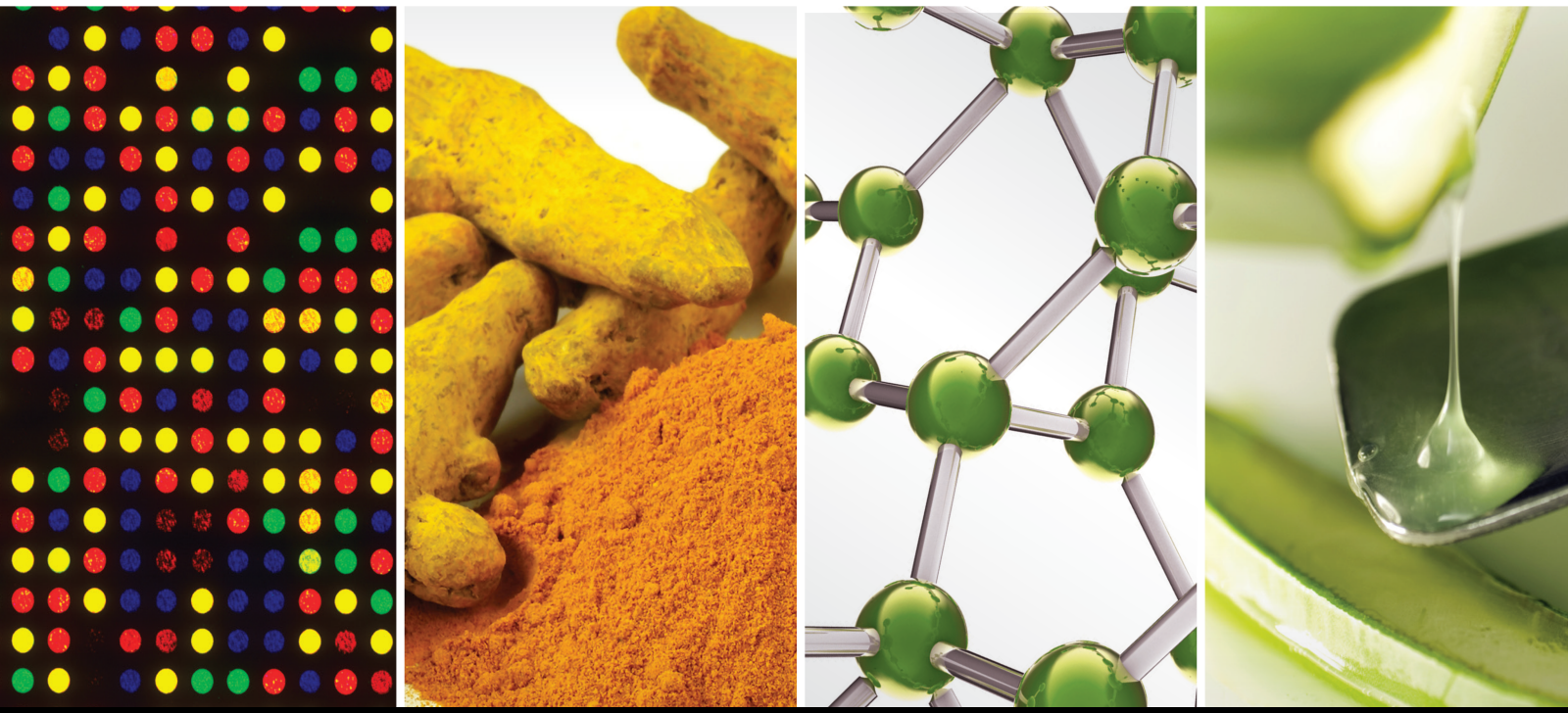
Evidence-Based Complementary and Alternative Medicine

Pharmacological Importance of the Mangroves

Lead Guest Editor: Jayanta Kumar Patra
Guest Editors: Hrudayanath Thatoi, Nabin K. Dhal, and Sergio Martinez-Luis



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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2019, Article ID 4305148, 21 pages
https://doi.org/10.1155/2019/4305148

Review Article

A Review on Antidiabetic Properties of Indian Mangrove Plants with Reference to Island Ecosystem

V. Sachithanandam, P. Lalitha, A. Parthiban, T. Mageswaran, K. Manmadhan, and R. Sridhar

Integrated Island Management Unit, Futuristic Research Division, National Centre for Sustainable Coastal Management, Ministry of Environment, Forests & Climate Change, Government of India, Chennai, Tamil Nadu 600025, India

Correspondence should be addressed to V. Sachithanandam; pondiunisachin@gmail.com and R. Sridhar; sridharaug@gmail.com

Received 9 May 2019; Revised 18 July 2019; Accepted 22 August 2019; Published 5 December 2019

Guest Editor: Jayanta Kumar Patra

Mangrove ecosystem has many potential species that are traditionally used by the coastal communities for their traditional cure for health ailments as evidenced by their extensive uses to treat hepatic disorders, diabetes, gastrointestinal disorders, anti-inflammation, anticancer, and skin diseases, etc. In recent times, the diabetes mellitus (DM), a serious physiological disorder all over the world, occur due to the relative or complete deficiency of insulin in the body, characterized by an abnormally high blood glucose level. India has a rich traditional knowledge on plant-based drug formulations that are protective and curative for many health ailments. In this context, we aimed to compile the works done on the antidiabetic activities of mangrove species from Indian coastal regions especially on Andaman and Nicobar Islands as well as some recent works reported from other countries. A total of 126 published articles and 31 mangrove species related pieces of information were gathered with reference to antidiabetic properties of mangroves. This review summarizes the chemical structures, molecular formula, molecular weight, and their biological activities with an aspiration that it might be helpful for the future bioprospecting industries who are interested in develop the natural drugs for DM.

1. Introduction

Mangrove species grow at the edge between the coastal and land area in subtropical and tropical regions of the world and are highly adapted to various temperatures, strong coastal winds, extreme tidal waves, salinity fluctuations, coastal water turbulence, river run-off, and anaerobic soil. No other wild species exhibit such physiological and morphological adaptations to the extreme conditions. The worldwide diversity of mangrove flora includes around 81 tree and shrub species of 30 genera from 17 families. Of these, Indian mangroves represent 46 true mangrove species (42 species and 4 natural hybrids) belonging to 14 families and 22 genera [1] (Figure 1). The unique ecology and ecosystem services, plant morphological characteristics, and traditional uses of mangrove plants have already drawn the attention to researchers over the years. Mangroves possess unique biochemical functions in their ecosystem and are considered as a source of novel natural/biological products. Mangroves are rich resources of compounds like polyphenols and tannins. Further, mangrove leaves also possess phenolic compounds, alkaloids, and flavonoids which serve as novel bioactive compounds.

