate extract on streptozotocin-induced diabetic rats," *Asian Pacific Journal of Tropical Biomedicine*, vol. 1, no. 5, pp. 386–390, 2011.

[56] A. Alqethami, J. A. Hawkins, and I. Teixidor-Toneu, "Medicinal plants used by women in Mecca: urban, Muslim and gendered knowledge," *Journal of Ethnobiology and Ethnomedicine*, vol. 13, no. 62, pp. 62–24, 2017.

[57] M. T. Goodarzi, I. Khodadadi, H. Tavilani, and E. Abbasi Oshaghi, "The role of Anethum graveolens L. (Dill) in the management of diabetes," *Journal of Tropical Medicine*, vol. 2016, Article ID 1098916, 11 pages, 2016.

[58] D. A. Mohamed, I. Mohamed Hamed, and K. Aly Fouda, "Antioxidant and anti-diabetic effects of cumin seeds crude ethanol," *Extract Journal of Biological Sciences*, vol. 18, no. 5, pp. 251–259, 2018.

[59] R. P. Singh, H. V. Gangadharappa, and K. Mruthunjaya, "Cuminum cyminum—a popular spice: an updated review," *Pharmacognosy Journal*, vol. 9, no. 3, pp. 292–301, 2017.

[60] S. Lodhi, G. Vadnere, V. K. Sharma, and M. Rageeb Usman, "Marrubium vulgare L.: a review on phytochemical and pharmacological aspects," *Journal of Intercultural Ethnopharmacology*, vol. 6, no. 4, pp. 429–452, 2017.

[61] H. A. S. Murad, H. M. Abdallah, and S. S. Ali, "Mentha longifolia protects against acetic-acid induced colitis in rats," *Journal of Ethnopharmacology*, vol. 190, pp. 354–361, 2016.

[62] B. Asgharia, G. Zenginb, M. Babak Bahadoric, and M. Abbas-Mohammadid, "Leila Dinparast Amylase, glucosidase, tyrosinase, and cholinesterases inhibitory, antioxidant effects, and GC-MS analysis of wild mint (Mentha longifolia var. calliantha) essential oil: a natural remedy," *European Journal of Integrative Medicine*, vol. 22, pp. 44–49, 2018.

[63] M. Eldesouky Zain, Amani Shafeek Awaad, and M. R. Al-Outhman, "Reham mostafa el-meligy antimicrobial activities of Saudi arabian desert plants," *Phytopharmacology*, vol. 2, no. 1, pp. 106–113, 2012.

[64] A.-A. A. Rasheed, A.-Q. T. Musbah, A. M. Sulaiman, S. M. Saed, and B. A. Mohammad, "Short-term feeding effects of origanum syriacum crude extract on blood constituents in rats," *International Journal of Research in Ayurveda and Pharmacy*, vol. 8, no. 1, pp. 118–120, 2017.

[65] N. Fatima, "A Review on *Teucrium oliveranum*, a plant found abundantly in Saudi Arabia," *Science International*, vol. 28, no. 2, pp. 1229–1231, 2016.

[66] A. A. Shahat, M. S. Alsaid, J. A. Khan, M. Higgins, and A. T. Dinkova-Kostova, "Chemical constituents and NAD(P)H:quinone oxidoreductase 1 (NQO1) inducer activity of Teucrium oliverianum Ging. ex Benth," *Indian Journal of Traditional Knowledge*, vol. 15, no. 2, pp. 232–236, 2016.

[67] M. Hossam, G. A. M. Abdallah, M. A. Farag, K. Z. Alshali, E. A. Alsherif, and S. A. Ross, "Volatile oil profile of some lamiaceous plants growing in Saudi Arabia and their biological activities," *Zeitschrift für Naturforschung C*, vol. 72, no. 1-2, pp. 35–41, 2017.

[68] S. Bahramikia and R. Yazdanparast, "phytochemistry and medicinal properties of Teucrium polium L. (Lamiaceae)," *Phytotherapy Research*, vol. 26, no. 11, pp. 1581–1593, 2012.

[69] H. M. Abdallah, A. B. Abdel-Naimc, O. M. Ashourd, I. A. Shehata, and E. A. Abdel-Sattar, "Anti-infl ammatory activity of selected plants from Saudi Arabia Z," *Naturforscher*, vol. 69c, pp. 1–9, 2014.

[70] R. Vijayaraj and R. Vidhya, "Biological activity of Achyranthes aspera linn.—a review," *Asian Journal of Biochemical and Pharmaceutical Research*, vol. 69, no. 1, pp. 2231–2560, 2016.

[71] M. A. Rahman, S. M. Jaber, M. S. Al-Said, and M. A. Al-Yahya, "Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families," *Fitoterapia*, vol. 75, pp. 149–161, 2004.

[72] K. S. Omotoso, F. R. Aigbe, O. A. Salako, M. C. Chijioke, and O. O. Adeyem, "Toxicological evaluation of the aqueous whole plant extract of Aerva lanata (l.) Juss. ex Schult (Amaranthaceae)," *Journal of Ethnopharmacology*, vol. 208, pp. 174–184, 2017.

[73] R. Rajesh, K. Chitra, and P. M. Paarakh, "Anti hyperglycemic and antihyperlipidemic activity of aerial parts of Aerva lanata Linn Juss in streptozotocin induced diabetic rats," *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 2, pp. S924–S929, 2012.

[74] W. M. El-Ghanim, L. M. Hassan, T. M. Galal, and A. Badr, "Floristic composition and vegetation analysis in Hail region north of central Saudi Arabia," *Saudi Journal of Biological Sciences*, vol. 17, no. 2, pp. 119–128, 2010.

[75] A. I. Hossain, M. Faisal, S. Rahman, R. Jahan, and M. Rahmatullah, "A preliminary evaluation of

antihyperglycemic and analgesic activity of Alternanthera sessilis aerial parts," *BMC Complementary and Alternative Medicine*, vol. 14, no. 169, pp. 1–5, 2014.

[76] H. Al-Youssef and W. Hassan, "Chemical constituents of carissa edulis vahl," *Arabian Journal of Chemistry*, vol. 10, pp. 109–113, 2017.

[77] J. S. Kaunda and Y.-J. Zhang, "The genus carissa: an ethnopharmacological, phytochemical and pharmacological review," *Natural Products and Bioprospecting*, vol. 7, pp. 181–199, 2017.

[78] E. K. F. Elbeshehy, H. S. M. AL-Zahrani, A. Y. Aldhebiani, and T. Elbeaino, "Viruses infecting periwinkle (catharanthus roseus L.) in western Saudi Arabia phytopathologia mediterranea," vol. 56, no. 3, pp. 479–485, 2017.

[79] W. M. Al-Shaqha, M. Khan, N. Salam, A. Azzi, and A. A. Chaudhary, "Anti-diabetic potential of Catharanthus roseus Linn. and its effect on the glucose transport gene (GLUT-2 and GLUT-4) in streptozotocin induced diabetic wistar rats," *BMC Complementary and Alternative Medicine*, vol. 15, no. 379, pp. 1–8, 2015.

[80] N. A. Bukhari, R. A. Al-Otaibi, and M. M. Ibhrahim, "Phytochemical and taxonomic evaluation of Rhazya stricta in Saudi Arabia," *Saudi Journal of Biological Sciences*, vol. 24, no. 7, pp. 1513–1521, 2017.

[81] N. Menon, J. Sparks, and F. Omoruyi, "Hypoglycemic and hypocholesterolemic activities of the aqueous preparation of Kalanchoe pinnata leaves in streptozotocin-induced diabetic rats," *Asian Pacific Journal of Tropical Biomedicine*, vol. 5, no. 1, pp. 3–9, 2015.

[82] A. A. Awaad, H. F. Alkanhal, R. M. El-Meligy et al., "Anti-ulcerative colitis activity of Calotropis procera Linn," *Saudi Pharmaceutical Journal*, vol. 26, no. 1, pp. 75–78, 2018.

[83] K. Bhagour, D. Arya, and R. S. Gupta, "A review: anti-hyperglycemic plant medicines in management of diabetes," *Acupuncture and Related Therapies*, vol. 4, no. 4, pp. 7–16, 2016.

[84] A. H. Alfarhan, T. A. Al-Turki and A. Y. Basahy, Flora of jizan region," Final Report Volume-1, 2005.

[85] C. Sharma, S. Rani, B. Kumar, A. Kumar, and V. Raj, "Plant opuntia dillenii: a review on its traditional uses, phytochemical and pharmacological properties," *EC Pharmaceutical Science*, vol. 1, no. 1, pp. 29–43, 2015.

[86] S. H. Hwang, I. J. Kang, and S. S. Lim, "Antidiabetic effect of fresh nopal (Opuntia ficus-indica) in low-dose streptozotocin-induced diabetic rats fed a high-fat diet," *Evidence-based Complementary and Alternative Medicine*, vol. 2017, Article ID 4380721, 8 pages, 2017.

[87] K. El-Mostafa, Y. El Kharrassi, A. Badreddine et al., "Nopal cactus (Opuntia ficus-indica) as a source of bioactive compounds for nutrition, health and disease," *Molecules*, vol. 19, no. 9, pp. 14879–14901, 2014.

[88] T. Gull, F. Anwar, B. Sultana, M. A. C. Alcayde, and W. Nouman, "Capparis species: a potential source of bioactives and high-value components: a review," *Industrial Crops and Products*, vol. 67, pp. 81–96, 2015.

[89] A. K. Al-Asmari, A. M. Al-Elaiwi, M. T. Athar, M. Tariq, A. Al Eid, and S. M. Al-Asmary, "A review of hepatoprotective plants used in Saudi traditional medicine," *Evidence-based Complementary and Alternative Medicine*, vol. 2014, Article ID 890842, 22 pages, 2014.

[90] A. U. Kabir, M. B. Samad, A. Ahmed et al., "Aqueous fraction of Beta vulgaris ameliorates hyperglycemia in diabetic mice due to enhanced glucose stimulated insulin secretion, mediated by acetylcholine and GLP-1, and elevated glucose uptake via increased membrane bound GLUT4 transporters," *PLoS One*, vol. 10, no. 2, Article ID e0116546, 2015.

[91] S. Gezginci-Oktayoglu, O. Sacan, S. Bolkent et al., "Chard (Beta vulgaris L. var. cicla) extract ameliorates hyperglycemia by increasing GLUT2 through Akt2 and antioxidant defense in the liver of rats," *Acta Histochemica*, vol. 116, no. 1, pp. 32–39, 2014.

[92] R. Ullah, M. S. Alsaid, A. A. Shahat et al., "Antioxidant and hepatoprotective effects of methanolic extracts of Zilla spinosa and Hammada elegans against carbon tetrachlorideinduced hepatotoxicity in rats," *Open Chemistry*, vol. 16, no. 1, pp. 133–140, 2018.

[93] J. P. Singh, V. S. Rathore, and M. M. Roy, "Notes about Haloxylon salicornicum (Moq.) Bunge ex Boiss., a promising shrub for arid regions," *Genetic Resources and Crop Evolution*, vol. 62, no. 3, pp. 451–463, 2015.

[94] G. Duraisamy, R. Ganesan, K. Manokaran, D. Kanakasabapathi, and U. Chandrasekar, "Protective effect of the whole plant extract of Evolvulus alsinoides on glycoprotein alterations in streptozotocin induced diabetic rats," *Journal of Acute Disease*, vol. 2, no. 2, pp. 148–150, 2013.

[95] P. Sundaramurthi and K. K. Packiam, "A review on pharmacognosy and pharmacology of Evolvulus alsinoides (l.) L," *International Research Journal of Pharmacy*, vol. 8, no. 7, pp. 1–4, 2017.

[96] Y. M. Al-Sodany, "A new record to the flora of Saudi Arabia: Ipomoea carneaJacq., Convolvulaceae," *World Journal of Research and Review*, vol. 3, no. 4, pp. 25–30, 2016.

[97] A. A. Sajak, A. Mediani, N. S. Dom et al., "Effect of Ipomoea aquatica ethanolic extract in streptozotocin (STZ) induced diabetic rats via1H NMR-based metabolomics approach," *Phytomedicine*, vol. 36, pp. 201–209, 2017.

[98] K. Hamid, M. O. Ullah, S. Sultana et al., "Evaluation of the leaves of Ipomoea aquatica for its hypoglycemic and antioxidant activity," *Journal of Pharmaceutical Sciences and Research*, vol. 3, no. 7, pp. 1330–1333, 2011.

[99] A. Amin, M. Tahir, and K. P. Lone, "Effect of Citrullus colocynthis aqueous seed extract on beta cell regeneration and intra-islet vasculature in alloxan induced diabetic male albino rats," *Journal of Pakistan Medical Association*, vol. 67, no. 5, pp. 715–721, 2017.

[100] Y. S. Masrahi, M. Remesh, and O. H. Sayed, "Enumeration of the flora of wadi lajab Saudi Arabia," *Ecology Environment and Conservation*, vol. 23, no. 1, pp. 115–123, 2017.

[101] F. Wang, H. Li, H. Zhao et al., "Antidiabetic activity and chemical composition of sanbai melon seed oil," *Evidence-based Complementary and Alternative Medicine*, vol. 2018, Article ID 5434156, 14 pages, 2018.

[102] S. Varghese, R. Narmadha, D. Gomathi, M. Kalaiselvi, and K. Devaki, "Evaluation of hypoglycemic effect of ethanolic seed extracts of Citrullus lanatus," *Journal of Phytopharmacology*, vol. 2, pp. 31–40, 2013.

[103] A. P. Attanayake, K. A. Jayatilaka, C. Pathirana, and L. K. Mudduwa, "Antihyperglycemic activity of *Coccinia grandis* (L.) Voigt in streptozotocin induced diabetic rats," *Indian journal of traditonal knowledge*, vol. 14, no. 3, pp. 376–381, 2015.

[104] H. A. Abdelgadir and J. Van Staden, "Ethnobotany, ethnopharmacology and toxicity of Jatropha curcas L.(Euphorbiaceae): a review," *South African Journal of Botany*, vol. 88, pp. 204–218, 2013.

[105] M. A. Gad-Elkareem, E. H. Abdelgadir, O. M. Badawy, and A. Kadri, "Potential antidiabetic effect of ethanolic and

aqueous-ethanolic extracts of Ricinus communis leaves on streptozotocin-induced diabetes in rats," *PeerJ*, vol. 7, pp. 1–15, Article ID e6441, 2019.

[106] K. Y. Khan, M. A. Khan, M. Ahmad et al., "Hypoglycemic potential of genus Ficus L.: a review of ten years of plant based medicine used to cure diabetes (2000–2010)," *Journal of Applied Pharmaceutical Science*, vol. 1, no. 6, pp. 223–227, 2011.

[107] O. A. Adoum, B. O. Micheal, and I. S. Mohammad, "Phytochemicals and hypoglycaemic effect of methanol stem-bark extract of Ficus sycomorus Linn (Moraceae) on alloxan induced diabetic Wistar albino rats," *African Journal of Biotechnology*, vol. 11, no. 17, pp. 4095–4097, 2012.

[108] T. A. Ibrahiem, "Beneficial effects of diet supplementation with Nigella sativa (Black Seed) and sesame seeds in Alloxan-Diabetic Rats," *International Journal of Current Microbiology and Applied Sciences*, vol. 5, pp. 411–423, 2016.

[109] R. Diez, J. J. García, M. J. Diez, M. Sierra, A. M. Sahagun, and N. Fernandez, "Influence of Plantago ovata husk (dietary fiber) on the bioavailability and other pharmacokinetic parameters of metformin in diabetic rabbits," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, p. 298, 2017.

[110] G. Sammaiah and R. S. Srivastava, "Hypoglycemic activity of Polygala erioptera (Whole plant) in normal and alloxan induced diabetic rats," *Asian Journal of Chemistry*, vol. 20, no. 1, pp. 107–112, 2008.

[111] H. M. Al-Hazimi and S. N. Haque, "A new naphthoquinone from Polygonum aviculare," *Natural Product Letters*, vol. 16, no. 2, pp. 115–118, 2002.

[112] Y. M. Al-Sodany, A. B. Salih, and H. A. Mosallam, "Medicinal plants in Saudi Arabia: I. Sarrwat mountains at taif, KSA," *Academic Journal of Plant Sciences*, vol. 6, pp. 134–145, 2013.

[113] B. Baharvand-Ahmadi, M. Bahmani, P. Tajeddini, N. Naghdi, and M. Rafieian-Kopaei, "An ethno-medicinal study of medicinal plants used for the treatment of diabetes," *Journal of nephropathology*, vol. 5, no. 1, pp. 44–50, 2015.

[114] M. Bahmani, A. Zargaran, M. Rafieian-Kopaei, and K. Saki, "Ethnobotanical study of medicinal plants used in the management of diabetes mellitus in the Urmia, Northwest Iran," *Asian Pacific journal of tropical medicine*, vol. 7, pp. S348–S354, 2014.

[115] A. A. Al-Ghamdi and A. A. Shahat, "Antioxidant, hypoglycemic and anti-diabetic activities of Ziziphus spina-christi (L) Willd (Rhamnacae) leaf extract," *Tropical Journal of Pharmaceutical Research*, vol. 16, no. 11, pp. 2601–2610, 2017.

[116] T. Inin, M. Mohsina, and R. Mohammed, "Bacopa monnieri: an evaluation of antihyperglycemic and antinociceptive potential of methanolic extract of whole plants," *Pakistan journal of pharmaceutical sciences*, vol. 28, no. 6, pp. 2135–2139, 2015.