1.1. Drugs from Traditional Knowledge. Traditional medicine has long been used as the primary source of therapeutic drugs. The attention on traditional medicine is escalating as there is substantial evidence that it can be a potential source for drugs to combat diseases. The scope and value of traditional medicine research assume greater importance in the realm of healthcare of humankind. In spite of the increased usage of synthetic drugs in modern medicine, half of the world's medicinal compounds are still derived from plants [2]. Some of the most important medicines which have

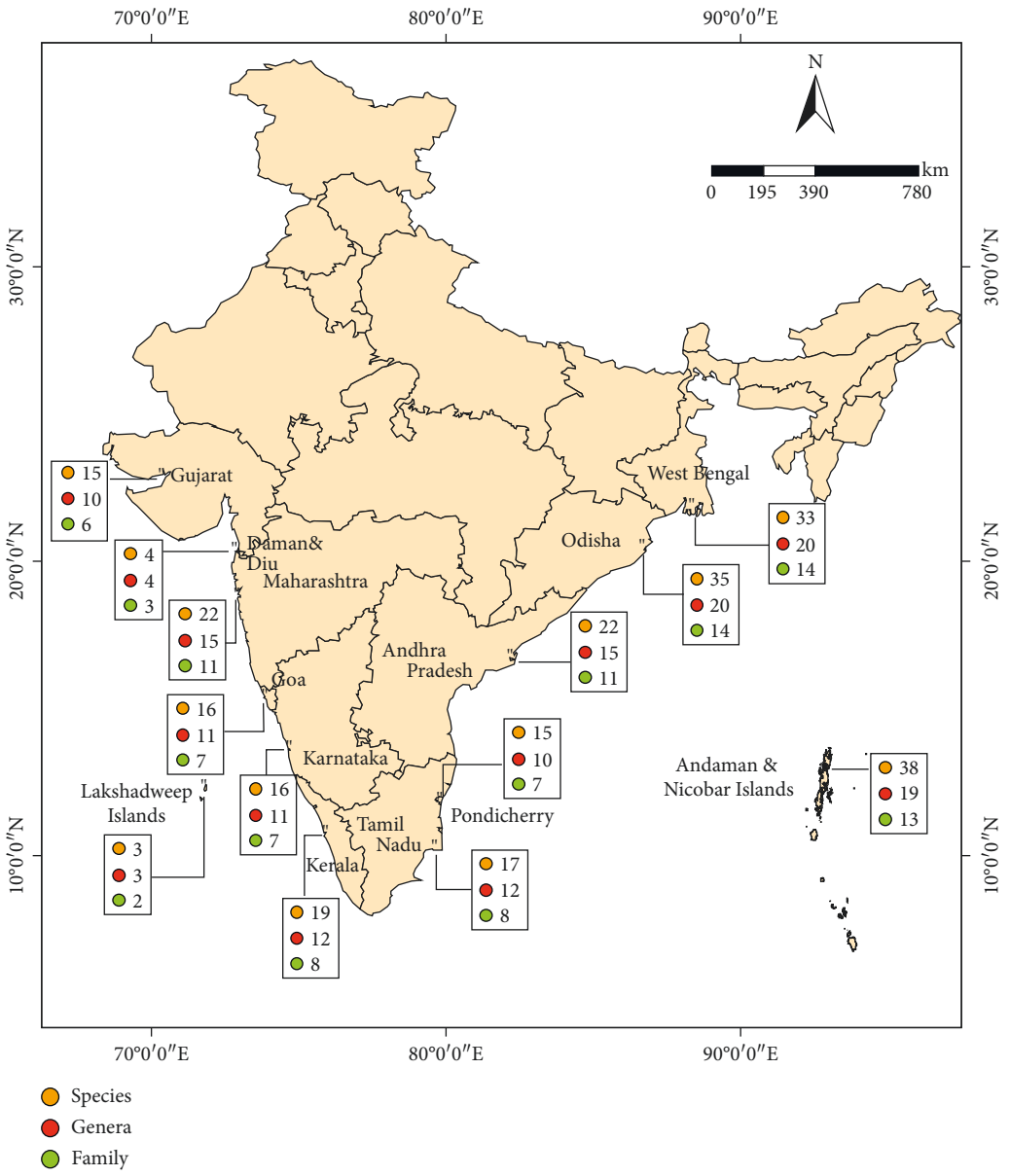


Figure 1

modernized into the modern medical systems have been isolated initially from the plants that were used by ancient society. These wonder drugs include the curare alkaloids, penicillin, and other antibiotics, cortisone [3], reserpine, veratrum alkaloids, podophyllotoxin, and other therapeutic agents [4] (Figure 2). Considering the fact that natural compounds isolated from plants are more economical and may have a holistic effect, assessing traditional knowledge for drug discovery is imperative. Morphine is a well-known product of natural plant medicine.

Drug discovery through traditional knowledge has numerous challenges due to the plethora of ancient and ethnobotanical texts describing myriads of applications of the medicinal plant [5]. Thus, a research program involving potential leads from traditional knowledge and screening based on computational methods like molecular docking, pharmacophore modeling, and molecular dynamics to identify the potential leads and final validation of potential leads through biological studies can be an economical and time-saving approach for drug discovery.

1.2. Traditional Knowledge Worldwide: A Brief. Traditional knowledge (TK) based traditional medicines (TM) are generally developed and practiced by primitive communities, based on their medicine experiences, success rate, and depending on numerous trail and errors basis [6]. World Health Organization (WHO) estimates that about 80% of the world population relies on traditional systems of medicine for primary health needs [6]. Scientific literatures from last decade [7–9] and many official factsheets published by WHO stated that, among Asian and African regions, about 85% of the people depend on TM derived by TK practitioners using various primary health care systems

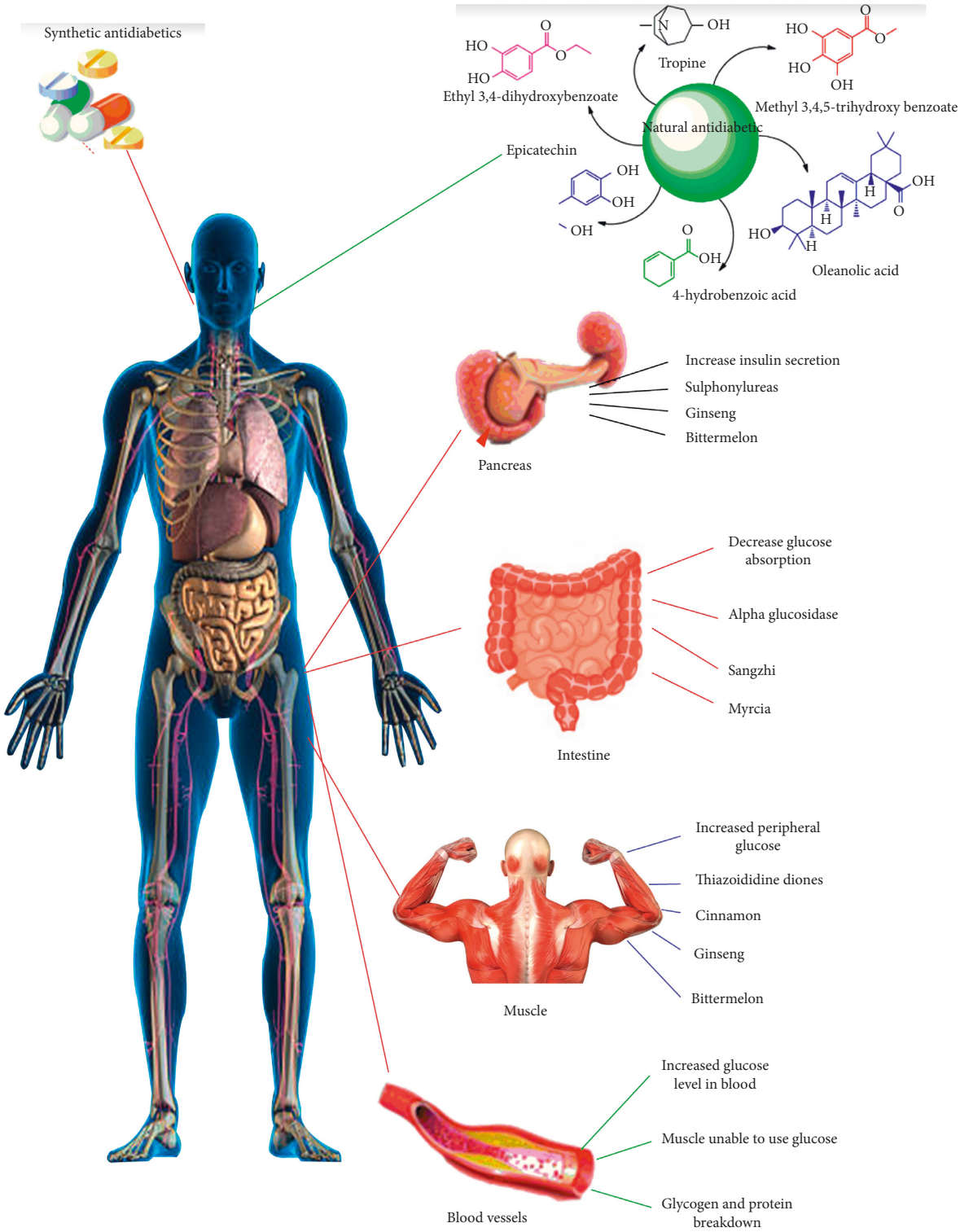


Figure 2

[10–12]. Among Southeast Asia, China alone accounts for about 40% depending on TM health care. The recently published data suggested that the use of TK in Asia and African countries is substantially declined due to the lack of documentation of TK since ancient time. The spectrum use of TM has resulted in traditional health use becoming a multinational business between the continents. For instance, in 2012, about 32 billion dollars were spent in the USA on supplementary foods originated from Southeast Asia and Africa, and this may escalate up to 80 billion dollars in 2025.

Every year, medicinal plant-based trade is growing rapidly and India's share in the global market of natural drugs is very low as it contributes only 0.5–1%, whereas demand for these products is increasing at a rapid rate [13, 14]. Developing countries and their traditional people have contributed considerably to the global drugs industry [15].

In India, the epic poems such as Vedas and others illustrated our culture, food, and habitat. The extensive use of TM in the Indian coastal region, composed mainly of plants based derivatives, has been linked to communities' composition and cultural aspects. This is why the WHO and World Bank promotes integrated approaches to documents TK and TM in their healthcare system. In the rural areas of India, 70% of the population is dependent on traditional medicines. Indigenous or traditional knowledge has developed from understanding and documenting the processes in nature. Since ethnobotany is a rapidly expanding science, beginning with the study of plants used by tribals for food, medicine, and shelter, now it includes studies like conservational practices of tribals, ethnopharmacology, ethnopharmacognosy, ethnomusicology, ethnogynaecology, etc. Ethnobotanical studies in relation to traditional communities like the tribal groups have been studied by several researchers in India including the island's ecosystem.

India is rich in its diverse natural resources and one of the seventeen biggest natural biodiversity countries of the world. It has rich vegetation of more than 45,000 plant species, out of which 15,000 to 20,000 plants are estimated to have medicinal values. Out of these, only 7,000 to 7,500 plants are used for the medicinal purpose by established communities [16, 17].

The marine biodiversity is an extremely rich resource for the development of a wide array of goods and services in the food web, pharmaceuticals, cosmetics, coastal protection, etc., but it has been extensively utilized for curing various ailments for many tribal and native communities inhabiting the coastal lines in different parts of the world. As a result of the close and respectful interaction with the marine ecosystem, the indigenous coastal communities possess marine life based rich traditional knowledge that also aids in the sustainable development of the community as well as the marine ecosystem. The valuable traditional knowledge (TK) has so far remained confined within their community and generally passed orally from one generation to another [18]. Various forms of TK, including TM knowledge, have been silently developing over the 19^{th} centuries, with the coastal tribes in nations across the world. Unfortunately, marine TK and TM have been underestimated both commercially and legally. It has still not gained its due importance at the international platform for sustainable use and development of new drugs [18, 19].

1.3. Traditional Knowledge of the Andaman and Nicobar Island. Andaman and Nicobar island (ANI), a union territory of the Republic of India, located in the Bay of Bengal, is blessed with the enchanting beauty of whitey sandy beaches, blueish coast, and unique tropical islands with biologically rich flora and fauna [20]. The Andaman and Nicobar archipelago consisting of about 525 islands and islets lies in the Bay of Bengal and forms an arched string stretching geologically from Arakan Yoma in Myanmar in the north to Sumatra in the south ($6^{o}4'$and 13^{o} $41'$N latitude and $92^{o}11'$ and $94^{o}10'$E longitude) with a land area of 8290 km^2. Floristically, there are 2654 species belonging to 1083 genera and 237 families, of which 308 species are endemic. The native people of these islands belong to two races, namely, Onges, Jarawas, Sentinelese, and Great Andamanese from Andaman Islands are probably the most primitive communities in India. The Nicobarese and the Shompen tribes are confined to Nicobar group of islands [20]. The forest resource of the islands has a rich repository of biodiversity of medicinal plants representing invaluable ethnobotanical wealth from the six indigenous tribes, namely, the Great Andamanese, Onges, Jarawas, Sentinelese, Nicobarese, and Shompens. Among them, the Great Andamanese, Onges, Jarawas, and Sentinelese originated from the Andaman Islands are probably the most primitive communities in India. The Nicobarese and the Shompen tribes are confined only to Nicobar group of islands [21].

The island's tropical forests represent nature's major storehouse of chemicals and pharmacodynamics compounds used in the perfumery, cosmetics, and pharmaceutical industries. The folklore medicinal uses of the tribes except for the Sentinels have been documented by various researchers [16, 17, 22–26]. Unfortunately, the traditional healing systems and knowledge of these aboriginals have largely eroded along with the natural resources, because of the lack of needed support and recognition, as well as the rapid destructions of their habitats through a series of unsustainable developmental activities such as urbanization, natural calamities, and sea level rise impact on small islands. Further, due to the exposure of these tribes with the outside people, modern lifestyle, education, and lack of time, they gradually forget their TK practices and culture and TM benefits over many decades [20]. Besides all these parameters, the aged TK practitioners who fully depend on the TM are putting their efforts in the transformation of knowledge from one generation to another, but due to the lack of interest in the younger generation, the values of TK and TM practices face the threat of getting vanished. In addition, ANI is located in the seismic hard zone of IV, which is more prone to the earthquake and tsunami waves. The 2004 Tsunami hit ANI very aggressively and 70% of coastal ecosystems were highly damaged, which led to the destruction of coastal habitats and more damages to inland areas.

In these circumstances, to preserve the genetic resources of the medicinal plants for the universal sustainable utilization, the IUCN has proposed a medicinal plant specialist group for making public awareness of ethnobotanical uses and for the conservation of these plant groups in the threating areas. The above statement clearly depicts that there is an urgent need to found out the entire uses of ethnomedicinal plants used by different tribes of the Indian

islands. It is also important that such bioresources are conserved and used sustainably, as the island inhabitants still source these plants from the wild for the treatment of ailments. Study of the biogeography of at least some of such medicinal plant species could be useful for further management practices. Although many research works have been carried out in the landward side for medicinal plant resources and its conservation, there is a need for the conservation and sustainable use of medicinal plants of the coastal forest areas. In this review, we attempt to cover research information of the documentation of indigenous knowledge on coastal plants and recent investigations on the biological activities of mangroves extracts especially on the antidiabetic properties, retrieved from different web sciences that focused on drug molecules identification, future perspective, research implication, and conservation of mangroves resources of ANI.

1.4. Mangrove Plant-Based Bioactive Studies. Traditionally, mangrove plants are used in folklore medicine for the treatment of several ailments including diabetes throughout worldwide [27]. Many plants are considered to be a rich source of potent antidiabetic drugs, and these herbal preparations are considered to be devoid of any side effects. Approximately, 400 plants and their secondary metabolites, namely, alkaloids, carotenoids, flavonoids, glycosides, polyphenolic, terpenoids, and tannins molecules, were used for treating DM [28, 29].