[117] H. Sher and M. N. Alyemeni, "Evaluation of anti-diabetic activity and toxic potential of Lycium shawii in animal models," *Journal of Medicinal Plants Research*, vol. 5, no. 15, pp. 3387–3395, 2011.

[118] M. S. Umamageswari, T. M. Karthikeyan, and Y. A. Maniyar, "Antidiabetic activity of aqueous extract of *Solanum nigrum* linn berries in alloxan induced diabetic wistar albino rats," *Journal of Clinical and Diagnostic Research: Journal of Clinical and Diagnostic Research*, vol. 11, no. 7, pp. FC16–FC19, 2017.

[119] T. Anwer, M. Sharma, K. K. Pillai, and M. Iqbal, "Effect of withania somnifera on insulin sensitivity in non-insulin-dependent diabetes mellitus rats," *Basic and Clinical Pharmacology and Toxicology*, vol. 102, no. 6, pp. 498–503, 2008.

[120] R. Udayakumar, S. Kasthurirengan, T. S. Mariashibu et al., "Hypoglycaemic and hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats," *International Journal of Molecular Sciences*, vol. 10, no. 5, pp. 2367–2382, 2009.

[121] M. Saxena, J. Saxena, and S. Khare, "A brief review on: therapeutical values of Lantana camara plant," *International Journal of Pharmacy & Life Sciences*, vol. 3, no. 3, pp. 1551–1554, 2012.

[122] G. Komeili, M. Hashemi, and M. Bameri-Niafar, "Evaluation of antidiabetic and antihyperlipidemic effects of peganum harmala seeds in diabetic rats," *Cholesterol*, vol. 2016, Article ID 7389864, 6 pages, 2016.

[123] S. Chhatre, T. Nesari, G. Somani, D. Kanchan, and S. Sathaye, "Phytopharmacological overview of Tribulus terrestris," *Pharmacognosy Reviews*, vol. 8, no. 15, p. 45, 2014.

[124] A. El-Shaibany, A. H. Molham, B. Al-Tahami, and S. Al-Massarani, "Anti-hyperglycaemic activity of Tribulus terrestris L aerial part extract in glucose-loaded normal rabbits," *Tropical Journal of Pharmaceutical Research*, vol. 14, no. 12, pp. 2263–2268, 2015.

[125] A. A. Shahat, A. Y. Ibrahim, E. Ezzeldin, and M. S. Alsaid, "Acetylcholinesterase inhibition and antioxidant activity of some medicinal plants for treating neuro degenarative disease," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 12, no. 3, pp. 97–103, 2015.

[126] A. Ahangarpour, M. Mohammadian, and M. Dianat, "Antidiabetic effect of hydroalcholic Urtica dioica leaf extract in male rats with fructose-induced insulin resistance," *Iranian Journal of Medical Sciences*, vol. 37, no. 3, pp. 181–186, 2012.

[127] S. A. Dar, F. A. Ganai, A. R. Yousuf, M. U. Balkhi, T. M. Bhat, and P. Sharma, "Pharmacological and toxicological evaluation of *Urtica dioica*," *Pharmaceutical Biology*, vol. 51, no. 2, pp. 170–180, 2013.

[128] M. Marrelli, V. Amodeo, G. Statti, and F. Conforti, "Biological properties and bioactive components of Allium cepa L.: focus on potential benefits in the treatment of obesity and related comorbidities," *Molecules*, vol. 24, no. 119, pp. 1–18, 2019.

[129] O. BashirLawal, K. Shittu, F. I. Oibiokpa, H. Mohammed, S. I. Umar, and G. M. Haruna, "Antimicrobial evaluation, acute and sub-acute toxicity studies of Allium sativum," *Journal of Acute Disease*, vol. 5, no. 4, pp. 296–301, 2016.

[130] A. Moufid and M. Eddouks, "Artemisia herba alba: A popular plant with potential medicinal propertie," *Pakistan Journal of Biological Sciences*, vol. 15, no. 24, pp. 1152–1159, 2012.

[131] G. Gani, T. Fatima, T. Qadri, N. J. Beenish, and O. Bashir, "Phytochemistry and pharmacological activities of fig (Ficus carica)," *A review nternational Journal of Research in Pharmacy and Pharmaceutical Sciences*, vol. 3, no. 2, pp. 80–82, 2018.

[132] G. E. Odo, J. E. Agwu, N. Newze et al., "Toxicity and effects of fig (Ficus carica) leaf aqueous extract on haematology and some biochemical indices of wistar albino rats (Rattus norvegicus)," *Journal of Medicinal Plants Research*, vol. 10, no. 22, pp. 298–305, 2016.

[133] A. I. Oliveira, C. Pinho, B. Sarmento, and A. C. P. Dias, "Neuroprotective activity of Hypericum perforatum and its major components," *Frontiers of Plant Science*, vol. 7, p. 1004, 2016.

[134] S. Jana and G. S. Shekhawat, "Anethum graveolens: an Indian traditional medicinal herb and spice," *Pharmacognosy Reviews*, vol. 4, no. 8, pp. 179–184, 2010.

[135] N. D. Swati and K. P. Dipak, "Acute and sub chronic toxicoloigcal evaluation of anethum graveolens seed extract," *Journal of Global bioseciences*, vol. 5, no. 6, pp. 4213–4220, 2016.

[136] M. H. Farzaei, R. Bahramsoltani, G. Ali, and F. Farzaei, "Fariba najafi pharmacological activity of mentha longifolia and its phytoconstituents," *Journal of Traditional Chinese Medicine*, vol. 37, no. 5, pp. 710–720, 2017.

[137] B. Lukas, C. Schmiderer, C. Franz, and J. Novak, "Composition of essential oil compounds from different Syrian populations of origanum syriacum L. (Lamiaceae)," *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 1362–1365, 2009.

[138] H. R. Rasekh, H. Yazdanpanah, and L. Hosseinzadeh, "Narges bazmohammadi and mohammad kamalinejad acute and subchronic toxicity of Teucrium polium total extract in rats," *Iranian Journal of Pharmaceutical Research*, vol. 4, pp. 245–249, 2005.

[139] C. C. Barua, A. Talukdar, S. Ara Begum et al., "In vivo wound-healing efficacy and antioxidant activity of Achyranthes aspera in experimental burns," *Pharmaceutical Biology*, vol. 50, no. 7, pp. 892–899, 2012.

[140] B. M. Dinnimath, S. S. Jalalpure, and U. K. Patil, "Anti-urolithiatic activity of natural constituents isolated from Aerva lanata," *Journal of Ayurveda and Integrative Medicine*, vol. 8, pp. 226–233, 2017.

[141] R. Sivakumar and D. Sunmathi, "Phytochemical screening and antimicrobial activity of ethanolic leaf extract of alternanthera sessilis (l.) r.br. ex dc and alternanthera philoxeroides (MART.) griseb," *European Journal of Pharmaceutical and Medical Research*, vol. 3, no. 3, pp. 409–412, 2016.

[142] J. Ya'u, B. A. Chindo, A. H. Yaro, S. E. Okhale, J. A. Anuka, and I. M. Hussaini, "Safety assessment of the standardized extract of Carissa edulis root bark in rats," *Journal of Ethnopharmacology*, vol. 147, no. 3, pp. 653–661, 2013.

[143] S. Das and A. B. Sharangi, "Madagascar periwinkle (Catharanthus roseus L.): diverse medicinal and therapeutic benefits to humankind," *Journal of Pharmacognosy and Phytochemistry*, vol. 6, no. 5, pp. 1695–1701, 2017.

[144] V. R. Vutukuri, M. C. Das, M. Reddy, S. Prabodh, and P. Sunethri, "Evaluation of acute oral toxicity of ethanol leaves extract of *Catharanthus roseus* in wistar albino rats," *Journal of Clinical and Diagnostic Research*, vol. 11, no. 3, pp. FF01–FF04, 2017.

[145] S. A. Gilani, A. Kikuchi, Z. Khan Shinwari, Z. I. Khattak, and N. Kazuo, "Watanabe phytochemical, pharmacological and ethnobotanical studies of rhazya stricta decne," *Phytotherapy Research*, vol. 21, pp. 301–307, 2007.

[146] G. Parihar and N. Balekar, "Calotropis procera: a phytochemical and pharmacological review," *Thai Journal of Pharmaceutical Sciences*, vol. 40, no. 3, 2016.

[147] G. G. Ouedraogo, M. Ouedraogo, A. Lamien-Sanou, M. Lompo, O. M. Goumbri-Lompo, and P. I. Guissou, "Acute and subchronic toxicity studies of roots barks extracts of "calotropis procera" (ait.) R. Br used in the treatment of sickle cell disease in Burkina Faso," *British Journal of Pharmacology and Toxicology*, vol. 4, no. 5, pp. 194–200, 2013.

[148] E. H. Han, M. K. Lim, S. H. Lee, M. M. Rahman, and Y. H. Lim, "An oral toxicity test in rats and a genotoxicity study of extracts from the stems of Opuntia ficus-indica var. saboten," *BMC Complementary and Alternative Medicine*, vol. 19, no. 1, p. 31, 2019.

[149] P. K. Dhakad, P. K. Sharma, and S. Kumar, "Effect of hydroalcoholic extract of Capparis decidua (forssk.) edgew on sexual behavior of male rats," *Advances in Pharmacology and Pharmacy*, vol. 6, no. 2, pp. 50–56, 2018.

[150] N. K. Jain and A. K. Singhai, "Protective role of Beta vulgaris L. leaves extract and fractions on ethanol-mediated hepatic toxicity," *Acta Poloniae Pharmaceutica*, vol. 69, no. 5, pp. 945–950, 2012.

[151] T. R. Chander and Y. N. Reddy, "Evaluation of hepatoprotective and antihepatotoxic activity of ethanolic extract of evolvulus alsinoides linn on CCL4 induced rats," *Asian Journal of Pharmacology and Toxicology*, vol. 2, no. 4, pp. 1–6, 2014.

[152] S. S. Alkiyumi, M. A. Abdullah, A. S. Alrashdi, S. M. Salama, S. I. Abdelwahab, and A. H. Hadi, "Ipomoea aquatica extract shows protective action against thioacetamide-induced hepatotoxicity," *Molecules*, vol. 17, no. 5, pp. 6146–6155, 2012.

[153] M. J. Khan, V. Saini, V. S. Bhati, M. S. Karchuli, and M. B. Kasturi, "Anxiolytic activity of Ipomoea aquatica leaves," *European Journal of Experimental Biology*, vol. 1, pp. 63–70, 2011.

[154] A. I. Hussain, H. A. Rathore, M. Z. Sattar, S. A. Chatha, S. D. Sarker, and A. H. Gilani, "Citrullus colocynthis (L.) Schrad (bitter apple fruit): a review of its phytochemistry, pharmacology, traditional uses and nutritional potential," *Journal of Ethnopharmacology*, vol. 155, no. 1, pp. 54–66, 2014.

[155] R. S. Rajasree, P. I. Sibi, F. Francis, and H. William, "Phytochemicals of Cucurbitaceae family—a review," *International Journal of Pharmacognosy and Phytochemical Research*, vol. 8, pp. 113–123, 2016.

[156] C. D. Deshmukh, A. Jain, and M. S. Tambe, "Phytochemical and pharmacological profile of Citrullus lanatus (THUNB)," *The Biologist*, vol. 3, no. 2, pp. 483–488, 2015.

[157] S. Pekamwar, T. Kalyankar, and S. Kokate, "Pharmacological activities of Coccinia grandis," *Journal of Applied Pharmaceutical Science*, vol. 3, pp. 114–119, 2013.

[158] A. P. Attanayake, K. A. Jayatilaka, C. Pathirana, and L. K. Mudduwa, "Efficacy and toxicological evaluation of Coccinia grandis (Cucurbitaceae) extract in male Wistar rats," *Asian Pacific Journal of Tropical Disease*, vol. 3, no. 6, pp. 460–466, 2013.

[159] S. Khan Marwat, E. A. Khan, M. S. Baloch et al., "Ricinus cmmunis: ethnomedicinal uses and pharmacological activities," *Pakistan Journal of Pharmaceutical Sciences*, vol. 30, no. 5, pp. 1815–1827, 2017.

[160] W. M. Abdul, N. H. Hajrah, J. S. Sabir et al., "Therapeutic role of Ricinus communis L. and its bioactive compounds in disease prevention and treatment," *Asian Pacific Journal of Tropical Medicine*, vol. 11, no. 3, pp. 177–185, 2018.

[161] S. Mawa, K. Husain, and I. Jantan, "Ficus carica L.(Moraceae): phytochemistry, traditional uses and biological activities," *Evidence-based Complementary and Alternative Medicine*, vol. 2013, Article ID 974256, 8 pages, 2013.

[162] S. Allahyari, A. Delazar, and M. Najafi, "Evaluation of general toxicity, anti-oxidant activity and effects of Ficus carica leaves extract on ischemia/reperfusion injuries in isolated heart of rat," *Advanced Pharmaceutical Bulletin*, vol. 4, no. 2, pp. 577–582, 2014.

[163] H. Raeisi-Dehkordi, M. Mohammadi, F. Moghtaderi, and A. Salehi-Abargouei, "Do sesame seed and its products affect body weight and composition? A systematic review and meta-analysis of controlled clinical trials," *Journal of Functional Foods*, vol. 49, pp. 324–332, 2018.

[164] S. Miraj and S. Kiani, "Bioactivity of Sesamum indicum: a review study," *Der Pharmacia Lettre*, vol. 8, no. 6, pp. 328–334, 2016.

[165] A. Ruckmani, V. Meti, R. Vijayashree et al., "Anti-rheumatoid activity of ethanolic extract of Sesamum indicum seed extract in Freund's complete adjuvant induced arthritis in Wistar albino rats," *Journal of Traditional and Complementary Medicine*, vol. 8, no. 3, pp. 377–386, 2018.

[166] D. Tewari and N. Anjum, "Tripathi phytochemistry and pharmacology of plantago ovata: a natural source of laxative medicine," *World Journal of Pharmaceutical Research*, vol. 3, no. 9, pp. 361–372, 2014.

[167] K. Haddadian, K. Haddadian, and M. Zahmatkash, "A review of Plantago plant," *Indian Journal of Tradational knowledge*, vol. 13, no. 4, pp. 681–685, 2014.

[168] G. Sammaiah, N. Narsaiah, G. Dayakar et al., "Constituents and cytotoxicity of *polygala erioptera*," *International Journal of Chemical Sciences*, vol. 6, no. 2, pp. 643–646, 2008.

[169] H. Shin, H. Chung, B. Park, and K. Y. Lee, "Identification of antioxidative constituents from Polygonum aviculare using LC-MS coupled with DPPH assay," *Natural Product Sciences*, vol. 22, no. 1, pp. 64–69, 2016.

[170] B. B. Shen, Y. P. Yang, S. Yasamin et al., "Analysis of the phytochemistry and bioactivity of the genus polygonum of polygonaceae," *Digital Chinese Medicine*, vol. 1, no. 1, pp. 19–36, 2018.

[171] A. Bozicevic, M. De Mieri, A. Di Benedetto, F. Gafner, and M. Hamburger, "Dammarane-type saponins from leaves of Ziziphus spina-christi," *Phytochemistry*, vol. 138, pp. 134–144, 2017.

[172] M. E. Karar, L. Quiet, A. Rezk et al., "Phenolic profile and in vitro assessment of cytotoxicity and antibacterial activity of Ziziphus spina-christi Leaf Extracts," *Medicinal Chemistry*, vol. 6, no. 3, pp. 143–156, 2016.

[173] A. O. Abdel-Zaher, S. Y. Salim, M. H. Assaf, and R. H. Abdel-Hady, "Antidiabetic activity and toxicity of Zizyphus spina-christi leaves," *Journal of Ethnopharmacology*, vol. 101, no. 1–3, pp. 129–138, 2005.

[174] S. Sireeratawong, S. Vannasiri, U. Nanna, T. Singhalak, and K. Jaijoy, "Evaluation of acute and chronic toxicities of the water extract from Ziziphus attopensis pierre," *ISRN pharmacology*, vol. 2012, Article ID 789651, 7 pages, 2012.

[175] A. E. Al-Snafi, "The pharmacology of Bacopa monniera. A review," *International Journal of Pharma Sciences and Research*, vol. 4, no. 12, pp. 154–159, 2013.

[176] S. Sireeratawong, K. Jaijoy, P. Khonsung, N. Lertprasertsuk, and K. Ingkaninan, "Acute and chronic toxicities of Bacopa monnieri extract in Sprague-Dawley rats," *BMC Complementary and Alternative Medicine*, vol. 16, no. 1, pp. 1–10, 2016.

[177] N. U. Rehman, H. Hussain, S. A. Al-Riyami, I. R. Green, and A. Al-Harrasi, "Chemical constituents isolated from lycium shawii and their chemotaxonomic significance," *Records of Natural Products*, vol. 12, no. 4, pp. 380–384, 2018.

[178] F. A. Ahmed, N. M. Abdallah, M. K. Ezz, and M. M. El-Azab, "Seasonal variations and identification of biologically active constituents of lycium shawii plant roem. & shult. (family solanaceae)," *International Journal of Innovative Science, Engineering & Technology*, vol. 4, no. 11, pp. 2348–7968, 2017.

[179] U. Saleem, S. Amin, B. Ahmad, H. Azeem, F. Anwar, and S. Mary, "Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OECD 425 TG," *Toxicology Reports*, vol. 4, pp. 580–585, 2017.

[180] T. M. Saleem, C. M. Chetty, S. Ramkanth et al., "Solanum nigrum Linn.—a review," *Pharmacognosy Reviews*, vol. 3, no. 6, pp. 342–345, 2009.