In the recent era, people from developed and developing countries are increasingly being diagnosed with diabetes. In 2016, WHO reported that approximately 400 million people globally suffer from diabetes disorder that caused about 1.6 million deaths in 2015. Further, the WHO has projected that the diabetes population will likely to be increased to 300 million in 2025 [30]. The current trends in India indicated that there is an alarming rise in the prevalence of diabetes which has gone beyond epidemic form to a pandemic one. Globally, diabetes outbreaks place an enormous amount of public health disorders. The occurrences and consequences associated with DM are found to be in high risk for countries like India (31.7%), China (20.8%), and the USA (17.7%) as reported by Balaraman et al. [6]. From this data, it is projected that by 2030, India, China, and the USA will have the largest number of people with DM [31]. Instead of being a single disorder, DM shows a series of disorders, characterized by increased fasting, postprandial glucose concentration, insulin deficiency or decreased insulin action and impaired glucose tolerance, and malfunction in lipid and protein metabolism. The long-term use of commercially available drugs for the cure of diabetes may also cause unwanted side effects. As a result, many studies are underway to find natural remedies that can effectively reduce the intensity of diabetes [32].

Therefore, the management of DM in recent times possesses a big challenge throughout the world. Not only insulin but also several types of drugs that act to reduce blood glucose (insulin secretagogues, insulin sensitizers, α-glucosidase inhibitors, peptide analogues, dipeptidyl peptidase-4 inhibitors, and glucagon-like peptide-1) have been developed by current medicinal scenario. However, these synthetic oral hypoglycemic agents possess characteristic profiles of serious side effects like hypoglycemia, weight gain, gastrointestinal discomfort (disorder), nausea, diarrhea, liver function disorder, jaundice, heart failure, etc. [33]. Therefore, alternative treatment way is the need of the hour.

A set of associated diseases (blood pressure etc.) in which the body cannot regulate the amount of sugar in the blood is called DM. The blood delivers glucose to provide the body with energy (sugar molecules) to perform a person's daily activities. The food a person eats is converted into glucose by the liver, thereby releasing the glucose into the bloodstream. In a healthy person, the blood glucose level is regulated by several hormones, primarily insulin secretion from pancreatic β-cell, a small organ between the stomach and liver. It also makes other important enzymes which are released directly into the gut and help digest food. Insulin allows glucose to exit from the blood into cells throughout the body, where it is used for fuel/energy. DM either does not produce enough insulin or cannot use insulin properly or both. In the disorder, blood glucose cannot move efficiently into cells, so blood glucose levels remain high. This not only starves all the cells that need glucose for fuel but also harms certain organs and tissues exposed to the high glucose levels [34]. Categorically, there are two types of DM recognized by the WHO, namely, Type 1 diabetes (insulin-dependent) and Type II diabetes (noninsulin-dependent). Treatment of diabetes is considered as the main global problem and successful treatment is yet to be discovered. Two major drugs like (i) insulin and (ii) oral hypoglycemic agents which are the first line of treatment for diabetes have some side effects and fail to significantly alter the course of diabetic complications [35].

Gurudeeban et al. showed that the crude extracts of *Citrullus colocynthis, Aegle marmelos,* and *Ipomoea pes-caprae* exhibited potential α-glucosidase inhibitory activity. These three plants can be exploited to treat diabetes [36]. Similarly, 5 different compounds (cysteine, phenylacetic acid, acrylamide, caprylone, and oleic acid) isolated from *Rhizophora mucronata* were evaluated for an inhibitory action on DPP IV inhibitors using in silico approach [37]. In 2014, methanolic extract of *Rhizophora apiculata* yielded 18 phytocompounds. The results of GC-MS identified 18 phytocompounds, among those major peaks were 1-adamantyl-*p*-methylbenzalimine, clivorine, 4-butyl pyridine, 1-oxide, acetamide, and *p*-aminodiethyl-amidine. These major compounds were subjected to in silico analysis on human peroxisome proliferator-activated receptor gamma protein determined by Auto DOCK 4.0 and identified as thiazolidinediones [38]. Selvaraj et al. reported that α-glucosidase is the key intestinal enzyme having clinical relevance in the treatment of DM. In the study, leaf extract of *R. apiculata* contains a huge amount of alkaloids and exhibited significant α-glucosidase inhibitory activity (250.53 ± 0.51 mg/g) [39].

Recently, it was reported that alkaloid compound like glycosine is derived from ethanolic extract of *R. apiculata*. It

showed antidiabetic/antihyperglycemic effect of glycosine in diabetic rats. The results showed that glycosine treatment significantly ($p < 0.01$) reduced the blood glucose level and increased the body weight and hemoglobin levels, high-density lipoprotein and insulin levels, protein, and the activity of hexokinase when compared to untreated rats. Decreased activities of liver function enzymes as well as the level of urea and creatinine were observed in glycosine treated rats [40]. Selvaraj et al. exhibited dichloromethane fraction (DCM-F) of *R. mucronata* on noninsulin-dependent diabetes mellitus. 100 mg/kg of DCM-F treatment in diabetes rats stimulates the action of β-cells to secrete insulin and improve antihyperglycemic conditions in NIDDM. This is also clearly evident from the carbohydrates lipid profile, plasma insulin, and marker enzymes present in the serum [41]. Selvaraj et al. revealed that *Aegiceras corniculatum* leaf extracts showed a moderate reduction in blood glucose (382 ± 34 to 105 ± 35), glycosylated hemoglobin, a decrease in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase, and an increase in activity of liver hexokinase active through the oral administration of extract of 100 mg/kg [42]. Satyavani et al. revealed that medicinally important mangrove species such as *Acanthus ilicifolius, Excoecaria agallocha, R. apiculata*, and *R. mucronata* were extracted for secondary metabolites with different solvents such as petroleum ether, diethyl ether and ethanol. A total of 135 chemical constituents were identified and compared with retention time in the NIST library in 2011. The chemical constituents were characterized into essential oils, higher alkanes, acid, alcohol, and esters. Major peaks indicated the presence of 8-pentadecane, 1, 2, 5-trimethylphyrrole, di-(2-ethylhexyl) phthalate, diethyl phthalate, epoxyhexobarbital, and cyclooctacosane [43].

Kaliamurthi et al. [44] reported on 33 medicinal plant species and documented the hypoglycemic and wound healing properties of plant species especially halophytes and its associates collected from the coastal village of Kodiyampalayam from Southeast coast of India. Kaliamurthi and Selvaraj [45] conducted several studies and elaborated the risk factors responsible for Type 2 DM including obesity, hypertension, smoking, physical inactivity, low education, dietary patterns, family history, and specific gene. Recently, researchers focused their interest on finding out the potential antidiabetic molecules from the medicinal plants to reduce the side effects caused by commercial drugs. Antinociceptive effects of *E. agallocha* against chemically and thermally induced nociception was studied on Albino mice which received a dosage of 10, 15, 20, or 25 mg/kg of alkaline chloroform fraction (Alk-CF). Compared with controls, Alk-CF decreased the writhing numbers ($p < 0.01$) in a dose-dependent manner [45]. Similarly, results showed an antinociceptive effect in mice of thillai flavonoid rutin [42].

2. Materials and Methods

This review was carried out by collecting information on relevant research findings with the help of Internet search engines like Google, Google Scholar, PubMed, ScienceDirect, and ResearchGate and other published articles, reports, and monographs. A total of 126 published articles have been reviewed and the related information was gathered for this current study with respect to antidiabetic research from Indian coastal region and from other countries.

2.1. Antidiabetic Agents from Mangrove Plants. Alkaloids brugine are 2-dithiolane (sulfur-containing) compounds, which have been isolated from *Bruguiera sexangula* (Table 1 (Sl. No. 1-2)). Three sulfur compounds along with an alkaloid brugine were reported from the stem and bark of *B. cylindrica* by Japanese scientists during 1975–1976 [50]. Similarly, the presence of acanthicifoline in *A. ilicifolius* and brugine (a sulfur containing alkaloid) in *B. sexangula* was reported by Katu and Takahashi and Richter et al., respectively [46, 47]. This study showed that *Bruguiera* sp. exhibited high anticancer activity and antidiabetic activities [48, 50].

Loder and Russell [50] identified the presence of alkaloids (tropine 2 and tropine esters of acetic acid, isobutyric acid, isovaleric acid, propionic acid, *n*- butyric acid, benzoic acid, and tropine esters of ethyl 3,4-dihydroxybenzoate) (Table 1 (Sl. No. 3–10)) in the stem and bark extracts of *B. sexangida* [50, 51]. Tropine alkaloids are medicinally useful natural products and their synthetic derivatives show anticancer, antiemetic drugs, antispasmodics, mydriatics, and cholinergic muscarinic antagonists [52].

Bioactive molecules of polysaccharides from *S. alba* are mainly derived from the seeds and have been reported to possess antidiabetic properties [53, 54]. In addition to that, complex polysaccharides show various biomedical applications such as antimicrobial, antiviral, and antihyperglycemic agent and proliferation activity for fibroblasts [55, 56]. Rutin, quercetin, kaempferol, catechin, and (-)-epicatechin in Table 1 (Sl. No. 11–15) represent flavonoids that are abundantly found in a mangrove plant species such as *R. apiculata* and *A. ilicifolius* [49]. *A. marina*, *Xylocarpus granatum,* and *B. sexangula* are reported to be rich in flavonoid compounds, namely, rutin, quercetin, kaempferol, catechin, and epicatechin that exhibited hypoglycemic activities and other biological activities such as antibacterial, antifungal antimycobacterial, antimalarial, antiretroviral, and antiviral activities [59–62].

As can be seen in Table 1 (Sl. No. 16–20), β-sitosterol (beta-sitosterol) **16**, β-amyrin **17**, α-amyrin **18**, ursolic acid **19**, and stigmasterol **20** are several phytosterols (plant sterols compounds) with chemical structures similar to that of cholesterol. These compounds (Figures D **16** and **17**) are derived from *B. gymnorhiza,* and *B. sexangula* [45, 63]. These sterols showed high anti-inflammatory activity, antidiabetic effects, inducing apoptosis, angiogenic effect, hypocholesterolemic activity, antioxidant effects, and anthelminthic and antimutagenic activities as reported by Soodabeh et al. [64].

Sun and Guo [69] first documented the presence of bartogenic acid **21** from the extract of stem, bark, and fruits of *Barringtonia racemosa* ethanolic extract. Further studies have verified the presence of bartogenic acid by K. R. Patil and C. R. Patil [70]. Bartogenic acid shows anti-DM,

Table 1: Antidiabetics agents from mangroves plants.

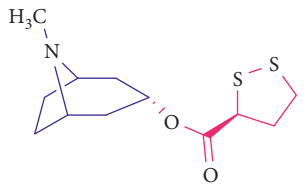
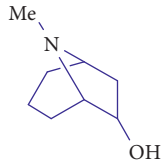
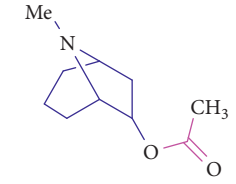
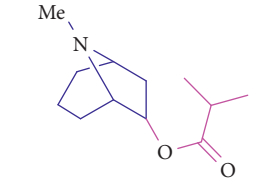
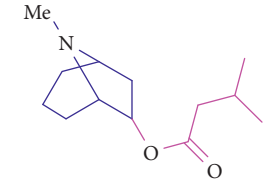
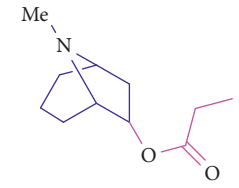
Sl. No.	Chemical structure	Description	Reference
1.	 Brugine	Source: *B. sexangula, B. cylindrica* (stem and bark) Mole. for: $C_{12}H_{19}NO_2S_2$ Mol. wt: 273.409 Biological activity: antidiabetic, anticancer	Katu and Takahashi [46] Richter et al. [47] Nebula et al. [48] Loder and Russell [50]
2.	 Tropine	Source: *B. sexangular* (stem and bark) Mole. for: $C_8H_{15}NO$ Mol. wt: 141.214 Biological activity: antidiabetic, anticancer	Loder and Russell [50] Brion et al. [51]
3.	 Tropine esters of acetic acid	Source: *B. sexangular* (stem and bark) Mole. for: $C_{10}H_{17}NO_2$ Mol. wt: 183.25 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]
4.	 Tropine esters of iosbutyric acid	Source: *B. sexangular* (stem and bark) Mole. for: $C_{12}H_2NO_2$ Mol. wt: 211.31 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]
5.	 Tropine esters of isolvaleric acid	Source: *B. sexangular* (stem and bark) Mole. for: $C_{13}H_{23}NO_2$ Mol. wt: 225.33 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]
6.	 Tropine esters of propionic acid	Source: *B. sexangular* (stem and bark) Mole. for: $C_{11}H_{19}NO_2$ Mol. wt: 197.28 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]

Table 1: Continued.

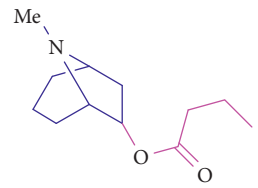
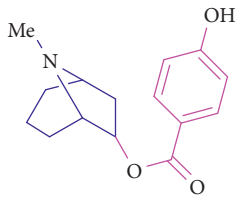
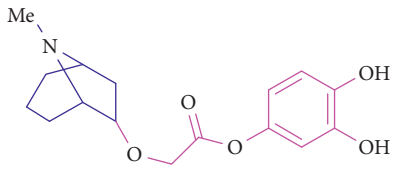
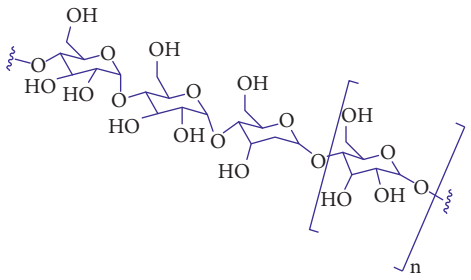
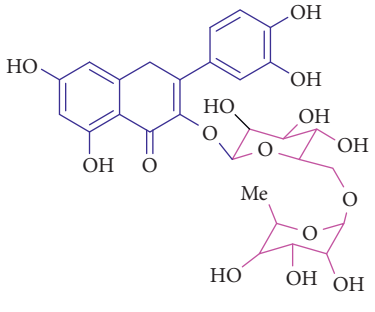
Sl. No.	Chemical structure	Description	Reference
7.	 Tropine esters of *n*-butyric acid	Source: *B. sexangular* (stem and bark) Mole. for: $C_{12}H_{21}NO_2$ Mol. wt: 211.31 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]
8.	 Tropine esters of 4-hydroybenzoic acid	Source: *B. sexangular* (stem and bark) Mole. For: $C_{15}H_{19}NO_3$ Mol. wt: 261.32 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]
9.	 Tropine esters of ethyl 3,4-dihydroxybenzoate	Source: *B. sexangular* (stem and bark) Mole. For: $C_{16}H_{21}NO_5$ Mol. wt: 307.35 Biological activity: antidiabetic, anticancer, antiemetic, antispasmodics, mydriatics	Loder and Russell [50] Brion et al. [51] Gronkiewicz and Gadzikowska [52]
10.	 Complex polysaccharides	Source: *Sonneratia alba* Mole. for: $C_X\,(H_2O)_Y$ Mol. wt: 213–277 kDa Biological activity: antimicrobial, antiviral, antihyperglycemic agent, proliferation activity for fibroblasts	Liu et al. [53] Premanathan et al. [54] Morada et al. [55] Das et al. [56]
11.	 Rutin	Source: *R. apiculata* and *A. ilicifolius, A. marina*, *X. granatum,* and *B. sexangula* Mole. for: $C_{27}H_{30}O_{16}$ Mol. wt: 610.52 Biological activity: antidiabetic, antimicrobial, antiviral, antihyperglycemic, and proliferation	Kim et al. [49] Bisht et al. [57] Ganeshpurkar and Saluja [58] Kreft et al. [59] Harborne [60] Bandaranayake [61] Cheng et al. [62] Nebula et al. [48]

Table 1: Continued.