[181] A. Patel, S. Biswas, M. H. Shoja, G. V. Ramalingayya, and K. Nandakumar, "Protective effects of aqueous extract of Solanum nigrum Linn. leaves in rat models of oral mucositis," *The Scientific World Journal*, vol. 2014, Article ID 345939, 10 pages, 2014.

[182] M. Rai, P. S. Jogee, G. Agarkar, and C. A. Santos, "Anticancer activities of Withania somnifera: current research, formulations, and future perspectives," *Pharmaceutical Biology*, vol. 54, no. 2, pp. 189–197, 2016.

[183] S. B. Patel, N. J. Rao, and L. L. Hingorani, "Safety assessment of Withania somnifera extract standardized for Withaferin A: acute and sub-acute toxicity study," *Journal of Ayurveda and Integrative Medicine*, vol. 7, no. 1, pp. 30–37, 2016.

[184] R. Delgado-Altamirano, R. I. López-Palma, L. Monzote et al., "Chemical constituents with leishmanicidal activity from a pink-yellow cultivar of *Lantana camara* var. *aculeata* (L.) collected in Central Mexico," *International Journal of Molecular Sciences*, vol. 20, no. 4, pp. 1–17, 2019.

[185] B. M. Pour, L. Y. Latha, and S. Sasidharan, "Cytotoxicity and oral acute toxicity studies of Lantana camara leaf extract," *Molecules*, vol. 16, no. 5, pp. 3663–3674, 2011.

[186] I. Jawonisi and G. Adoga, "Acute toxicity, haematological and histopathological assessment of the ethanolic extract of Lantana camara leaf," *Journal of Pharmacy & Bioresources*, vol. 14, no. 1, pp. 45–50, 2017.

[187] M. T. Moradi, A. Karimi, F. Fotouhi, S. Kheiri, and A. Torabi, "In vitro and *in vivo* effects of *Peganum harmala* L. seeds extract against influenza A virus," *Avicenna Journal of Phytomedicine*, vol. 7, no. 6, pp. 519–530, 2017.

[188] X. Shang, X. Guo, B. Li et al., "Microwave-assisted extraction of three bioactive alkaloids from Peganum harmala L. and their acaricidal activity against Psoroptes cuniculi in vitro," *Journal of Ethnopharmacology*, vol. 192, pp. 350–361, 2016.

[189] A. Majid, "A review study of the chemical constituents and therapeutic effects of Peganum harmala L," *Global Journal of Pure and Applied Chemistry Research*, vol. 6, no. 2, pp. 12–19, 2018.

[190] N. M. Ammar, S. S. EL-Hawary, D. A. Mohamed, M. S. Afifi, D. M. Ghanem, and G. Awad, "Phytochemical and biological studies of Tribulus terrestris L. growing in Egypt," *International Journal of Pharmacology*, vol. 14, no. 2, pp. 248–259, 2018.

[191] R. Ullah, I. Hussain, and S. Ahmad, "Diocanol; one new phenol derivative isolated and characterized from Urtica dioica," *Arabian Journal of Chemistry*, vol. 10, pp. S1284–S1286, 2017.

[192] C. Bourgeois, É. A. Leclerc, C. Corbin et al., "Nettle (Urtica dioica L.) as a source of antioxidant and anti-aging phytochemicals for cosmetic applications," *Comptes Rendus Chimie*, vol. 19, no. 9, pp. 1090–1100, 2016.

[193] U. J. Vajic, J. Grujic-Milanovic, Z. Miloradovic et al., "Urtica dioica L. leaf extract modulates blood pressure and oxidative stress in spontaneously hypertensive rats," *Phytomedicine*, vol. 46, pp. 39–45, 2018.

[194] S. K. Bharti, S. Krishnan, A. Kumar, and A. Kumar, "Antidiabetic phytoconstituents and their mode of action on

metabolic pathways," *Therapeutic Advances in Endocrinology and Metabolism*, vol. 9, no. 3, pp. 81–100, 2018.

[195] R. Ponnulakshmi, B. Shyamaladevi, P. Vijayalakshmi, and J. Selvaraj, "In silico and in vivo analysis to identify the antidiabetic activity of beta sitosterol in adipose tissue of high fat diet and sucrose induced type-2 diabetic experimental rats," *Toxicology Mechanisms and Methods*, vol. 29, no. 4, pp. 276–290, 2019.

[196] G. Arumugam, P. Manjula, and N. Paari, "A review: anti diabetic medicinal plants used for diabetes mellitus," *Journal of Acute Disease*, vol. 2, no. 3, pp. 196–200, 2013.

[197] P. P. Jaya, "Herbal medicine for diabetes mellitus," *A Review International Journal of Phytopharmacy*, vol. 3, no. 1, pp. 1–22, 2013.

[198] S. Jeeva and S. Y. Anlin, "A review of antidiabetic potential of ethnomedicinal plants," *Medicinal & Aromatic Plants*, vol. 3, no. 4, pp. 1–8, 2014.

[199] H. Azaizeh, S. Fulder, K. Khalil, and O. Said, "Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region," *Fitoterapia*, vol. 74, no. 1-2, pp. 98–108, 2003.

[200] G. E. El-Ghazali, K. S. Al-Khalifa, G. A. Saleem, and E. M. Abdallah, "Traditional medicinal plants indigenous to Al-Rass province, Saudi Arabia," *Journal of Medicinal Plants Research*, vol. 4, no. 24, pp. 2680–2683, 2010.

[201] M. F. Amal and M. M. Masarrat, "Migahid ethnobotanical survey of plants used in the treatment of diabetes mellitus in Tabuk region," *Saudi Arabia International Journal of Current Microbiology and Applied Sciences*, vol. 5, no. 6, pp. 258–270, 2016.

[202] M. S. Abdel-Kader, A. M. Hazazi, O. A. Elmakki, and S. I. Alqasoumi, "A survey on traditional plants used in Al Khobah village," *Saudi Pharmaceutical Journal*, vol. 26, no. 6, pp. 817–821, 2018.

[203] N. A. Ali, S. S. Al Sokari, A. Gushash, S. Anwar, K. Al-Karani, and A. Al-Khulaidi, "Ethnopharmacological survey of medicinal plants in Albaha region, Saudi Arabia," *Pharmacognosy Research*, vol. 9, no. 4, p. 401, 2017.

[204] R. J. Marlesa and N. R. Farnsworth, "Antidiabetic plants and their active constituents," *Phytomedicine*, vol. 2, no. 2, pp. 137–189, 1995.

[205] S. Hasani-Ranjbar, B. Larijani, and M. Abdollahi, "A systematic review of Iranian medicinal plants useful in diabetes mellitus," *Archives of Medical Science*, vol. 4, no. 3, pp. 285–292, 2008.

[206] G. B. Kavishankar, N. Lakshmidevi, S. M. Murthy, H. S. Prakash, and S. R. Niranjana, "Diabetes and medicinal plants—a review," *International Journal of Pharma Bio Sciences*, vol. 2, no. 3, pp. 65–80, 2011.

[207] S. L. Chen, H. Yu, H. M. Luo, Q. Wu, C. F. Li, and A. Steinmetz, "Conservation and sustainable use of medicinal plants: problems, progress, and prospects," *Chinese Medicine*, vol. 11, p. 37, 2016.

[208] E. Robertson, *Medicinal Plants at Risk. Nature's Pharmacy, Our Treasure Chest: Why We Must Conserve Our Natural Heritage*, Cent. Biol. Divers., Tucson, AZ, USA, 2008.

[209] K. Annan, R. A. Dickson, I. K. Amponsah, and I. K. Nooni, "The heavy metal contents of some selected medicinal plants sampled from different geographical locations," *Pharmacognosy Research*, vol. 5, no. 2, p. 103, 2013.

[210] M. Bedassa, A. Abebaw, and T. Desalegn, "Assessment of selected heavy metals in onion bulb and onion leaf (Allium cepa L.), in selected areas of central rift valley of Oromia region Ethiopia," *Journal of Horticulture and Forestry*, vol. 4, p. 217, 2017.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 3509706, 9 pages
https://doi.org/10.1155/2022/3509706

Research Article

The Effect of Broccoli Extract in Arsenic-Induced Experimental Poisoning on the Hematological, Biochemical, and Electrophoretic Parameters of the Liver and Kidney of Rats

Mahdieh Raeeszadeh,[1] Pouria Karimi,[2] Nadia Khademi,[2] and Pejman Mortazavi[3]

[1]*Department of Basic Sciences, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran*
[2]*Graduate of Faculty of Veterinary Sciences, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran*
[3]*Department of Pathobiology Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran*

Correspondence should be addressed to Mahdieh Raeeszadeh; vet_mr@yahoo.com

Received 14 November 2021; Revised 12 December 2021; Accepted 23 December 2021; Published 5 January 2022

Academic Editor: Rajadurai Murugan

Heavy metals such as arsenic contribute to environmental pollution that can lead to systemic effects in various body organs. Some medicinal plants such as broccoli have been shown to reduce the harmful effects of these heavy metals. The main aim of the present study is to evaluate the effects of broccoli extract on liver and kidney toxicity, considering hematological and biochemical changes. The experimental study was performed in 28 days on 32 male Wistar rats classified into four groups: the control group (C), a group receiving 5 mg/kg oral arsenic (AS), a group receiving 300 mg/kg broccoli (B), and a group receiving arsenic and broccoli combination (AS + B). Finally, blood samples were taken to evaluate the hematological and biochemical parameters of the liver and kidney, as well as serum proteins' concentration. Liver and kidney tissue were fixed and stained by H&E and used for histopathological diagnosis. The results demonstrated a significant decrease in white blood cells (WBC), red blood cells (RBC), and hemoglobin (Hb) in the AS group compared to other groups. However, in the B group, a significant increase in RBC and WBC was observed compared to the AS and C groups ($P < 0.05$). Moreover, RBC and WBC levels increased significantly in the AS + B group compared to the AS group ($P = 0.046$). However, in the AS group, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine levels increased, while total protein, albumin, and globulin decreased. This can be a result of liver and kidney damage, which was observed in the AS group. Furthermore, the increase in the concentration of albumin and globulin in the AS + B group was higher than that in the AS group. Infiltration of inflammatory cells and necrosis of the liver and kidney tissue in the pathological evaluation of the AS group were significantly higher than other groups. There was an increase in superoxide dismutases (SOD), glutathione peroxidase (GPx), and total antioxidant capacity (TAC); however, a decrease in malondialdehyde (MDA) concentration was seen in the AS + B group compared to the AS group. It seems that broccoli is highly effective at reducing liver and kidney damage and improving the hematological and biochemical factors in arsenic poisoning conditions.

1. Introduction

Arsenic ranks second (after lead) among the significant heavy metals, while arsenic contamination is one of the major global environmental problems [1]. This poisonous substance endangers humans' and animals' life. Because of the extensive human industrial activities and the abundance of chemical pollutants, pesticides, and herbicides, this heavy metal in the ecosystem is increasing progressively [2]. The most common cause of arsenic contamination is eating and drinking, followed by inhalation and dermal absorption [3]. Arsenic poisoning changes the hematological, biochemical, and oxidative stress parameters and leads to tissue damage (mainly in the liver and kidney). This is supported by evidence from carcinogenicity in animals and humans [4].

Various mechanisms have been reported for arsenic poisoning, including induction of oxidative stress, inhibition of enzymes, and changes in the mitochondrial function.

Moreover, binding with sulfhydryl (-SH) groups is another pathway for arsenic damage [5].

Experimental studies on the liver (being the most vulnerable organ to arsenic) have reported that arsenic poisoning can change the concentration of liver enzymes. Liver damage is mainly related to ROS production and oxidative stress [6]. Chronic arsenic poisoning also increases the acute-phase protein and C-reactive protein (CRP) in the liver and kidney and raises proinflammatory cytokines [7].

Oxidative stress and inflammation increase transcription factor activation and the gene expression of proinflammatory cytokines as a complex cycle [8]. Decreased phagocytic activity in leukocytes under the influence of oxidative damage can further amplify inflammatory reactions [9].

Recently, researchers have focused on the safe and available treatment methods and prevention of heavy metal poisoning. The use of chelating agents such as dimercaptopropane and binding to antioxidant compounds such as vitamins C and E have been suggested [10, 11]. On the other hand, increasing the concentration of intracellular antioxidants can also be helpful in preventing arsenic poisoning. Protection against calcium homeostasis and mitochondrial integrity that regulates apoptosis is another effective mechanism that has a positive impact on arsenic poisoning [12].

Vegetables as a source of antioxidants with almost no side effects for physiological systems are essential for human health [13]. Broccoli (*Brassica oleracea* L. var. *Italica*) contains a variety of polyphenols, so it greatly benefits the body. The natural antioxidants in broccoli include beta-carotene, vitamins C and E, which may directly and/or indirectly help reduce free radicals. Other antioxidants of broccoli such as iron, selenium, calcium, manganese, phosphorus, zinc, and potassium help regulate the body's ionic balance [14].

Considering the importance of controlling and preventing arsenic poisoning and the lack of research emphasis in this area, the aim of this study was to investigate the effect of broccoli extract on changes caused by arsenic poisoning in blood and the biochemical parameters in the liver and kidney of rats.

2. Materials and Methods

2.1. Subjects. The study was performed on 32 male Wistar rats weighing 200–230 g. The animals were housed in standard conditions, with temperatures between 20 and 22°C, humidity ranging from 45 to 50%, 12 hours of light, and 12 hours of darkness in the animal house of Sanandaj Veterinary Medicine university. The study followed the rules and regulations of animal ethics and the approval of the Ethics Committee of Kurdistan Medical Sciences (IR.MUK.REC.1400.6089).

2.2. Experimental Protocol. The animals were randomly divided into four groups (8 in each group): the control group (C), receiving no treatment; the arsenic group (AS), receiving sodium arsenate, CAS Number 10048-95-0, at 5 mg/kg/body weight by means of oral gavage; the broccoli group (B), receiving broccoli extract at a dose of 300 mg/kg (based on the best functional dose in previous studies) intraperitoneal (IP) injection; and the AS + B group (AS + B), receiving 5 mg/kg sodium arsenate and 300 mg/kg broccoli alcoholic extract [15, 16].

The study lasted 28 days, with blood samples taken from animals under anesthesia on the last day. EDTA-anticoagulant blood was used to measure hematological parameters such as red blood cells (RBC), white blood cells (WBC), hematocrit (HCT), neutrophils, lymphocytes, monocytes, eosinophils, and hemoglobin (HGB). Serum was separated by centrifugation. It was used to estimate hepatic and renal biochemical parameters including aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and urea. Furthermore, oxidative stress levels glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), and malondialdehyde (MDA) were measured. Moreover, changes in serum proteins regarding albumin and globulin were evaluated by electrophoresis.

2.3. Hematological Parameters. Hemoglobin concentration was determined by the cyanmethemoglobin method. The RBC and WBC counts were measured using a Neubauer hemocytometer. The differential leukocyte counts were measured by exploiting standard methods [4].

2.4. Serum Biochemical Analysis. ALP, AST, ALT, urea, and creatinine levels were measured using a Pars test kit and an autoanalyzer [4].

2.5. Stress Oxidative Biomarkers. Trichloroacetic acid (TCA) was measured using the Ferric reducing antioxidant power (FRAP) method suggested by Benzi et al. [17].

Thiobarbituric acid (TBA) reactivity was measured using malondialdehyde (MDA). 1 ml of reagent [10% trichloroacetic acid (TCA), 0.67% thiobarbituric acid (TBA), and 0.25 M hydrochloric acid (HCl)] was added to 500 μl of serum sample. This mixture was then placed in a bain-marie for 15 minutes at 95°C. After cooling, the mixture was centrifuged (10 min, 1000 g), and the obtained supernatant absorbance was determined at 535 nm [18].

The catalase enzyme decomposes hydrogen peroxide into water and oxygen, measured spectrophotometrically at a wavelength of 240 nm.

The superoxide dismutase enzyme was measured at 505 nm [19]. To this end, xanthine and xanthine oxidase (XOD) were used to produce superoxide radicals, in which the reaction with the combination phenyl tetrazolium chloride-5-(nitrophenol-4)-3-(iodophenol-4)-2 creates red dye color.

Glutathione peroxidase was measured using the method described by Paglia and Valentine. The glutathione peroxidase enzyme catalyzes the oxidation reaction of glutathione (GSH) via cumene hydroperoxide that can be measured by light absorption at a wavelength of 340 nm [20].

2.6. Serum Protein Electrophoresis (SPEP). SPEP was measured by electrophoresis on cellulose acetate paper. The steps are as follows:

(a) Cellulose acetate paper was placed in barbital buffer for 10 minutes at pH = 8.6

(b) Cellulose acetate paper damping was done between two sheets of filter paper

(c) Ten microliters of serum from each sample was poured on each template platform

Sampling was done 4–6 times by the applicator at a distance of 3 cm from the edge of the cellulose acetate paper

(d) The gel was placed on the tank and electrophoresis was performed for 30 minutes at 160 volts and a current between 5–7 mA

(e) At the end of the electrophoresis time, the plate(s) was (were) removed from the chamber. They were placed in 40–50 mL of Ponceau S Staining sufficient volume to cover the plate(s) for 5–7 minutes of shaking

(f) Destaining was done in three consecutive containers of 5% acetic acid, each for 2 to 3 minutes.