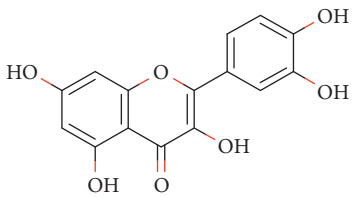
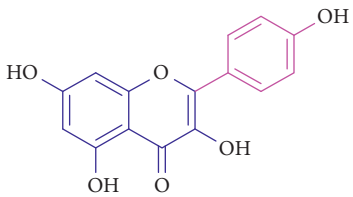
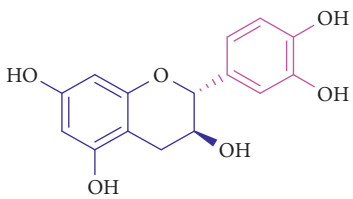
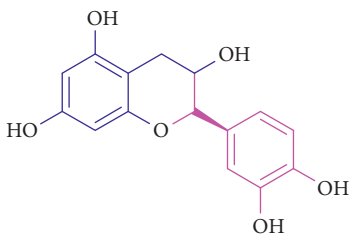
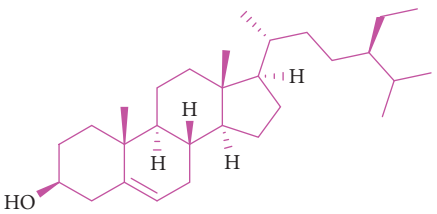
Sl. No.	Chemical structure	Description	Reference
12.	 Quercetin	Source: *R. apiculata* and *A. ilicifolius, A. marina*, *X. granatum,* and *B. sexangula* Mole. for: $C_{15}H_{10}O_7$ Mol. wt: 302.238 Biological activity: antidiabetic, antibacterial, antifungal, antimycobacterial, antimalarial, antiretroviral, antiviral	Kim et al. [49] Bisht et al. [57] Ganeshpurkar and Saluja [58] Kreft et al. [59] Harborne [60] Bandaranayake [61] Cheng et al. [62] Nebula et al. [48]
13.	 Kaempferol	Source: *R. apiculata* and *A. ilicifolius, A. marina*, *X. granatum,* and *B. sexangula* Mole. for: $C_{15}H_{10}O_6$ Mol. wt: 286.239 Biological activity: antidiabetic, anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic, and antiatherosclerotic	Kim et al. [49] Bisht et al. [57] Ganeshpurkar [58] Kreft et al. [59] Harborne [60] Bandaranayake [61] Cheng et al. [62] Nebula et al. [48]
14.	 Catechin	Source: *R. apiculata, A. ilicifolius, A. marina*, *X. granatum,* and *B. sexangula* Mole. for: $C_{15}H_{14}O_6$ Mol. wt: 290.26 Biological activity: antidiabetic, antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antiosteoporotic, estrogenic/antiestrogenic, anxiolytic, analgesic	Kim et al. [49] Bisht et al. [57] Ganeshpurkar and Saluja [58] Kreft et al. [59] Harborne [60], Bandaranayake [61] Cheng et al. [62] Nebula et al. [48]
15.	 (-)-Epicatechin	Source: *R. apiculata, A. ilicifolius, A. marina*, *X. granatum,* and *B. sexangula* Mole. for: $C_{15}H_{14}O_6$ Mol. wt: 290.271 Biological activity: antidiabetic, antioxidant, anti-inflammatory, antimutagenic, cardiovascular disease	Kim et al. [49] Bisht et al. [57] Ganeshpurkar and Saluja [58] Kreft et al. [59] Harborne [60] Bandaranayake [61] Cheng et al. [62] Nebula et al. [48]
16.	 Beta-sitosterol	Source: *B. gymnorrhiza*, *B. sexangular* Mole. for: $C_{29}H_{50}O$ Mol. wt: 414.71 Biological activity: antidiabetic, brain disorders, endothelial dysfunction, hypertension, neuroprotection	Nebula et al. [48] Vázquez et al. [63] Soodabeh et al. [64]

Table 1: Continued.

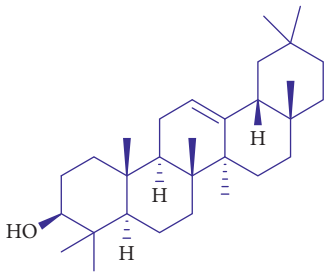
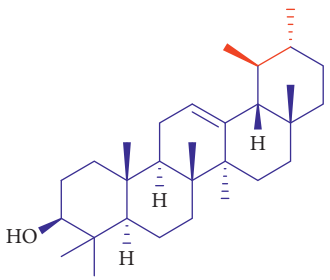
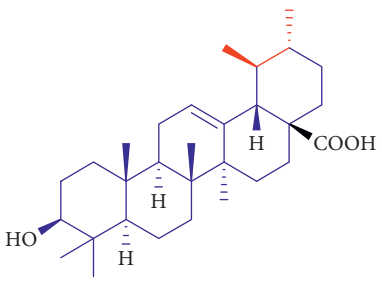
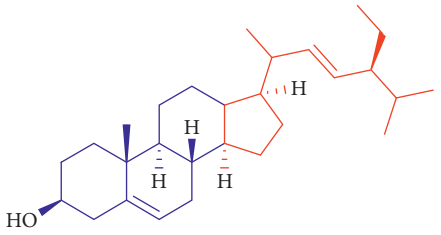
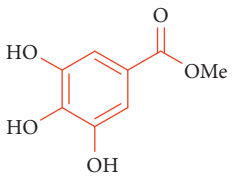
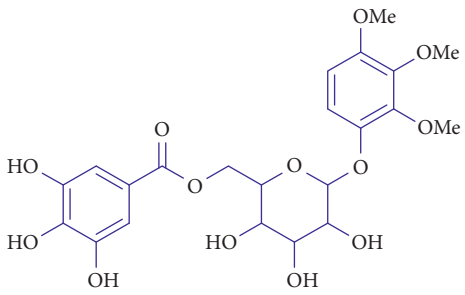
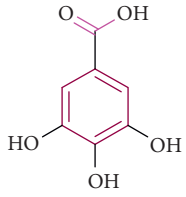
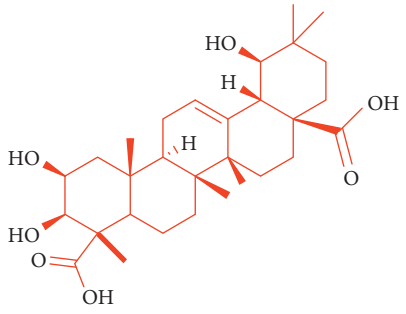
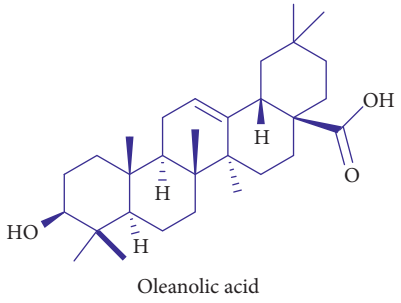
Sl. No.	Chemical structure	Description	Reference
17.	 Beta-amyrin	Source: *B. gymnorrhiza*, *B. sexangular* Mole. for: $C_{30}H_{50}O$ Mol. wt: 426.729 Biological activity: antidiabetic, antioxidant, anti-inflammatory, and anticancer	Nebula et al. [48] Vázquez et al. [63] Soodabeh et al. [64]
18.	 Alpha-amyrin	Source: *B. gymnorrhiza*, *B. sexangular* Mole. for: $C_{30}H_{50}O$ Mol. wt: 426.729 Biological activity: antidiabetic, antinociceptive, anti-inflammatory	Nebula et al. [48] Vázquez et al. [63] Soodabeh et al. [64]
19.	 Ursolic acid	Source: *B. gymnorrhiza*, *B. sexangular* Mole. for: $C_{30}H_{48}O_3$ Mol. wt: 456.7 Biological activity: antidiabetic, antinociceptive, and anti-inflammatory	Nebula et al. [48] Vázquez et al. [63] Soodabeh et al. [64]
20.	 Stigmasterol	Source: *B. gymnorrhiza*, *B. sexangular* Mole. for: $C_{29}H_{48}O$ Mol. wt: 412.69 Biological activity: antidiabetic, anticancer, anti-inflammatory, antioxidant, antihyperlipidemic, antihyperglycemic	Nebula et al. [48] Vázquez et al. [63] Soodabeh et al. [64]
21.	 Methyl 3,4,5, trihydroxybenzoate	Source: *B. racemosa, Rhizophora* sp. Mole. for: $C_8H_8O_5$ Mol. wt: 184.147 Biological activity: antidiabetic, anticancer, antidiabetes, anti-inflammation, antiosteoporosis, antipsoriasis, hepatoprotection, and hypolipidemic activity	Kabir et al. [65] Shahriar et al. [66] Saeki et al. [67] Li et al. [68]