(g) Dehydration was done in pure methanol for 2 minutes

(h) The plate was placed in the clearing solution for 5–10 minutes

(i) The plate was placed in a drying oven at 70°C for 10 minutes or until dry

(j) The cleared film, when cooled, was ready for the densitometer scanner

(k) Charts were drawn and printed [21, 22]

2.7. Histopathological Evaluation of the Liver and Kidney. Liver and kidney tissue were fixed in 10% formalin buffer after separation. Then, tissue sections were prepared. Hematoxylin-eosin (H and E) staining was performed.

Liver and kidney tissue sections were analyzed based on the severity and weakness of tissue lesions at a magnification of 400 in 5 microscopic fields.

2.8. Preparation of Alcoholic Extract of Broccoli. Due to the higher antioxidant of alcoholic broccoli extract than aqueous extract, methanolic extract was considered. Therefore, after drying and grinding the broccoli, 50 g of powder was kept in 250 ml of 80% methanol by shaking for 48 hours. Then, after filtering the extract, it was placed in a rotary vacuum to let the dissolvent be evaporated. Then, the extract was dried into a powder and stored in refrigeration for administration at a dose of 300 mg/kg [16, 23, 24].

2.9. Data Analysis. After checking the normality of the data using the Kolmogorov–Smirnov test, the descriptive data were reported as mean ± standard deviation. After that, the results were analyzed using one-way analysis of variance (ANOVA), followed by Tukey post hoc test. The significance level was set at $P < 0.05$.

3. Results

According to Table 1, the hematological analysis showed that the highest mean of WBC is in the B group, and the lowest is in the AS group. The WBC in the B and AS + B groups was significantly different from other groups. The lowest mean of the RBC was shown in the AS group, and the highest mean was reported in the B group. Moreover, the increased hemoglobin concentration in the B group is significantly higher than in other groups except for the C group. On the other hand, the PCV percentage was not significantly different between groups. Additionally, the levels of neutrophils, lymphocytes, monocytes, and eosinophils did not show a significant difference between groups.

The biochemical changes in the liver and kidney parameters are shown in Table 2. The concentration of ALT, as a liver-specific enzyme, was 74.3 ± 1.90 in the AS group, which was the highest value reported between groups, but after treatment with broccoli, it reduced to 58.60 ± 6.60. The difference between the S group and other groups was significant. Serum AST and ALP levels were highest in the AS group. However, the enzyme values did not differ significantly between the B and C groups. The enzyme levels decreased in the AS + B group, although AST concentration in this group was not significantly different from the control group.

Creatinine concentration in AS-induced renal injury has the highest value among groups (1.42 ± 0.18) and is significantly different from other groups. Urea showed the lowest values in the C group and the highest values in the AS group. The serum urea concentration in the AS group was significantly dissimilar to other groups except for the AS + B group. There was also a significant difference in the serum urea level between the AS and B groups.

Table 3 shows the changes in oxidative stress biomarkers. The level of CAT enzyme was different between the B and AS groups, but the differences between the other groups were not significant. GPx enzyme showed the highest serum levels in the C and B groups, and the lowest value was found in the AS group. There was a statistically significant difference between the AS group and other experimental groups. Furthermore, SOD enzyme levels were highest in the C and B groups. However, the antioxidant enzyme levels increased after treatment with broccoli extract compared to the AS group. The highest concentration of MDA was observed in the AS group (12.45 ± 1.20 nmol/ml), which showed a significant difference with the other groups. Evaluation of TAC showed the highest values in the B group (858.47 ± 25.49) compared to the lowest values in the AS group (220.29 ± 69.53). These two groups were significantly different from other experimental groups.

The highest globulin levels were reported in the C and B groups (2.80 ± 0.05 and 2.31 ± 0.47, respectively), and the lowest levels were reported in the AS group (1.02 ± 0.31). The dissimilarity between the AS, AS + B, and control groups was significant. Albumin showed the highest values in the control group. The AS and AS + B groups were significantly different from the C group; moreover, there was a significant difference between the B and AS groups. The highest A/G ratio was observed in the AS group, and the lowest ratio was observed in the control group. Plasma total protein showed

Table 1: Changes in hematology parameters in the different groups of the study.

Parameters	Groups				
	Control	AS	B	AS + B	*P* value
WBC (per mm^3)	7980.9 ± 950.3	7180.45 ± 322.6	1340 ± 230.64[a,b]	11548 ± 451.7[a,b]	0.034
RBC ($\times 10^6/\mu L$)	5.30 ± 0.81	4.37 ± 0.99[a]	6.03 ± 1.08[a,b]	5.56 ± 0.42[b,c]	0.049
HGB (g/dL)	14.88 ± 0.65	12.86 ± 1.43[a]	14.50 ± 1.10[b]	13.68 ± 1.00	0.043
PCV (%)	43.22 ± 3.76	41.62 ± 3.03	44.16 ± 1.57	41.56 ± 1.40	0.356
Neutrophils (%)	37.40 ± 1.67	42.60 ± 12.89	39.60 ± 6.18	39.80 ± 14.46	0.883
Lymphocytes (%)	58.80 ± .04	54.80 ± 12.35	55.00 ± 10.93	56.40 ± 15.91	0.942
Monocytes (%)	2.00 ± 0.00	1.60 ± 1.94	1.60 ± 0.54	1.00 ± 0.5	0.585
Eosinophils (%)	1.80 ± 0.44	0.84 ± 0.43	1.80 ± 0.83	1.60 ± .041	0.492

All values are presented as mean ± SEM. The letter (a) indicates a comparison with the control group, the letter (b) indicates a comparison with the AS group, and the letter (c) indicates a comparison with the B group. All data were analyzed using one-way ANOVA, and a post hoc test was performed by Tukey test ($n = 8$, $P < 0.05$).

Table 2: Evaluation changes in the biochemical parameters of the liver and kidney between groups.

Parameters	Groups				
	Control	AS	B	AS + B	*P* value
ALT (U/L)	28.4 ± 2.87	74.3 ± 1.90[a]	30.20 ± 4.25[b]	58.60 ± 6.60[a,b,c]	0.034
AST (U/L)	68.20 ± 4.80	142.25 ± 8.63[a]	62.60 ± 4.07	78.38 ± 12.38	0.049
ALP (U/L)	170.50 ± 12.24	245.82 ± 18.39[a]	183.22 ± 11.15[b]	190.34 ± 20.70[b]	0.043
Creatinine (mg/dl)	0.37 ± 0.12	1.42 ± 0.18[a]	0.52 ± 0.25[b]	0.97 ± 0.26[a,b]	0.036
Urea (mg/dl)	31.30 ± 8.50	50.18 ± 7.80[a]	32.10 ± 6.54 α,[b]	42.07 ± 11.03	0.048

All values are presented as mean ± SEM. The letter (a) indicates a comparison with the control group, the letter (b) indicates a comparison with the AS group, and the letter (c) indicates a comparison with the B group. All data were analyzed using one-way ANOVA, and a post hoc test was performed by Tukey test ($n = 8$, $P < 0.05$).

Table 3: Investigation of changes in oxidative stress biomarkers.

Parameters	Groups				
	Control	AS	B	AS + B	*P* value
CAT (nmol/ml)	7.31 ± 3.24	5.96 ± 0.88	9.38 ± 3.28	6.63 ± 1.69	0.197
GPx (nmol/ml)	5.12 ± 0.67	1.84 ± 0.97[a]	4.22 ± 2.04[a,b]	2.95 ± 1.6[a,b,c]	0.049
SOD (nmol/ml)	66.16 ± 7.49	23.22 ± 10.44[a]	64.11 ± 10.05[b]	32.45 ± 5.66[a,b,c]	≤0.01
MDA (nmol/ml)	4.73 ± 1.76	12.45 ± 1.20[a]	3.32 ± 1.96[b]	5.25 ± 2.64[b,c]	≤0.01
TAC (μmol/ml)	450.57 ± 37.58	220.29 ± 69.53[a]	858.47 ± 25.49[a,b]	628.40 ± 54.71[a,b,c]	≤0.01

All values are presented as Mean ± SEM. The letter (a) indicates a comparison with the control group, the letter (b) indicates a comparison with the AS group, and the letter (c) indicates a comparison with the B group. All data were analyzed using one-way ANOVA, and a post hoc test was performed by Tukey test ($n = 8$, $P < 0.05$).

the highest value in the B group (6.5 ± 0.42) and the lowest value in the AS group (4.78 ± 0.38). In addition, alpha globulins showed higher values in the arsenic group than other groups ($P = 0.032$) (Table 4 and Figure 1).

Pathological changes were compared between the different groups based on quantitative criteria. Infiltration of the inflammatory cells was highest in the AS group and lowest in the B group. The highest central portal venous hyperemia was in the AS group, with a mean of 3.74, while the lowest amount was in the control group. The highest cytoplasm vacuolation was seen in the AS group, and the lowest was in the B group. Moreover, vacuolation showed a significant decrease in the AS + B group compared to the AS group. With a mean of 2.67, hepatocyte necrosis was highest in the AS group and lowest in the B group. In the AS + B group, this reduction was not significantly different from the control group (Table 5 and Figure 2).

Table 6 shows all the pathological changes in the kidney tissue. There are four criteria, that is, the penetration of inflammatory cells, hyperemia, hemorrhage, and interstitial space in the Bowman capsule. The highest mean of infiltration of the inflammatory cells was in the AS group (3.69 ± 0.49), while the lowest was in the control group (0.03 ± 0.02). Vascular hyperemia has the greatest amount in the AS and AS + B groups, showing significant differences from the other groups. Moreover, the highest interstitial space in the Bowman capsule and its abnormal structure were in the AS group; however, this value was in a normal range in the control and B groups. The highest mean of bleeding was reported in the AS group (3.26), and the lowest was in the control group (0.03) (Figure 3).

4. Discussion

Due to the increased spread of agricultural and metallic pesticides in the environment, the amount of arsenic in the ecosystem is increasing. Pollution caused by these heavy metals has a chronic and long-term poisonous effect on

Table 4: Serum protein values by electrophoresis in the studied animals.

Parameters	Groups				
	Control	AS	B	AS + B	*P* value
Globulin (g/dl)	2.80 ± 0.05	1.02 ± 0.31[a]	2.31 ± 0.47[b]	1.25 ± 0.36[a,c]	≤0.01
Albumin (g/dl)	4.81 ± 0.07	3.25 ± 0.51[a]	4.56 ± 0.36[b]	3.58 ± 0.37	≤0.01
A/G	1.71 ± 0.65	3.18 ± 0.49[a]	1.9 ± 0.28[b]	2.86 ± 0.89[a,b]	≤0.01
Total protein (g/dl)	6.25 ± 0.48	4.78 ± 0.38[a]	6.50 ± 0.42[b]	5.69 ± 0.37[a,c]	≤0.01
α-Globulin (g/dl)	0.50 ± 0.12	0.68 ± 0.35[a]	0.58 ± 0.04	0.52 ± 0.12	0.032
β-Globulin (g/dl)	0.62 ± 0.26	0.58 ± 0.14	0.70 ± 0.26	0.76 ± 0.04	0.215
γ-Globulin (g/dl)	0.84 ± 0.05	0.72 ± 0.03	0.70 ± 0.03	0.75 ± 0.02	0.453

All values are presented as Mean ± SEM. The letter (a) indicates a comparison with the control group, the letter (b) indicates a comparison with the AS group, and the letter (c) indicates a comparison with the B group. All data were analyzed using one-way ANOVA, and a post hoc test was performed by Tukey test ($n = 8$, $P < 0.05$).

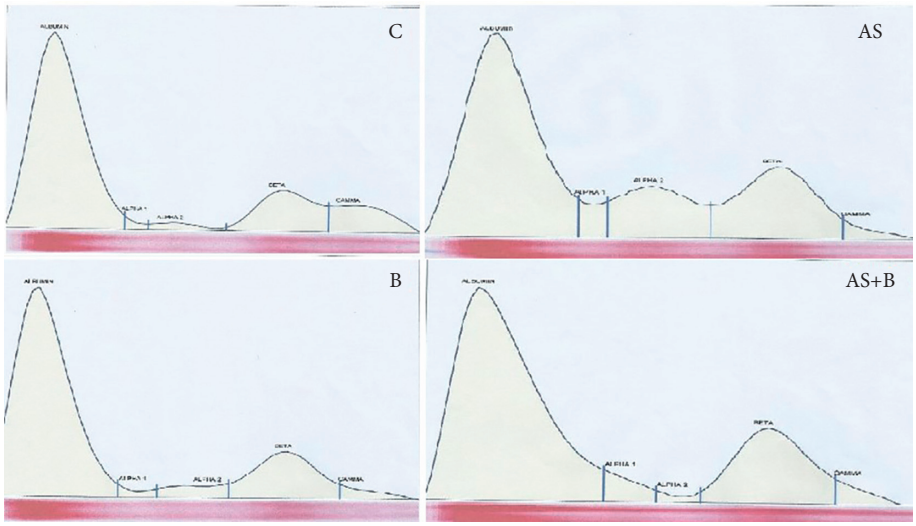


Figure 1: Electrophoretic changes of serum proteins in different groups.

Table 5: Evaluation of pathological changes in liver tissue in the experimental groups.

Parameters	Groups				
	Control	AS	B	AS + B	*P* value
Inflammatory infiltration	0.32 ± 0.01	4.5 ± 0.23[a]	0.03 ± 0.00[b]	2.38 ± 1.40[a,b,c]	0.047
Central venous congestion	0.06 ± 0.00	3.74 ± 0.67[a]	0.32 ± 0.05[b]	0.07 ± 0.6[b]	0.053
Cytoplasmic vacuolation	0.16 ± 0.09	2.22 ± 0.44[a]	0.11 ± 0.08[b]	1.12 ± 0.64[a,b,c]	≤0.01
Hepatocyte necrosis	0.73 ± 0.05	2.67 ± 1.32[a]	0.04 ± 0.03	1.02 ± 0.7[a,b,c]	0.034

All values are presented as Mean ± SEM. The letter (a) indicates a comparison with the control group, the letter (b) indicates a comparison with the AS group, and the letter (c) indicates a comparison with the B group. All data were analyzed using one-way ANOVA, and a post hoc test was performed by Tukey test ($n = 8$, $P < 0.05$).

wildlife [25]. Therefore, the use of chelator and antioxidant compounds is recommended to decrease the poisoning effects of heavy metals [26]. The present study confirmed that arsenic poisoning could cause changes in the hematological and biochemical parameters of the liver and kidneys, affecting the functions of these organs. On the other hand, liver damage can affect the total protein and electrophoretic pattern of other blood proteins. In addition, due to the oxidative function of arsenic in the body, we observed a decrease in enzymatic antioxidants and increase in oxidative damage to macromolecules. In this regard, the results indicate the positive performance of broccoli in controlling the hematological, biochemical, and enzymatic damage of arsenic.

The decreased levels of WBC and RBC were more severe in the AS group than in the other groups. Previous studies have shown that the concentration and route of administration of arsenic can decrease or increase the number of red blood cells [27].

Arsenic reduces the number of blood cells through inhibiting cell activity, antimycotic properties, stimulating oxidative stress, reducing cellular antioxidants, and increasing cell involvement in immune processes [28].

In other studies, the effect of arsenic on blood parameters has been implicated as a disorder of the Heme synthesis pathway, which is the beginning of the systemic effects of arsenic poisoning [29].

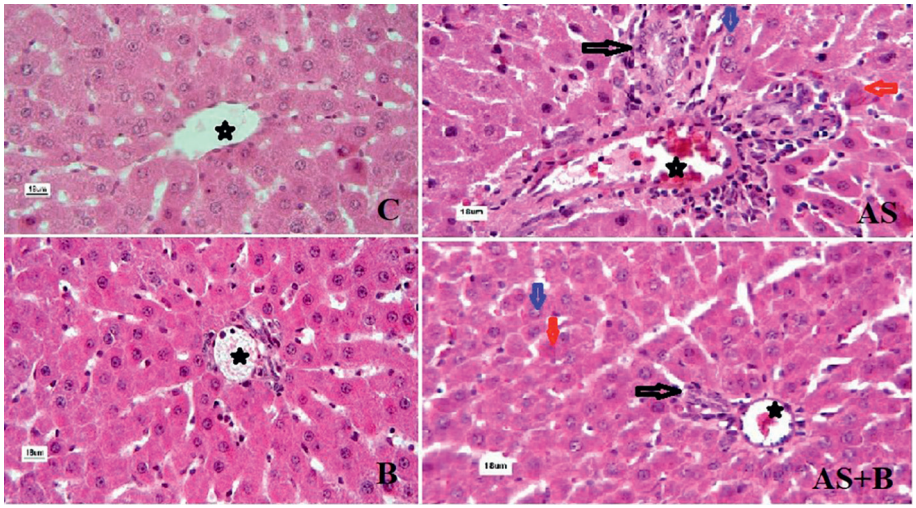


Figure 2: Histopathological changes of liver tissue in different groups. The star shows the central venous location, a black arrow pointing to the inflammatory cells, a blue arrow indicating to the cytoplasm vacuolation, and a red marker pointing the hepatocyte necrosis. magnification 400; H and E staining.

Table 6: Evaluation of the histopathological structure of kidney tissue in the studied groups.