Table 1: Continued.

Sl. No.	Chemical structure	Description	Reference
22.	 3,4,5-trimethoxy phenyl 1-F(6-galloyl)-glucopyranoside	Source: *B. racemosa, Rhizophora* sp. Mole. for: $C_{22}H_{26}O_{13}$ Mol. wt: 498.44 Biological activity: antidiabetic, anticancer, antidiabetes, anti-inflammation, antiosteoporosis, antipsoriasis, hepatoprotection, and hypolipidemic activity	Kabir et al. [65] Shahriar and Robin [66] Saeki et al. [67] Li et al. [68]
23.	 Gallic acid	Source: *B. racemosa, Rhizophora* sp. Mole. for: $C_7H_6O_5$ Mol. wt: 170.12 Biological activity: antidiabetic, antiherpetic, antioxidant	Kabir et al. [65] Shahriar and Robin [66] Saeki et al. [67] Li et al. [68]
24.	 Bartogenic acid	Source: *B. racemosa* (stem, bark, and fruits) Mole. for: $C_{30}H_{46}O_7$ Mol. wt: 518.691 Biological activity: antidiabetic, antiosteoarthritic, antihypercholesterolemic, cytotoxicity, antitumor, hypoglycaemic, antimutagenic, antioxidant, anti-inflammatory, and CNS effects	Sun and Guo [69] Patil and Patil [70] Patil et al. [71]
25.	 Oleanolic acid	Source: *S. hydrophylacea* Mole. for: $C_{30}H_{48}O_3$ Mol. wt: 456.711 Biological activity: antidiabetic, antiarthritic activity, antitumor, antinociceptive, antibacterial, and antifungal activities and anti-inflammatory drugs	Samarakoon et al. [72] Babalola et al. [73] Kurek et al. [74]

antiarthritic activity, antitumor, antinociceptive, antibacterial, and antifungal activities and anti-inflammatory drugs [69, 71].

Hexane and chloroform extracts of the leaves of *Scyphiphora hydrophylacea* yielded compound, namely, oleanolic acid **22**. Oleanolic acid has been isolated for the first time in Sri Lanka and demonstrated in vitro cytotoxic effects in estrogen receptor-positive (MCF-7) and nonsmall lung cancer (NCI-H-292) cells [72]. Triterpenoid has been reported to show anti-inflammatory, antitumor, hepatoprotective, antidiabetic, and antibacterial properties [72–74].

Methyl 3,4,5-trihydroxy benzoate **23**, gallic acid **24**, and 3,4,5-trimethoxy phenyl 1-OF (6- galloyl)-glucopyranoside **25** are phenolic compounds obtained from *B. racemosa, Rhizophora* sp., and their biological activities such as antidiabetic, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, and anticancer activities [65–68, 74]. This review highlights the traditional knowledge of mangroves plants that possess numerous bioactive compounds which are summarized in Table 1.

2.2. Studies on Antidiabetic Activities of Mangroves Plants

2.2.1. Antidiabetic Activity of Mangrove Extracts and Their Phytochemicals. Mangrove plants are considered to be a rich source of potent antidiabetic agents and are considered to be devoid of side effects. It is estimated that more than 400 plants and their secondary metabolites such as glycosides, alkaloids, terpenoids, flavonoids, carotenoids, tannins, polyphenolic, aliphatic alcohols, acids, amino acids, carbohydrates, hydrocarbons (including polyunsaturated fatty acids), lipids, pheromones, phorbol esters, and steroid derivatives are being used for the management of diabetes mellitus across the globe [27, 28]. Mangroves are woody plants growing at the interface between the land and sea in tropical and subtropical latitudes, where they exist under conditions of high salinity, extreme tides, strong winds, high temperatures, and muddy, anaerobic soils. Asia is the richest region of mangrove species diversity with 44 species reported [49]. Some of the important mangrove plants along with their phytochemical constituents and their mechanism of antidiabetic achievement are shown in Table 2.

2.2.2. Acanthaceae. Acanthaceae is represented by two genera, namely, *Acanthus* and *Avicennia*. The genus *Acanthus* is represented by three species in India, namely, *A. ilicifolius*, *A. ebracteatus,* and *A. volubilis*. Of these, *A. ilicifolius* is commonly distributed, while *A. ebracteatus* is found only in Kerala, Puducherry, and ANI at confined locations [1]. Venkataiah et al. reported that ethanolic root extract of *A. ilicifolius* at a dose of 200 and 400 mg/kg body weight significantly reduced the blood sugar level in normal glucose-fed hyperglycemic and alloxan-induced diabetic rats. Regeneration of β-cells has also been reported in diabetic rats [78]. Flavonoids, alkaloids, terpenoids, tannins, and steroids are present in the root extracts of this plant and this may play an important role in their hypoglycemic activities [56].

2.2.3. Arecaceae. Arecaceae family is represented by two species, namely, *Nypa fruticans* and *Phoenix paludosa*. In India, both are distributed in Sundarbans and ANI. TK of *N. fruticans* and its uses for different ailments by the local practitioners/coastal communities of southern regions of Bangladesh and ANI has been well documented [16]. This species has very limited distribution in the Indian coast. However, the methanolic leaf and stem extracts of *N. fruticans* (500 mg/kg) have been reported to show their significant antihyperglycemic effect in diabetic mice [114].