Parameters	Groups				
	Control	AS	B	AS + B	*P* value
Inflammatory infiltration cells	0.03 ± 0.02	3.69 ± 0.49[a]	0.04 ± 0.02[b]	2.38 ± 1.40[a,b,c]	0.037
Hyperemia	0.14 ± 0.01	2.12 ± 0.28[a]	0.17 ± 0.03[b]	2.04 ± 0.58[a,c]	0.051
Space of Bowman's capsule membrane	0.16 ± 0.09	3.10 ± 0.44[a]	0.28 ± 0.04[b]	2.95 ± 1.10[a,c]	0.01
Hemorrhage	0.03 ± 0.01	3.26 ± 0.98[a]	0.05 ± 0.04	1.48 ± 0.38[a,b,c]	0.027

All values are presented as Mean ± SEM. The letter (a) indicates a comparison with the control group, the letter (b) indicates a comparison with the AS group, and the letter (c) indicates a comparison with the B group. All data were analyzed using one-way ANOVA, and a post hoc test was performed by Tukey test ($n = 8$, $P < 0.05$).

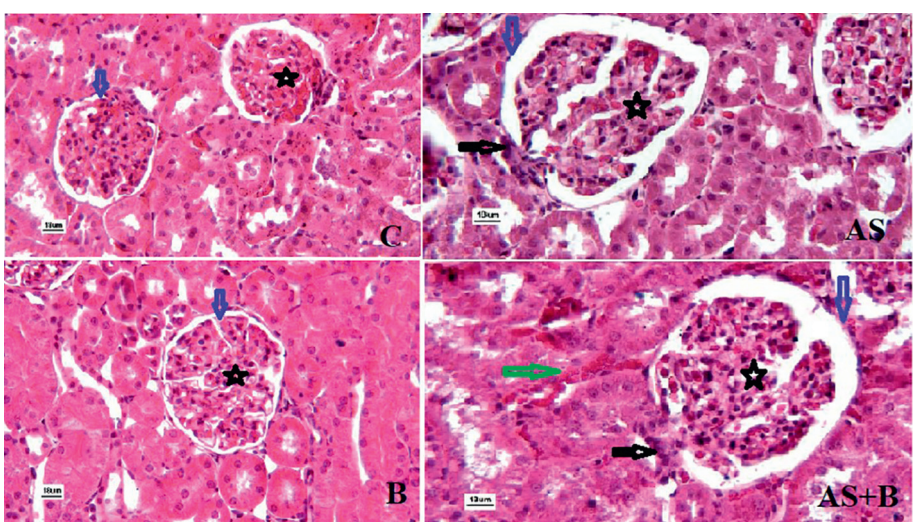


Figure 3: Histopathological assessments of renal tissue in different groups. Star is representing glomeruli, black arrow showing inflammatory cells, a blue arrow pointing the distance between Bowman's capsule membrane, and the green arrow showing hyperemia.

However, in a recent study, it was reported that doses of 300, 2000, and 4000 mg/kg of broccoli extract in 28 days showed no signs of poisoning by measuring the hematological, biochemical, and hepatic parameters [30]. Moreover, it is suggested that broccoli enhances the hematological parameters [31]. In another study, reduced production of RBC and WBC [32], oxidative stress in the liver, and alterations in the hematological parameters were reported to be caused by arsenic poisoning in fish [33]. Accordingly, the liver is one of the most important and active sites in the storage, redistribution, and detoxification of pollutants [34], and there are several tests to evaluate the liver. AST and ALT

are used for checking the hepatocyte's damage, and they are also indicators of the liver's biosynthetic capacity. ALT is a particular indicator for hepatocyte necrosis. Not only did the AST increase in liver injury, but also it was involved in damage to other tissues, such as the heart, bones, skeletal muscles, and kidneys. AST and ALT levels increased when drugs and toxins caused liver necrosis [35]. Increased levels of AST and ALT have been reported in acute and chronic liver poisoning [35]. In parallel pathological studies, inflammation, necrosis, and apoptosis of the liver following arsenic poisoning in mice have confirmed liver damage. On the other hand, oxidative damage is one of the significant concerns in arsenic poisoning cases. Increasing the antioxidant potential of arsenic contaminants is one of the most important strategies to prevent damage to this toxic metal. In addition, the nuclear factor (erythroid-2 related), factor 2 (Nrf2), and the Nrf2-regulated signaling pathway are among the most important ways to control oxidative damage that can maintain cellular redox homeostasis against metal poisoning [36]. Hence, activation of the Nrf2 transcription gene can cause an upregulated number of antioxidant genes and control oxidative damage [37]. According to the action mechanism of arsenic [36], and in our case, broccoli, due to its special polyphenolic and strong antioxidant potential, may activate this pathway. This process may control oxidative damage to the liver and kidney and reduce ASL and ALT levels.

Since total protein, albumin, and globulin are produced by the liver, its damage can alter the synthesis of these compounds. Any condition causing hepatocellular damage reduces the level of albumin and globulin, like what happens in arsenic poisoning [38]. It is worth noting that the α_1 fraction is mainly related to α_1 antitrypsin. Arsenic-induced inflammation in liver damage causes an increase in α_1 antitrypsin as an acute-phase reactant. Moreover, the α_2 region contains α_2 macroglobulin and haptoglobin [39]. The increase in α_2 macroglobulin in the nephrotic syndrome, as well as the increase in haptoglobin in the inflammation of the liver, occurred as a result of arsenic poisoning [40].

Furthermore, the induction of oxidative stress and the binding of arsenic to protein groups containing Sh-thiol can reduce the synthesis and function of proteins in the body. The results showed that reducing oxidative stress using antioxidant-rich substances such as broccoli could increase the number of serum proteins [41].

The kidney damage induced by arsenic poisoning inhibits the antioxidant system functions and results in immunosuppression [42]. Moreover, arsenic poisoning of the kidney affects the regulation of proteins responsible for renal reabsorption and secretion, which causes elicited tubular damage [43, 44]. Elevated creatinine and urea concentration are the result of inflammatory and pathological damage in the glomerulus structures due to arsenic poisoning in the kidney. Because of the potential of quercetin in the detoxification of acetaminophen poisoning [45], concomitant administration of broccoli in reducing liver, kidney, and hematology arsenic poisoning can be helpful.

Although inorganic arsenic is more toxic than the organic one, oxidative damage mediated by ROS species is a common mechanism of poisoning in both substances. Moreover, cascading mechanisms of superoxide-free radical formation and decreased glutathione levels increase the susceptibility of cells to arsenic poisoning. During human and animal contact with arsenic, ROS/RNS production increases oxidative stress and causes damage to macromolecules, including lipids, which can increase MDA levels [46–48]. The overall result of this damage is different diseases of the liver, kidneys, nervous system, gastrointestinal tract, and reproductive system [49, 50]. Antioxidant substances such as vitamins C and E and quercetin, extracted from broccoli, increase the concentration of antioxidant enzymes responsible for preventing oxidative damage [46, 51]. Therefore, the results showed that in arsenic poisoning conditions, broccoli extract causes an increase in CAT, SOD, and GPx while decreasing MDA, thus reducing liver and kidney damage. Moreover, the results of the present study were in line with the research conducted by Sharma and Sangha, in which the multimechanistic protective effects (especially the antioxidant actions) of broccoli extract on Triazophos neurosplenic toxicity were shown [52].

5. Conclusion

Following the fact that the most dangerous effect of arsenic poisoning is increased oxidative stress, we showed a significant decrease in glutathione peroxidase and superoxide dismutase as well as an increase in MDA in the AS group. There was also an increase in the pathological damage to the liver and kidney tissue and the levels of AST, ALT, urea, and creatinine enzymes. On the other hand, due to the importance of the liver and kidneys in metabolism and excretion, their damage reduces the level of albumin and globulin. Our findings also showed reduced hemoglobin and red and white blood cells in the hematological parameters. Overall, we were able to reduce the antioxidant potentials of broccoli and prevent arsenic poisoning at the molecular level. Molecular studies on the action mechanisms of broccoli in alleviating the effects of arsenic poisoning are recommended to reveal the fundamental basics behind the results of the present study.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to sincerely appreciate the Research Vice-Chancellor at the Islamic Azad University, Sanandaj Branch. In addition, the authors thank Dr. Ekradi for performing histological evaluations.

References

[1] J. Briffa, E. Sinagra, and R. Blundell, "Heavy metal pollution in the environment and their toxicological effects on humans," *Heliyon*, vol. 6, no. 9, p. e04691, 2020.

[2] J.-Y. Chung, S.-D. Yu, and Y.-S. Hong, "Environmental source of arsenic exposure," *Journal of Preventive Medicine and Public Health*, vol. 47, no. 5, pp. 253–257, 2014.

[3] I. Palma-Lara, M. Martínez-Castillo, J. C. Quintana-Pérez et al., "Arsenic exposure: a public health problem leading to several cancers," *Regulatory Toxicology and Pharmacology*, vol. 110, p. 104539, 2020.

[4] M. Al-Forkan and S. Islam, "A sub-chronic exposure study of arsenic on hematological parameters, liver enzyme activities, histological studies and accumulation pattern of arsenic in organs of Wistar albino rats," *Journal of Cytology & Histology*, vol. 7, no. 2, 2016.

[5] S. J. Flora, S Bhadauria, G. M Kannan, and N Singh, "Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: a review," *Journal of Environmental Biology*, vol. 28, no. 2, pp. 333–347, 2007.

[6] N. Das, S. Paul, D. Chatterjee et al., "Arsenic exposure through drinking water increases the risk of liver and cardiovascular diseases in the population of West Bengal, India," *BMC Public Health*, vol. 12, no. 1, p. 639, 2012.

[7] N. Singh, D. Kumar, K. Lal, S. Raisuddin, and A. P. Sahu, "Adverse health effects due to arsenic exposure: modification by dietary supplementation of jaggery in mice," *Toxicology and Applied Pharmacology*, vol. 242, no. 3, pp. 247–255, 2010.

[8] M. Raeeszadeh, P. Mortazavi, and R. Atashin-Sadafi, "The antioxidant, anti-inflammatory, pathological, and behavioural effects of medicago sativa L.(Alfalfa) extract on brain injury caused by nicotine in male rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 6694629, 2021.

[9] S. Wang and X. Shi, "Molecular mechanisms of metal toxicity and carcinogenesis," *Molecular Mechanisms of Metal Toxicity and Carcinogenesis*, vol. 222, no. 1, pp. 3–9, 2001.

[10] C. Rajak, N. Singh, and P. Parashar, "Metal toxicity and natural antidotes: prevention is better than cure," *Environmental Science and Pollution Research*, vol. 27, no. 35, pp. 1–17, 2020.

[11] S. Berenjian and M. Raeeszadeh, "Prescription of antibiotics before and after surgery at the surgical wards of isfahan amiralmomenin hospital compared with the standard guidelines," *Health Research*, vol. 1, no. 3, pp. 133–140, 2016.

[12] M. N. Rana, J. Tangpong, and M. M. Rahman, "Toxicodynamics of Lead, Cadmium, Mercury and Arsenic-induced kidney toxicity and treatment strategy: a mini review," *Toxicology Reports*, vol. 5, pp. 704–713, 2018.

[13] C. N. Amadi, S. J. Offor, C. Frazzoli, and O. E. Orisakwe, "Natural antidotes and management of metal toxicity," *Environmental Science and Pollution Research*, vol. 26, no. 18, pp. 18032–18052, 2019.

[14] H. A. R. El-Hendy and H. H. A. R. El-Hendy, "Effect of broccoli intake on antioxidants in the liver and kidneys tissues of hyperglycemic rats," *Alexandria Science Exchange Journal*, vol. 34, pp. 379–390, 2013.

[15] M. Raeeszadeh, "The study of the effect of hydroalcholic extracts of broccoli induced oxidative stress by lead in kidney of mice," *Razi Journal of Medical Sciences*, vol. 25, no. 9, pp. 17–25, 2018.

[16] M. Raeeszadeh, A. Nadia khademi, and A. Akbari, "The effects of broccoli and caraway extracts on serum oxidative markers, testicular structure and function, and sperm quality before and after sperm cryopreservation," *Cryobiology*, vol. 99, pp. 11–19, 2021.

[17] I. F. F. Benzie and J. J. Strain, "[2] Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration," *Oxidants and Antioxidants Part A*, vol. 299, pp. 15–27, 1999.

[18] Q. Tang, Y. W. Su, and C. J. Xian, "Determining oxidative damage by lipid peroxidation assay in rat serum," *Bio-Protocol*, vol. 9, no. 12, p. e3263, 2019.

[19] S. F. Farzan, C. G. Howe, M. S. Zens et al., "Urine arsenic and arsenic metabolites in U.S. Adults and biomarkers of inflammation, oxidative stress, and endothelial dysfunction: a cross-sectional study," *Environmental Health Perspectives*, vol. 125, no. 12, p. 127002, 2017.

[20] D. E. Paglia and W. N. Valentine, "Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase," *The Journal of Laboratory and Clinical Medicine*, vol. 70, no. 1, pp. 158–169, 1967.

[21] J. Zaias, M. Mineau, C. Cray, D. Yoon, and N. H. Altman, "Reference values for serum proteins of common laboratory rodent strains," *Journal of the American Association for Laboratory Animal Science: Journal of the American Association for Laboratory Animal Science*, vol. 48, no. 4, pp. 387–390, 2009.

[22] S. Ramanathan and C. N. Srinivas, "Serum protein electrophoresis and its clinical applications," in *Biochemical Testing-Clinical Correlation and Diagnosis*IntechOpen, London, UK, 2019.

[23] J.-H. Hwang and S.-B. Lim, "Antioxidant and anti-inflammatory activities of broccoli florets in LPS-stimulated RAW 264.7 cells," *Preventive Nutrition and Food Science*, vol. 19, no. 2, pp. 89–97, 2014.

[24] A. K. Jaiswal, N. Abu-Ghannam, and S. Gupta, "A comparative study on the polyphenolic content, antibacterial activity and antioxidant capacity of different solvent extracts of *Brassica oleracea* vegetables," *International Journal of Food Science and Technology*, vol. 47, no. 2, pp. 223–231, 2012.

[25] V. Lenoble, D. Omanović, C. Garnier et al., "Distribution and chemical speciation of arsenic and heavy metals in highly contaminated waters used for health care purposes (Srebrenica, Bosnia and Herzegovina)," *The Science of the Total Environment*, vol. 443, pp. 420–428, 2013.

[26] J.-J. Kim, Y.-S. Kim, and V. Kumar, "Heavy metal toxicity: an update of chelating therapeutic strategies," *Journal of Trace Elements in Medicine and Biology*, vol. 54, pp. 226–231, 2019.

[27] Y. Yi, S. Gao, J. Xia et al., "Study of the accumulation and distribution of arsenic species and association with arsenic toxicity in rats after 30 days of oral realgar administration," *Journal of Ethnopharmacology*, vol. 247, p. 111576, 2020.

[28] Y. Gazitt and C. Akay, "Arsenic trioxide: an anti cancer missile with multiple warheads," *Hematology*, vol. 10, no. 3, pp. 205–213, 2005.

[29] F. Fazio, C. Saoca, V. Ferrantelli, G. Cammilleri, G. Capillo, and G. Piccione, "Relationship between arsenic accumulation in tissues and hematological parameters in mullet caught in Faro Lake: a preliminary study," *Environmental Science and Pollution Research*, vol. 26, no. 9, pp. 8821–8827, 2019.

[30] S. S. Saha and M. Ghosh, "Protective effect of conjugated linolenic acid isomers present in vegetable oils against arsenite-induced renal toxicity in rat model," *Nutrition*, vol. 29, no. 6, pp. 903–910, 2013.

[31] D. Pisuttharachai, W. Nalinanon, and N. Areechon, "Effects of dietary supplementation with broccoli sprouts (*Brassica oleracea*) on the hematology and immunological responses of nile tilapia (*Oreochromis niloticus*)," *Journal of Fisheries and Environment*, vol. 44, no. 1, pp. 65–75, 2020.

[32] C. O. Abernathy, D. J. Thomas, and R. L. Calderon, "Health effects and risk assessment of arsenic," *Journal of Nutrition*, vol. 133, no. 5, pp. 1536S–1538S, 2003.

[33] A. Bhattacharya and S. Bhattacharya, "Induction of oxidative stress by arsenic in *Clarias batrachus*: involvement of peroxisomes," *Ecotoxicology and Environmental Safety*, vol. 66, no. 2, pp. 178–187, 2007.

[34] J. C. Akan, M. Salwa, B. S. Yikala, and Z. M. Chellube, "Study on the distribution of heavy metals in different tissues of fishes from River Benue in Vinikilang, Adamawa State, Nigeria," *British Journal of Applied Science & Technology*, vol. 2, no. 4, pp. 311–333, 2012.

[35] S. Roy and S. Bhattacharya, "Arsenic-induced histopathology and synthesis of stress proteins in liver and kidney of Channa punctatus," *Ecotoxicology and Environmental Safety*, vol. 65, no. 2, pp. 218–229, 2006.

[36] S. Gao, X. Duan, X. Wang et al., "Curcumin attenuates arsenic-induced hepatic injuries and oxidative stress in experimental mice through activation of Nrf2 pathway, promotion of arsenic methylation and urinary excretion," *Food and Chemical Toxicology*, vol. 59, pp. 739–747, 2013.

[37] I. E. Britannica, *Encyclopedia Britannica Almanac 2008*, Encyclopaedia Britannica, Chicago, IL, USA, 2009.

[38] S. Tripathi and A. Kumar, "Effect of acute and chronic exposure of sodium arsenite (Na_3AsO_3) on total protein, albumin, and globulin in serum of Oryctolagus cuniculusL," *Toxicological and Environmental Chemistry*, vol. 93, no. 2, pp. 307–313, 2011.

[39] J. T. Busher, "Serum albumin and globulin," *Clinical Methods: The History, Physical, and Laboratory Examinations*, vol. 3, pp. 497–499, 1990.

[40] S. Banerjee, A. Mahanty, S. Mohanty, D. G. Mazumder, P. Cash, and B. P. Mohanty, "Identification of potential biomarkers of hepatotoxicity by plasma proteome analysis of arsenic-exposed carp Labeo rohita," *Journal of Hazardous Materials*, vol. 336, pp. 71–80, 2017.

[41] B. Chen, Q. Liu, A. Popowich et al., "Therapeutic and analytical applications of arsenic binding to proteins," *Metallomics*, vol. 7, no. 1, pp. 39–55, 2015.

[42] Y.-Y. Xu, Q.-B. Zeng, M.-L. Yao, C. Yu, J. Li, and A.-H. Zhang, "A possible new mechanism and drug intervention for kidney damage due to arsenic poisoning in rats," *Toxicology Research*, vol. 5, no. 2, pp. 511–518, 2016.

[43] C. Sosa, N. Guillén, S. Lucea, and V. Sorribas, "Effects of oral exposure to arsenite on arsenic metabolism and transport in rat kidney," *Toxicology Letters*, vol. 333, pp. 4–12, 2020.

[44] M. Raeeszadeh, M. Moradi, P. Ayar, and A. Akbari, "The antioxidant effect of medicago sativa L.(Alfalfa) ethanolic extract against mercury chloride (HgCl2) toxicity in rat liver and kidney: an in vitro and in vivo study," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 8388002, 2021.

[45] M. I. Yousef, S. A. M. Omar, M. I. El-Guendi, and L. A. Abdelmegid, "Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat," *Food and Chemical Toxicology*, vol. 48, no. 11, pp. 3246–3261, 2010.

[46] K. Jomova, Z. Jenisova, M. Feszterova et al., "Arsenic: toxicity, oxidative stress and human disease," *Journal of Applied Toxicology: JAT*, vol. 31, no. 2, pp. 95–107, 2011.

[47] D. K. Gupta, M. Inouhe, M. Rodríguez-Serrano, M. C. Romero-Puertas, and L. M. Sandalio, "Oxidative stress and arsenic toxicity: role of NADPH oxidases," *Chemosphere*, vol. 90, no. 6, pp. 1987–1996, 2013.

[48] M. Raeeszadeh, B. Karimfar, A. Akbar Amiri, and A. Akbari, "Protective effect of nano-vitamin C on infertility due to oxidative stress induced by lead and arsenic in male rats," *Journal of Chemistry*, vol. 2021, Article ID 9589345, 2021.

[49] "Leaf-nosed bat," in *Encyclopædia Britannica*Encyclopædia Britannica, Chicago, IL, USA, 2009.

[50] M. Raeeszadeh and M. Fallah, "The comparison of the effect of origanum vulgar aqueous extract and vitamin C on the control of cadmium chloride damage in testicular tissue in male rats," *Journal of Babol University of Medical Sciences*, vol. 20, no. 8, pp. 44–50, 2018.

[51] M. Qaderi Forough, M. Raeeszadeh, and A. Amiri, "Dose-response changes of *Brassica oleracea* var. italica hydro-alcholic extract in the control of oxidative stress by induction of diazinon on the cells of testicular tissue in male adult rat," *Journal of Rafsanjan University of Medical Sciences*, vol. 16, no. 7, pp. 593–604, 2017.

[52] D. Sharma and G. K. Sangha, "Triazophos induced neuro-splenic toxicity and evaluation of antioxidative potential of aqueous Broccoli extract in Wistar albino rats," *Journal of Applied and Natural Science*, vol. 13, no. 2, pp. 616–626, 2021.

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2021, Article ID 6140378, 11 pages
https://doi.org/10.1155/2021/6140378

Research Article

Structure-Based Virtual Screening of Benzaldehyde Thiosemicarbazone Derivatives against DNA Gyrase B of *Mycobacterium tuberculosis*

Andiyappan Kistan,[1] Balakrishnan Anna Benedict,[1] Sundaramoorthy Vasanthan,[1] Alphonse PremKumar,[1] Malathi Kullappan,[2] Jenifer Mallavarpu Ambrose,[2] Vishnu Priya Veeraraghavan,[3] Gayathri Rengasamy,[3] and Krishna Mohan Surapaneni[4]

[1]*Department of Chemistry, Panimalar Institute of Technology, Poonamallee, Chennai 600 123, Tamil Nadu, India*
[2]*Department of Research, Panimalar Medical College Hospital & Research Institute, Varadharajapuram, Poonamallee, Chennai 600 123, Tamil Nadu, India*
[3]*Department of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai 600 077, Tamil Nadu, India*
[4]*Departments of Biochemistry, Molecular Virology, Research, Clinical Skills & Simulation, Panimalar Medical College Hospital & Research Institute, Varadharajapuram, Poonamallee, Chennai 600 123, Tamil Nadu, India*

Correspondence should be addressed to Krishna Mohan Surapaneni; krishnamohan.surapaneni@gmail.com

Received 16 October 2021; Accepted 24 November 2021; Published 13 December 2021

Academic Editor: Saravanan Ramachandran

Emergence of antibiotic-resistant *Mycobacterium tuberculosis* (*M. tuberculosis*) restricts the availability of drugs for the treatment of tuberculosis, which leads to the increased morbidity and mortality of the disease worldwide. There are many intrinsic and extrinsic factors that have been reported for the resistance mechanism. To overcome such mechanisms, chemically synthesized benzaldehyde thiosemicarbazone derivatives were screened against *M. tuberculosis* to find potential inhibitor for tuberculosis. Such filtering process resulted in compound 13, compound 21, and compound 20 as the best binding energy compounds against DNA gyrase B, an important protein in the replication process. The ADMET prediction has shown the oral bioavailability of the novel compounds.

1. Introduction

Tuberculosis (TB) is a potentially serious communicable disease caused by the bacillus *Mycobacterium tuberculosis* (*M. tuberculosis*) [1]. TB was found with increased mortality, and it spreads from person to person through tiny droplets released into the air via coughs and sneezes [2]. According to the World Health Organization (WHO) report, worldwide, around 10 million people were affected with TB [3]. Here, 56% were men, 32% were women, and children accounted for 12%. Also, 1.2 million deaths were reported in HIV-negative people and 208000 deaths were reported among HIV-positive people. The largest number of new TB cases occurred in the Southeast Asian region with 44% of new cases, followed by the African region with 25% of new cases and Western Pacific with 18%. Eight countries accounted for two-thirds of the new TB cases including India (26%), Indonesia (8.5%), China (8.4%), Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), Bangladesh (3.6%), and South Africa (3.6) [3]. *M. tuberculosis* does not have any particular virulence factor, but it perseveres long-term in the human body without causing any significant damage and transmission, if not, the immune system of the host is compromised. It secrets effector proteins to complicate the immune system,

thereby, stimulating its intracellular survival in granulomas throughout the latency period of infection [4]. *M. tuberculosis* can develop in different conditions such as pulmonary and extrapulmonary TB (pleural, lymphadenitis, skeletal, gastrointestinal, and ocular) [5]. Once *M. tuberculosis* entered into the lungs, alveolar macrophages engulf the organism, and it was captured by phagosomes. Finally, it was delivered to lysosomes and get degraded. However, in most of the instances, to persist in human alveolar macrophages, the organism inhibits the acidification and phagosomes maturation. Also, to escape from the immune system recognition and hypoxic condition, the organism remains in a "quiescent status" (in nongrowing state but metabolically active) in 90% of the diseased individuals [6]. The innate (antimycobacterial elements, IFNγ and TNFα) as well as adaptive immunity (T cells, Th17, $CD4^+$, and $CD8^+$) controls *M. tuberculosis* activity in the latent phase of the infection. Hence, it is known as the conditional pathogenic bacterium [7]. If the host immune system is compromised, the organism gets activated and initiate replication. This promotes the diseased macrophage necrosis and, thereby, discharges the intracellular bacteria, which infect novel cells and invade other tissues [8]. Recent reports suggest that TB is also related with many other human complications, namely, autoimmune diseases (sarcoidosis: *M. tuberculosis* activates toll-like receptors, thereby, promoting pulmonary sarcoidosis [9]; systemic lupus erythematosus: immunosuppressive therapy and several immune abnormalities cause reactivation and diffusion of TB [10]), metabolic syndromes (diabetes mellitus: promotes the proliferation of mycobacterium [11]; atherosclerosis: mycobacterium rushes the development of atherosclerosis [12]), and pulmonary complications (pneumonia: TB infection rises the risk of secondary bacterial infection in children [13]; chronic obstructive pulmonary disease: pulmonary TB may alter the lung architecture [14]; lung cancer: TB is one of the risk factors for the development of lung cancer [15, 16]).

Treatment options available for drug-susceptible TB involve first-line drugs (isoniazid, rifampicin, ethambutol, and pyrazinamide) for six months. The indiscriminate use of the antibiotics leads to the development of resistance. The WHO classifies the resistance as multidrug resistance (MDR), extensive drug resistance (XDR), and total drug resistance (TDR). The rise of resistance to the first-line drugs termed as multidrug resistance (MDR) and resistance to the fluoroquinolones, one of the second-line drugs (capreomycin, kanamycin, and amikacin) tends to the occurrence of XDR. Both resistance to the first and second-line drugs leads to the development of TDR [3]. The U.S Food and Drug Administration (FDA) has developed bedaquiline, a novel drug against MDR *M. tuberculosis*. Over the 40 years, bedaquiline have been in use to treat TB. Unfortunately, resistance to bedaquiline antibiotic has been reported in recent years [17, 18]. The extrinsic factors associated with antibiotic resistance are social elements of TB in inhabitants and the eminence of prevention and control services of TB. Gygli et al., in 2017, explained the mechanism of intrinsic drug resistance in *M. tuberculosis*, such as decreased cell wall permeation, secretion of drug inactivating enzymes, mutation in the cell wall efflux system, bacterial drug target modification, and overexpression of drug targets. Here, isoniazid prodrug is activated by catalase or peroxidase (gene: katG), and it functions through enoyl acyl carrier protein reductase (gene: inhA). Rifampicin acts against *M. tuberculosis* through binding to the RNA polymerase β subunit (gene: rpoB). Ethambutol inhibits arabinosyl transferase (gene: embB) involved in the biosynthesis of cell wall arabinogalactan. Pyrazinamide, a nicotinamide analog, requires pyrazinamidase or nicotinamidase (gene: pncA) to get converted into pyrazinoic acid, an active form. For second-line drugs, fluoroquinolones inhibit the topoisomerase II (DNA gyrase), an essential enzyme involved in the replication process (genes: gyrA and gyrB). The role of kanamycin and amikacin modifies the range of 16S rRNA (gene: rrs) and, thereby, inhibits the protein synthesis. The capreomycin is found to inhibit the translation process in mycobacterium. The target gene tlyA participates in the rRNA ribose specific 2′-O-methylation. Here, the development of resistance to these first-line and second-line drugs is due to the mutations in the drug-targeting genes [19–23]. Such situation necessitates the design and development of novel drug with high antitubercular activity. In the recent study, Volynets et al., in 2019, have experimentally proved the activity of benzaldehyde thiosemicarbazone against *M. tuberculosis*. Hence, in the present study, we have retrieved the synthesized benzaldehyde thiosemicarbazone derivatives from the literature and screened against one of the main target DNA gyrases to find a potential inhibitor for *M. tuberculosis* [24] through molecular modelling methods. Our study may give an idea to the researchers who are designing drug against *M. tuberculosis* in the molecular level.

2. Materials and Methods

2.1. Protein Structure Preparation. The 3-dimensional (3D) structure of the target protein DNA gyrase was obtained from the protein data bank (PDB: 6GAU) [25, 26]. The PDB structure is a homodimer, which has two chains, chain A (DNA gyrase subunit B) and B (DNA gyrase subunit A) (Figure 1). For our virtual screening and molecular docking studies, we have utilized chain A. The sequence length of the subunit is 1179 amino acids. The 3D structure was crystalized by the X-ray diffraction method. The nonamino acid structures cocrystallized with DNA gyrase, phosphoaminophosphonic acid-adenylate ester, magnesium ions, and water molecules were detached from the structure.

2.2. Active Site Prediction. The active site of the DNA gyrase subunit B was predicted through the DoGSiteScorer [27]. Finding the low molecular weight ligand molecule by the target protein is the source for maintenance of the biological system. Here, active site is crucial for the function of an enzyme [28]. Usually, the active site prediction helps to ensure the protein function, druggability, and family classification. Such prediction is carried out by the DoGSite server, a structure-based prediction method, which functions on the basis of the difference of Gaussian (DoG) method. Most of the computational techniques available are

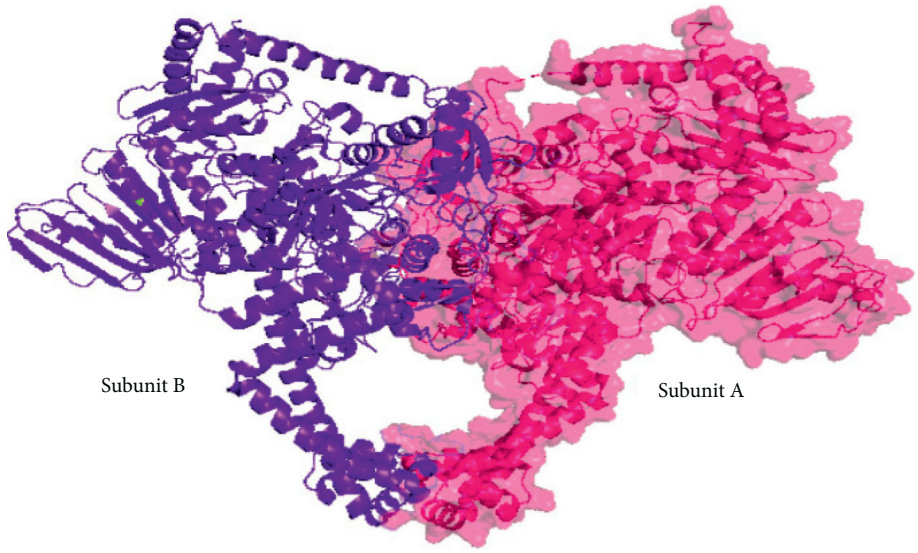


Figure 1: Structure of *M. tuberculosis* DNA gyrase.

geometry-based, energy-based, evolutionary-based, and combine method predictions. All of these approaches have disadvantages [29]. In contrast, DoGSite uses the pattern recognition method to predict the active sites. This locates the active site regions by sieving the grid of the protein using the DoG filter, and it finds the spherically designed structures called DoG cores in the grid. Finally, these detected DoG cores are gathered to form pockets, where the ligand molecules can fit firmly.

2.3. Ligand Preparation. The synthesized 30 benzaldehyde thiosemicarbazone (Figure 2) derivatives were obtained from the literature (9). The structures were drawn with the help of ChemSketch software and optimized (10) (Table 1).

2.4. Virtual Screening. A total of 30 benzaldehyde thiosemicarbazone derivatives were screened against gyrB using the python prescription virtual screening tool (PyRx) [30], a structure-based virtual screening process, which usually screens compound libraries against protein targets. Using the OpenBabel tool combined with the PyRx server, the ligand molecules were added and subjected to energy minimization with the help of the universal force field (UFF) by the conjugate gradient algorithm. Both protein and ligand structures were converted into PDBQT format. The docking procedure was carried out through the AutoDock Vina of PyRx Tool. From the 30 compounds, best binding energy was selected and passed to the next procedure, docking studies to get final best compounds.

2.5. Molecular Docking Studies. Prediction of binding orientation of ligand with the protein is carried out through the molecular docking studies; in the current study, AutoDock version 4.2.6 was utilized [31]. It works on the basis of the Lamarckian genetic algorithm [32]. The target protein DNA gyrase B 3D structure was added with polar hydrogen atoms

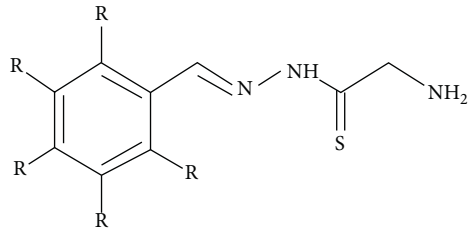


Figure 2: Structure of benzaldehyde thiosemicarbazone.

using AutoDock Tool (ADT). Both ligand and the protein structures were converted into PDBQT format. The grid was fixed with 80 × 80 x 80 size, which covers the active site of the protein. The desolvation map and grid map was created by the AutoGrid program. The algorithm was set with energy evaluation of 2500000, population of 150, 27000 generations, 0.02 mutation rate, and 0.8 crossover rate. Next, the AutoDock run was simulated, and the binding energy of protein-ligand affinity was checked to identify the best binding affinity. AutoDock produces empirical scoring functions; it is the combination of hydrogen bonding, van der Waals, electrostatic, hydrophobicity, entropy, and desolvation energies. The unit of binding energy is kcal/mol. The higher binding affinity between the complexes reflects the increased intermolecular forces. The lowest binding energy stabilizes the complex. The hydrophobic interactions were also explored using LigPlot+ v.2.2 analysis.

2.6. Absorption, Distribution, Metabolism, Excretion, and Toxicity Prediction. The pharmacokinetic properties such as absorption (A), distribution (D), metabolism (M), excretion (E), and toxicity (T) were predicted through the online server ADMET structure activity relationship (admetSAR). The database has structure and text search options, and also, the database collects, curates, and holds ADMET-related properties data from the available literature. The database

Table 1: PyRx (AutoDock Vina) virtual screening results for benzaldehyde thiosemicarbazone derivatives against DNA gyrase subunit B.

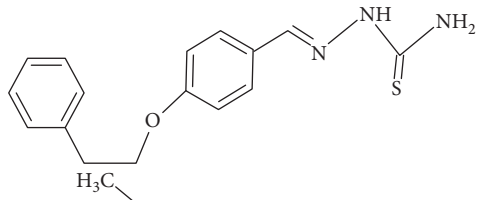
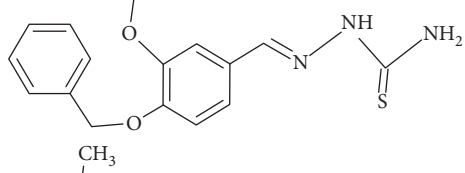
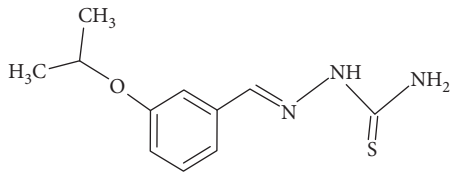
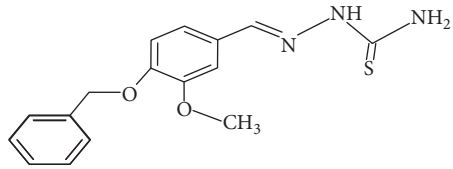
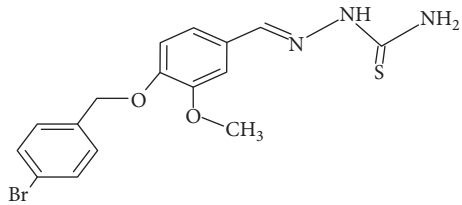
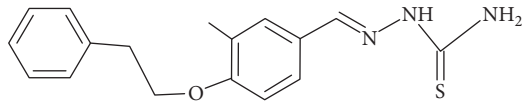
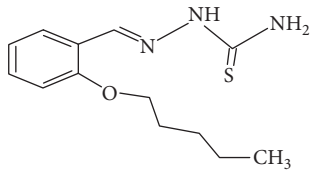
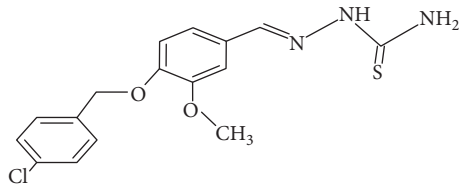
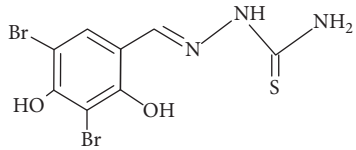
S. no.	Derivatives	Structure	Binding affinity (kcal/mol)
1	Compound 13		−8.5
2	Compound 21		−7.8
3	Compound 20		−7.7
4	Compound 5		−6.9
5	Compound 7		−6.9
6	Compound 1		−6.7
7	Compound 26		−6.7
8	Compound 6		−6.7
9	Compound 28		−6.6

Table 1: Continued.

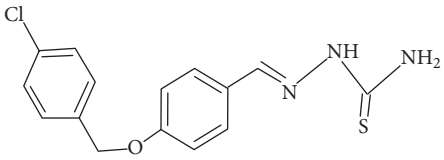
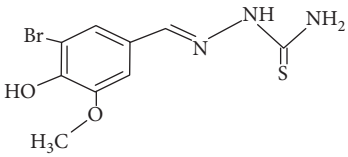
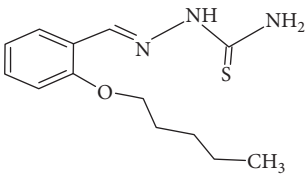
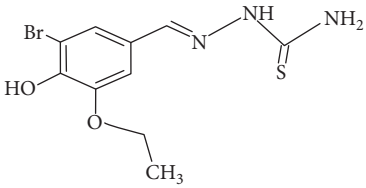
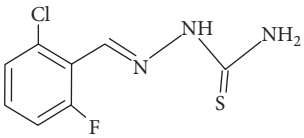
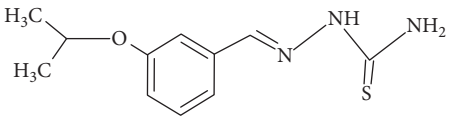
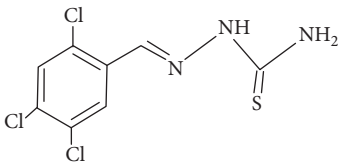
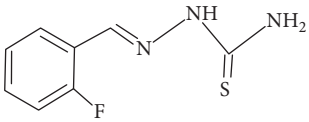
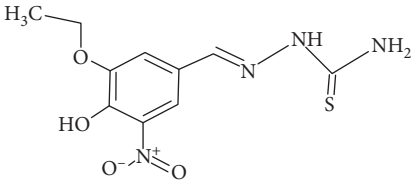
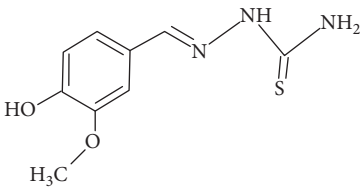
S. no.	Derivatives	Structure	Binding affinity (kcal/mol)
10	Compound 15		−6.6
11	Compound 9		−6.5
12	Compound 27		−6.5
13	Compound 10		−6.4
14	Compound 25		−6.3
15	Compound 19		−6.2
16	Compound 29		−6.2
17	Compound 24		−6.2
18	Compound 30		−6.1
19	Compound 8		−6.1

Table 1: Continued.

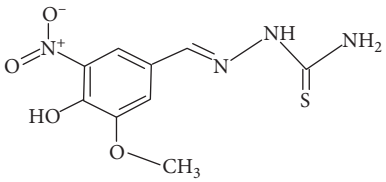
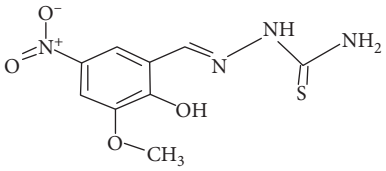
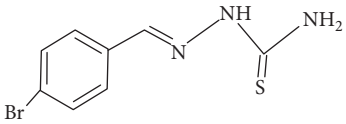
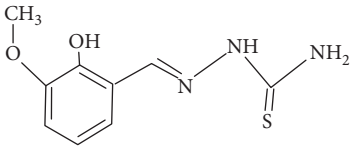
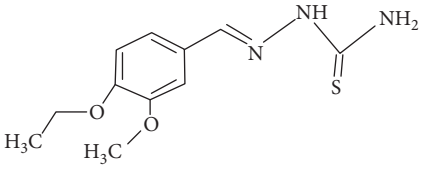
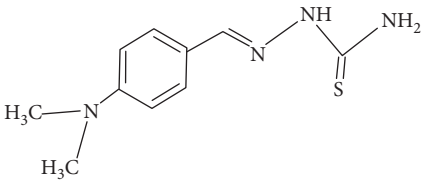
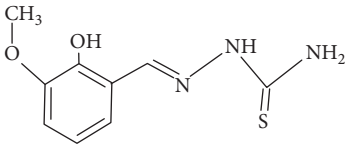
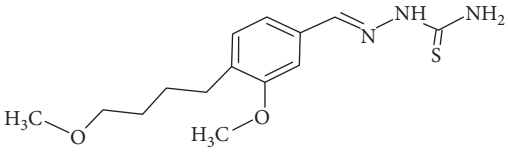
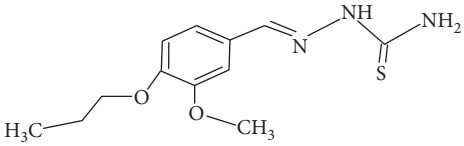
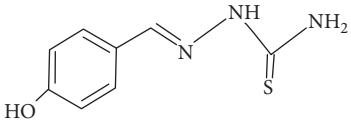
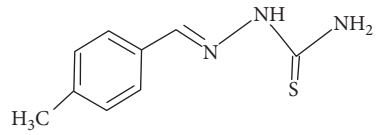
S. no.	Derivatives	Structure	Binding affinity (kcal/mol)
20	Compound 11		−6.1
21	Compound 12		−6.1
22	Compound 17		−6.1
23	Compound 22		−6.0
24	Compound 4		−5.9
25	Compound 14		−5.9
26	Compound 23		−5.9
27	Compound 2		−5.8
28	Compound 3		−5.7
29	Compound 18		−5.7

Table 1: Continued.

S. no.	Derivatives	Structure	Binding affinity (kcal/mol)
30	Compound 16		−5.6

includes 210,000 curated data for greater than 96,000 ligand molecules with 45 different ADMET-related properties, species, and proteins. The admetSAR interface is to search particular ligand profile, common name, and analog search. It has 5 quantitative regression models and 22 qualitative classifications with increased prediction accuracy. ADMET prediction is a distinctive interdisciplinary linking between the biologist, medicinal chemist, formulators, toxicologist, and regulators across India. The ADMET prediction of drug candidates has majorly reduced the drug failure in clinical trials [33].

3. Results and Discussion

TB, malaria, and acquired immune deficiency syndrome (AIDS) are the top most fatal infectious disease becoming a worldwide public health threat. The development of TB causes increased morbidity and mortality [34]. Different combinations of drugs have been given in treatment. There are several factors that have been reported for the failure of TB treatment, namely, delay in diagnosis, lack of effective drug administration, decreased accessibility of inexpensive, low toxic and effective drugs, prolong intake of drugs, lack of adherence to drug regimen, and rise of drug-resistant TB strains. Hence, to overcome the resistance mechanism, novel compounds were synthesized against the *M. tuberculosis*, and molecular modelling studies were carried out to find the potential drug candidate. The DoGSite server predicted the binding site pocket on the DNA gyrase B, which covered the area of 2834.65 $Å^3$, surface 3418.38 $Å^2$, and the drug score is 0.81. The drug score falls between 0 and 1, and the higher drug score reveals the high potential of the predicted pocket on the protein (Figure 3).

Virtual screening of 30 benzaldehyde thiosemicarbazone derivatives against DNA gyrase B reveals the high binding affinity of compound 13 with the binding energy of −8.5 kcal/mol (Table 1), followed by compounds 20 and 21 with the binding energies of −7.7 kcal/mol and −7.8 kcal/mol. Here, both compounds 20 and 21 have more or less similar binding energies. Other compounds, namely, 1, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 19, 22, 24, 25, 26, 27, 28, 29, and 30 have their binding energies ranging from −6.0 to −6.9 kcal/mol. Also, the binding energies of compounds 2, 3, 4, 14, 16, 18, and 23 range from −5.6 to −5.9 kcal/mol. From Table 1, 10 best binding energy compounds were selected and passed through the molecular docking simulation studies using the AutoDock Tool.

Molecular docking studies of 10 best compounds against DNA gyrase B result in compound 13 as the best compound with the binding energy of −8.2 kcal/mol, which has formed four amino acid interactions with the bond distances of

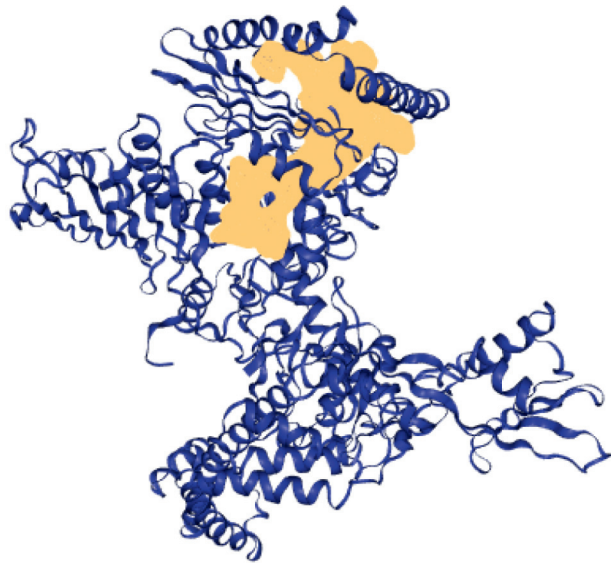


Figure 3: Binding pocket of DNA gyrase B.

ILE493: 2.0 Å, SER413: 1.9 Å, LYS409: 2.8 Å, and SER12: 2.6 and 2.1 Å, and 11 hydrophobic interactions, namely, LYS409, SER413, ALA416, GLY296, TYR297, ARG492, ALA491, SER298, HIS514, GLU352, and PHE551 (Figure 4). Next, compound 21 is with −7.5 kcal/mol of binding energy, 3 hydrogen bond interactions such as GLY612 (2.1 Å), ARG634 (1.8 Å), and PRO567 (2.0 Å), and 12 hydrophobic interactions of MET616, LYS611, LEU613, PRO566, ALA533, TYR610, GLN538, SER541, ALA531, ALA564, LEU563, and LEU529 (Figure 5). Here, compounds 20 and 21 have only slight variations in their binding energies (Table 2). Compound 20 has the binding energy of −6.9 kcal/mol. The amino acids, namely, GLY612 (2.1 Å), ARG634 (1.8 Å), and PRO567 (2.0 Å) have been found in the hydrogen bond interaction. In hydrophobic interaction, the amino acids MET616, LYS611, LEU613, PRO566, ALA533, TYR610, GLN538, SER541, ALA531, ALA564, LEU563, and LEU529 have been found in the interaction (Figure 6). The higher the hydrophobic interaction, the lower the hydrogen bond interaction and vice versa. Our findings are similar to that of the experimental studies carried out by Volynets et al. [24]. The in vitro studies on benzaldehyde thiosemicarbazone derivatives against *M. tuberculosis* results in the minimal inhibitory concentration (MIC) of 0.68 μM for compound 13 and 0.74 μM for compound 20. In our findings, compound 21 is the second most high binding energy compound. Also, compounds 20 and 21 slightly vary in their binding energies.

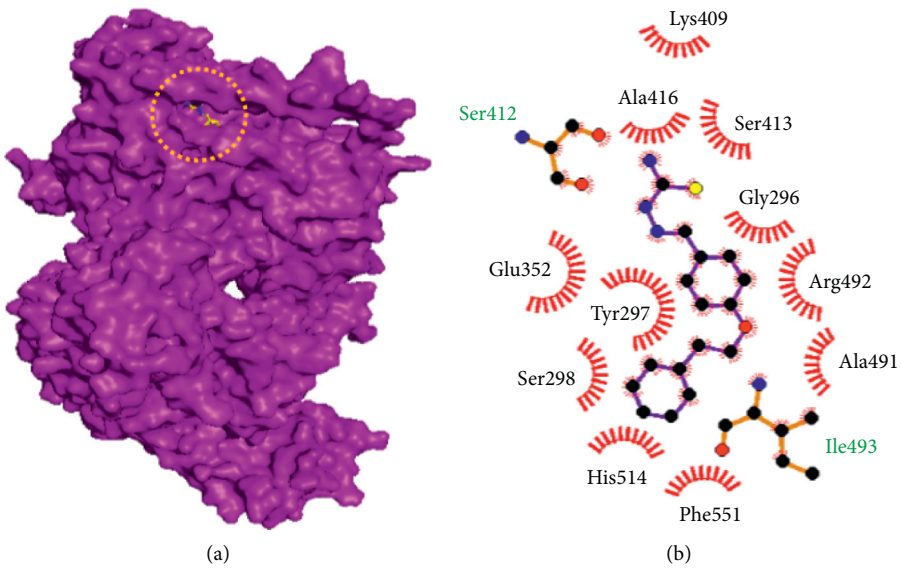


Figure 4: Binding mode of DNA gyrase B with the benzaldehyde thiosemicarbazone derivative compound 13. (a) Binding mode of compound 13 in the active site of DNA gyrase B. (b) Hydrophobic interactions of DNA gyrase B and compound 13.

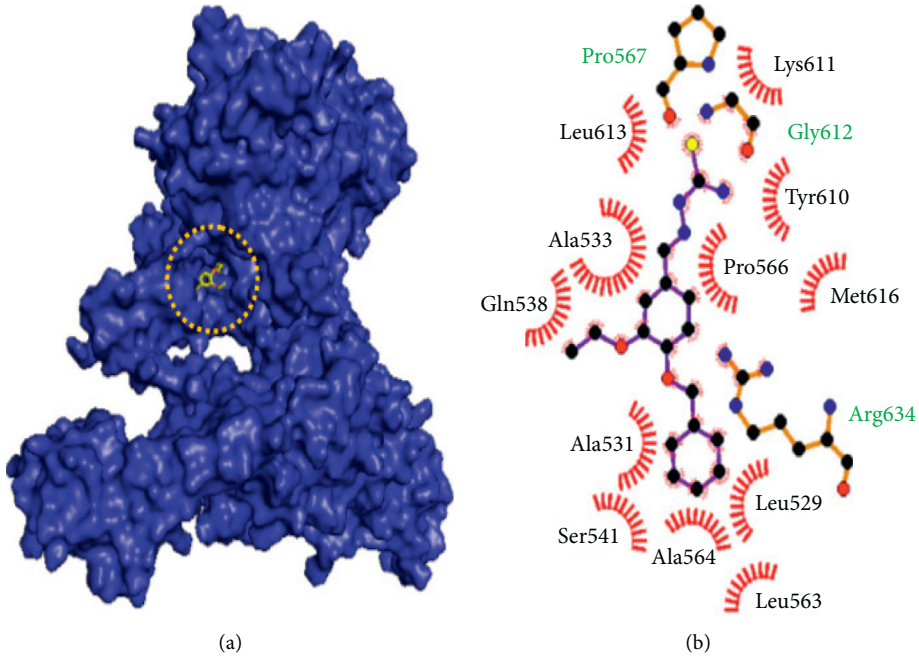


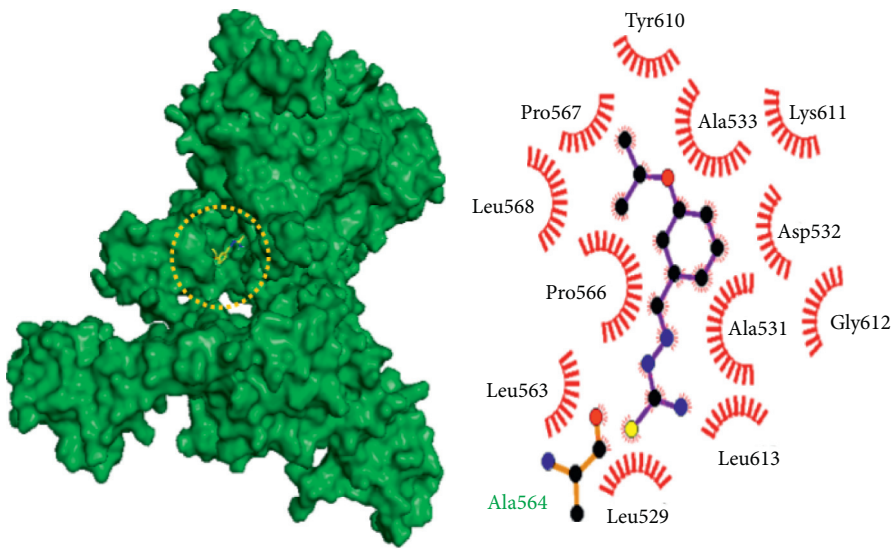
Figure 5: Binding mode of DNA gyrase B with the benzaldehyde thiosemicarbazone derivative compound 21. (a) Binding mode of compound 21 in the active site of DNA gyrase B. (b) Hydrophobic interactions of DNA gyrase B and compound 21.

Similar computational studies have been reported in the screening of antimicrobial compounds. Pertersen et al. [35] have used the virtual screening techniques to identify novel inhibitor for *M. tuberculosis* 3-dehydroquinate. Similarly, another study has employed pharmacophore-based virtual screening to identify the pyrazolo [1,5-*a*] pyrimidine derivatives against InhA of *M. tuberculosis* [36, 37].

Here, the first-line antimicrobial drugs rifampicin and isoniazid have lower energies −6.7 kcal/mol and −5.0 kcal/mol than the benzaldehyde thiosemicarbazone derivatives

Table 2: AutoDock results for benzaldehyde thiosemicarbazone derivatives against DNA gyrase subunit B.

S. no:	Compounds	Binding energy (kcal/mol)	Amino acid interactions and distances (Å)	Hydrophobic interactions
1	Compound 13	−8.2	ILE493 (2.0 Å), SER413 (1.9 Å), LYS409 (2.8 Å), and SER12 (2.6 and 2.1 Å)	LYS409, SER413, ALA416, GLY296, TYR297, ARG492, ALA491, SER298, HIS514, GLU352, and PHE551
2	Compound 21	−7.5	GLY612 (2.1 Å), ARG634 (1.8 Å), and PRO567 (2.0 Å)	MET616, LYS611, LEU613, PRO566, ALA533, TYR610, GLN538, SER541, ALA531, ALA564, LEU563, and LEU529
3	Compound 20	−6.9	ALA564 (1.9 Å) and ALA531 (2.0 Å)	TYR610, LEU613, ALA533, GLY612, PRO567, LEU568, PRO566, ASP532, LYS611, LEU529, LEU563, and ALA531
4	Levofloxacin	−7.0	ASN309 (2.5 Å) and ARG40 (2.0 Å)	LEU200, THR307, PHE304, GLU196, MET197, ASP639, TRP47, ASP640, ARG193, ASN309, ILE308, and HIS44
5	Rifampicin	−6.7	THR250 (2.3 Å), ASP259 (2.1 Å and 2.2 Å), and LYS262 (2.0 Å)	VAL301, ARG550, ASP348, HIS514, GLU317, HIS311, GLU312, PRO554, ASN558, and GLU557
6	Isoniazid	−5.0	SER1027 (2.7 Å), ALA531 (2.0 Å), LEU529 (2.1 Å), and ALA564 (2.2 Å)	ALA564, MET530, ARG634, LEU563, SER541, GLY537, GLU1023, and SER1027



(a) (b)

Figure 6: Binding mode of DNA gyrase B with the benzaldehyde thiosemicarbazone derivative compound 20. (a) Binding mode of compound 20 in the active site of DNA gyrase B. (b) Hydrophobic interactions of DNA gyrase B and compound 20.

Table 3: ADMET prediction for lead compounds.

S. no.	Models	Compound 13	Compound 21	Compound 20
		Absorption		
1	Blood-brain barrier	BBB+	BBB+	BBB+
2	Human intestinal absorption	HIA+	HIA+	HIA+
3	Caco-2 permeability	Caco-2	Caco-2	Caco-2
4	P-Glycoprotein substrate	Nonsubstrate	Nonsubstrate	Nonsubstrate
5	P-Glycoprotein inhibitor	Noninhibitor	Noninhibitor	Noninhibitor
6	Renal organic cation transporter	Inhibitor	Noninhibitor	Noninhibitor
		Metabolism		
7	CYP450 2C9 substrate	Nonsubstrate	Nonsubstrate	Nonsubstrate
8	CYP450 2D6 substrate	Nonsubstrate	Nonsubstrate	Nonsubstrate
9	CYP450 3A4 substrate	Nonsubstrate	Nonsubstrate	Nonsubstrate
10	CYP450 1A2 inhibitor	Inhibitor	Inhibitor	Inhibitor
11	CYP450 2C9 inhibitor	Inhibitor	Inhibitor	Inhibitor
12	CYP450 2D6 inhibitor	Noninhibitor	Noninhibitor	Noninhibitor
13	CYP450 2C19 inhibitor	Noninhibitor	Inhibitor	Inhibitor

Table 3: Continued.

S. no.	Models	Compound 13	Compound 21	Compound 20
14	CYP450 3A4 inhibitor	Noninhibitor	Noninhibitor	Noninhibitor
		Toxicity		
15	AMES toxicity	Non-AMES toxic	Non-AMES toxic	Non-AMES toxic
16	Carcinogens	Noncarcinogens	Noncarcinogens	Noncarcinogens

compounds 13, 20, and 21, respectively. The second-line drug levofloxacin is −7.0 kcal/mol.

From the virtual screening and molecular docking studies, it is confirmed that compound 13 could serve as a potential inhibitor for *M. tuberculosis*. Further preclinical studies have to be conducted to confirm the antimicrobial activity of the compound.

3.1. ADMET Prediction. ADMET prediction of benzaldehyde thiosemicarbazone derivatives using admetSAR server results in intestinal absorption capacity of the compounds 13, 21, and 20. These compounds also act as a nonsubstrate and noninhibitor for P-glycoprotein. Compound 13 serves as an inhibitor for renal organic cation transporter and other two compounds 20 and 21 as a noninhibitor. In the metabolism, for cytochrome P450 2C9, 2D6, and 3A4, the compounds 13, 21, and 20 possess nonsubstrate property and for cytochrome P450 1A2, 2C9, 2D6, 2C19, and 3A4, it results in noninhibitory activity. Toxicity prediction reveals the non-AMES toxicity and noncarcinogenic activity. Overall, the ADMET prediction further confirms the bioavailability of the compounds (Table 3).

4. Conclusion

The molecular modelling studies on 30 benzaldehyde thiosemicarbazone derivatives reveals the best binding energy of compound 13, compound 21, and compound 20 against DNA gyrase B of *M. tuberculosis*. To further confirm the activity of these compounds, preclinical studies have to be conducted.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

AK., ABB., VA., PKA., JMA., MK., and SKM conceptualized the study and wrote the original draft of the article. AK., ABB., MK., JMA., and SKM developed methodology, performed formal analysis and investigation. AK., ABB., VA., PKA., JMA., MK., VPV., GR., and SKM wrote and reviewed the article. VPV., GR., and SKM acquired fund and collected resources. SKM supervised the study.

Acknowledgments

MK, JMA, and SKM would like to thank the management of PMCHRI for providing the necessary facilities to carry out this research work.

References

[1] M. Pai, M. A. Behr, D. Dowdy et al., "Tuberculosis," *Nature Reviews Disease Primers*, vol. 2, p. 16076, 2016.

[2] C. J. L. Murray and G. T. Collaborators, "The global burden of tuberculosis: results from the Global Burden of Disease Study 2015," *The Lancet Infectious Diseases*, vol. 18, pp. 261–284, 2018.

[3] WHO, *Global Tuberculosis Report 2019*, WHO, Geneva, Switzerland, 2019, https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf.

[4] M. I. Groschel, F. Sayes, R. Simeone, L. Majlessi, and R. Brosch, "ESX secretion systems: mycobacterial evolution to counter host immunity," *Nature Reviews Microbiology*, vol. 14, no. 11, pp. 677–691, 2016.

[5] M. Shah and N. Chida, "Extrapulmonary tuberculosis," in *Handbook of Tuberculosis*, J. H. Grosset and R. E. Chaisson, Eds., Springer International Publishing, Cham, Switzerland, pp. 91–118, 2017.

[6] S. Bhavanam, G. R. Rayat, M. Keelan, D. Kunimoto, and S. J. Drews, "Understanding the pathophysiology of the human TB lung granuloma using in vitro granuloma models," *Future Microbiology*, vol. 11, pp. 1073–1089, 2016.

[7] C. J. Cambier, S. Falkow, and L. Ramakrishnan, "Host evasion andexploitation schemes of Mycobacterium tuberculosis," *Cell*, vol. 159, pp. 1497–1509, 2014.

[8] N. K. Dutta and P. C. Karakousis, "Latent tuberculosis infection: myths, models, and molecular mechanisms," *Microbiology and Molecular Biology Reviews*, vol. 78, pp. 343–371, 2014.

[9] M. I. Gabrilovich, J. Walrath, J. van Lunteren et al., "Disordered toll-like receptor 2 responses in the pathogenesis of pulmonary sarcoidosis," *Clinical and Experimental Immunology*, vol. 173, pp. 512–522, 2013.

[10] J. E. Yun, S. W. Lee, T. H. Kim et al., "The incidence and clinical characteristics of *Mycobacterium tuberculosis* infection among systemic lupus erythematosus and rheumatoid arthritis patients in Korea," *Clinical & Experimental Rheumatology*, vol. 20, pp. 127–132, 2002.

[11] N. P. Kumar, P. J. George, P. Kumaran, C. K. Dolla, T. B. Nutman, and S. Babu, "Diminished systemic and antigen-specific type 1, type 17, and other proinflammatory cytokines in diabetic and prediabetic individuals with latent *Mycobacterium tuberculosis* infection," *The Journal of Infectious Diseases*, vol. 210, pp. 1670–1678, 2014.

[12] C. Wick, E. Onestingel, E. Demetz, H. Dietrich, and G. Wick, "Oral tolerization with mycobacterial heat shock protein 65 reduces chronic experimental atherosclerosis in aged mice," *Gerontology*, vol. 64, pp. 36–48, 2017.

[13] J. N. Oliwa, J. M. Karumbi, B. J. Marais, S. A. Madhi, and S. M. Graham, "Tuberculosis as a cause or comorbidity of childhood pneumonia in tuberculosis-endemic areas: a systematic review," *Lancet Respiratory Medicine*, vol. 3, pp. 235–243, 2015.

[14] T. S. Jordan, E. M. Spencer, and P. Davies, "Tuberculosis, bronchiectasis and chronic airflow obstruction," *Respirology*, vol. 15, pp. 623–628, 2010.

[15] D. M. Shin, C. S. Yang, J. Y. Lee et al., "*Mycobacterium tuberculosis* lipoprotein-induced association of TLR2 with protein kinase C zeta in lipid rafts contributes to reactive oxygen species-dependent inflammatory signalling in macrophages," *Cellular Microbiology*, vol. 10, pp. 1893–1905, 2008.

[16] L. Bell and M. Noursadeghi, "Pathogenesis of HIV-1 and *Mycobacterium tuberculosis* co-infection," *Nature Reviews Microbiology*, vol. 16, pp. 80–90, 2018.

[17] K. Andries, C. Villelas, N. Coeck et al., "Acquired resistance of Mycobacterium tuberculosisto bedaquiline," *PLoS One*, vol. 9, Article ID e102135, 2014.

[18] H. Hoffmann, T. A. Kohl, S. Hofmann-Thiel et al., "Delamanid and bedaquiline resistance inMycobacterium tuberculosis ancestral Beijing genotype causing XDR-TB in a tibetian refugee," *American Journal of Respiratory and Critical Care Medicine*, vol. 193, pp. 337–340, 2016.

[19] P. Lempens, C. J. Meehan, K. Vandelannoote et al., "Isoniazid resistance levels of *Mycobacterium tuberculosis* can largely be predicted by high-confidence resistance-conferring mutations," *Scientific Reports*, vol. 8, p. 3246, 2018.

[20] P. Ma, T. Luo, L. Ge et al., "Compensatory effects of *M. tuberculosis* rpoB mutations outside the rifampicin resistance-determining region," *Emerging Microbes & Infections*, vol. 10, no. 1, pp. 743–752, 2021.

[21] K. Li, Z. Yang, J. Gu, M. Luo, J. Deng, and Y. Chen, "Characterization of *pncA* mutations and prediction of PZA resistance in *Mycobacterium tuberculosis* clinical isolates from chongqing, China," *Frontiers in Microbiology*, vol. 11, Article ID 594171, 2021.

[22] I. Chaoui, A. Oudghiri, and M. El Mzibri, "Characterization of gyrA and gyrB mutations associated with fluoroquinolone resistance in *Mycobacterium tuberculosis* isolates from Morocco," *Journal of Global Antimicrobial Resistance*, vol. 12, pp. 171–174, 2018.

[23] A. Sowajassatakul, T. Prammananan, A. Chaiprasert, and S. Phunpruch, "Molecular characterization of amikacin, kanamycin and capreomycin resistance in M/XDR-TB strains isolated in Thailand," *BMC Microbiology*, vol. 14, p. 165, 2014.

[24] G. P. Volynets, M. A. Tukalo, V. G. Bdzhola et al., "Benzaldehyde thiosemicarbazone derivatives against replicating and nonreplicating *Mycobacterium tuberculosis*," *Journal of Antibiotics*, vol. 72, pp. 218–224, 2019.

[25] S. K. Burley, H. M. Berman, G. J. Kleywegt, J. L. Markley, H. Nakamura, and S. Velankar, "Protein data bank (PDB): the single global macromolecular structure archive," *Methods in Molecular Biology*, vol. 1607, pp. 627–641, 2017.

[26] S. Petrella, E. Capton, B. Raynal et al., "Overall structures of *Mycobacterium tuberculosis* DNA gyrase reveal the role of a corynebacteriales GyrB-specific insert in ATPase activity," *Structure*, vol. 27, pp. 579–589, 2019.

[27] A. Volkamer, A. Griewel, T. Grombacher, and M. Rarey, "Analyzing the topology of active sites: on the prediction of pockets and sub-pockets," *Journal of Chemical Information and Modeling*, vol. 50, no. 11, pp. 2041–2052, 2010.

[28] A. Shulman-Peleg, R. Nussinov, and H. Wolfson, "Recognition offunctional sites in protein structures," *Journal of Molecular Biology*, vol. 339, pp. 607–633, 2004.

[29] M. Nayal and B. Honig, "On the nature of cavities on protein surfaces: application to the identification of drug-binding sites," *Proteins*, vol. 63, pp. 892–906, 2006.

[30] S. Dallakyan and A. J. Olson, "Small-molecule library screening by docking with PyRx," *Methods in Molecular Biology*, vol. 1263, pp. 243–250, 2015.

[31] G. M. Morris, R. Huey, W. Lindstrom et al., "AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility," *Journal of Computational Chemistry*, vol. 30, pp. 2785–2791, 2009.

[32] J. Fuhrmann, A. Rurainski, H. P. Lenhof, and D. Neumann, "A new Lamarckian genetic algorithm for flexible ligand-receptor docking," *Journal of Computational Chemistry*, vol. 31, no. 9, pp. 1911–1918, 2010.

[33] K. Tsaioun, B. J. Blaauboer, and T. Hartung, "Evidence-based absorption, distribution, metabolism, excretion (ADME) and its interplay with alternative toxicity methods," *Altex*, vol. 33, no. 4, pp. 343–358, 2016.

[34] V. Lohrasbi, M. Talebi, A. Z. Bialvaei et al., "Trends in the discovery of new drugs for *Mycobacterium tuberculosis* therapy with a glance at resistance," *Tuberculosis*, vol. 109, pp. 17–27, 2018.

[35] G. O. Petersen, S. Saxena, J. Renuka et al., "Structure-based virtual screening as a tool for the identification of novel inhibitors against *Mycobacterium tuberculosis* 3-dehydroquinate dehydratase," *Journal of Molecular Graphics and Modelling*, vol. 60, pp. 124–131, 2015.

[36] P. Modi, S. Patel, and M. T. Chhabria, "Identification of some novel pyrazolo [1,5-a] pyrimidine derivatives as InhA inhibitors through pharmacophore-based virtual screening and molecular docking," *Journal of Biomolecular Structure and Dynamics*, vol. 37, no. 7, pp. 1736–1749, 2019.

[37] S. M. Gygli, S. Borrell, A. Trauner, and S. Gagneux, "Antimicrobial resistance in *Mycobacterium tuberculosis*: mechanistic and evolutionary perspectives," *FEMS Microbiology Reviews*, vol. 41, no. 3, pp. 354–373, 2017.