2.2.4. Avicenniaceae. The family Avicenniaceae is represented with genus *Avicennia* by three species, namely, *A. alba*, *A. marina*, and *A. officinalis,* and all are commonly populated in the mangrove habitats of India. Recent studies reveal that the ethanolic extract of *A. marina* leaf has antihyperglycemic activity in alloxan-induced diabetic rats. In addition, when the ethanolic leaf extract (250 and 500 mg/kg) was used for the treatment of the diabetic rats for 15 days, it resulted in a significant reduction in the blood glucose levels along with an increase in total hemoglobin (Hb), total protein, and serum insulin. The leaf extract can reduce the level of serum urea that confirms the capacity to protect vital tissues, kidney, liver, and pancreas. In addition, it also improved the biochemical parameters such as serum phosphorus, albumin, and globulin. The possible mechanism underlying the antihyperglycemic action of *A. marina* is attributed to the stimulation of surviving β-cells that is releasing more insulin [118]. Mahera et al. [119] reported that methanolic extract of pneumatophores (aerial roots) of *A. marina* exhibits antihyperglycemic effect, which might be due to the inhibition of AGEs. Aljaghthmi et al. [79] elucidated the antidiabetic properties present in *A. marina*.

2.2.5. Euphorbiaceae. Two species, namely, *E. agallocha* and *E. indica,* belonging to the genus *Excoecaria* representing Euphorbiaceae are recorded in Indian mangroves. *E. agallocha* is commonly distributed in back mangrove areas where there is lower salinity, whereas *E. indica* is found in Sundarbans, Odisha, and Kerala. Recently Ragavan et al. reported *E. indica* species from mangrove ecosystem of ANI [1].

The methanolic stem extract of this plant has shown to reduce serum glucose levels in doses of 200 to 400 mg/kg [88]. This activity was significantly lower compared with glibenclamide (10 mg/kg·bw), an antidiabetic drug closely related to sulfonamide antibiotics. *E. agallocha* has also been found to contain *b*-amyrin acetate which is thought to be responsible for its antidiabetic activity [120]. Thirumurugan et al. [89] revealed that ethanolic leaf extracts of *E. agallocha* species possessed significant hypoglycemic activity. Flavonoids, triterpenoids, and phenolic compounds are the bioactive principles responsible for antibiotic and antidiabetic potential compounds which are present in *E. agallocha* species.

2.2.6. Malvaceae. Malvaceae is represented by two genera, namely, *Brownlowia* and *Heritiera* in Indian mangroves.

Table 2: Mangrove plants with phytochemical constituents and antidiabetic mechanism from different ecosystem (worldwide).

Sl.No.	Mangrove species	Phytochemical constituents	Antidiabetic mechanism	References
1.	*A. corniculatum*	Flavonoids, tannins, polyphenols Alkaloids, tannins, benzofurans Saponins	Utilization of glucose either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion Presence of antidiabetic properties	Gurudeeban et al. [36] Ishibashi et al. [76]
2.	*Acrosathe annulata*	Amino acids inorganic salts	Presence of antidiabetic properties	Popp [77]
3.	*A. ilicifolius*	Flavonoids	Regeneration of β-cells of the pancreas Presence of flavonoids	Venkataiah et al. [78] Li et al. [68]
4.	*A. marina*	Saponins Yet to be analysed	Stimulation of β-cells to release more insulin antiglycation activity Presence of antidiabetic properties	Babuselvam et al. [118] Aljaghthmi et al. [79]
5.	*B. cylindrical*	Flavonoids, phenolic acids, sterols/ triterpenoid, alkaloids, tannins, anthocyanins	Stimulation of β-cells to release more insulin	Das et al. [56]
6.	*Bruguiera* sp.	Alkaloids Flavonoids	Presence of antidiabetic properties	Cheng et al. [62] Li et al. [68]
7.	*B. racemosa*	Flavonoids, tannins, saponins Phenolic compounds	α-glucosidase and α-amylase inhibitory property Presence of antidiabetic properties	Gowri et al. [80] Kabir et al. [65] Li et al. [68]
8.	*B. gymnorrhiza*	Flavonoids, tannins, saponins, polyphenols, glycosides	Presence of antidiabetic properties	Nebula et al. [48]
9.	*B. rumphii*	Tannins, triterpenes	Presence of antidiabetic properties	Rollet [81]
10.	*B. parviflora*	Phenolic compounds	Presence of antidiabetic properties	Seshadri and Venkataramani [82]
11.	*B. sexangula*	Phenolic, steroids Alkaloids, tannins Saponins	Presence of antidiabetic properties	Hogg and Gillan [83] Nebula et al. [48]
12.	*C. decandra*	Flavonoids, tannins, saponins, polyphenols, glycosides	Presence of antidiabetic properties; stimulation β-cells to release more insulin The increased hexokinase activity	Seshadri and Trikha [84] Nabeel et al. [32]
13.	*C. tagal*	Flavonoids, tannins, saponins, polyphenols	The inhibition against PTPase enzyme activity	Tiwari et al. [85] Tamrakar et al. [86] Lawag et al. [87]
14.	*E. agallocha*	Flavonoids, tannins, saponins, polyphenols	Pancreatic secretion of insulin, uptake of glucose	Rahman et al. [88] Thirumurugan et al. [89]
15.	*K. candel*	Flavonoid, glycosides, triterpenoids, tannins, saponins, polyphenols	Presence of antidiabetic	Habeebulla and Velraj [90]
16.	*R. annamalayana*	Alkaloids, tannins steroids	Improved level of insulin secretion and its action	Ali et al. [91] Nabeel et al. [92]
17.	*R. apiculata*	Tannin, steroids, triterpenes, phenolic compounds	Improved level of insulin secretion and its action; insulin mimetic activity; cell protection	Lakshmi et al. [93] Sur et al. [55, 57, 58, 61, 75–77, 80–84, 90, 92, 94–113] Nabeel et al. [92]
18.	*R. mangle*	Tannins, triterpenes	Presence of antidiabetic properties	Willians [109]
19.	*R. mucronata*	Tannin, steroids, triterpenes, phenolic compounds	Improved level of insulin secretion and its action; insulin mimetic activity; α-glucosidase inhibitory	Nabeel et al. [92] Adhikari et al. [110] Aljaghthmi et al. [79]
20.	*R. racemosa*	Tannins, triterpenes	Presence of antidiabetic properties	Padmakumar and Ayyakkannu [111]
21.	*R. conjugate*	Anthocyanins, steroids Tannins, triterpenes	Presence of antidiabetic properties	Majumdar and Patra [112]

Table 2: Continued.

Sl.No.	Mangrove species	Phytochemical constituents	Antidiabetic mechanism	References
22.	*R. stylosa*	Inositols Steroids	Presence of antidiabetic properties	Ravi and Kathiresan [113]
23.	*N. fruticans*	Alkaloids glycosides tannins Sterols	Utilization of glucose	Reza et al. [114]
24.	*S. alba*	Tannins Phenolic Polysaccharides	Modifying glucose pathway Presence of antidiabetic properties	Morada et al. [55] Bandaranayake [61]
25.	*S. apetala*	Triterpenes steroids flavonoids alkaloids	Enhanced insulin-releasing activity; enhance transport of blood glucose to the peripheral tissue	Hossain et al. [101] Patra et al. [115]
26.	*S. brachiata*	Steroids Triterpenes	Presence of antidiabetic properties	Padmakumar et al. [116]
27.	*S. caseolaris*	Steroids glycosides	Intestinal α-glucosidase inhibitory activity; potentiation pancreatic secretion of insulin	Tiwari et al. [98] Hasan et al. [100]
28.	*S. ovata*	Steroids	Presence of antidiabetic properties	Bhosle et al. [117]
29.	*V. adenantha*	Yet to be analysed		Habeebulla and Velraj [90]
30.	*X. granatum*	Alkaloids steroids tannins triterpenes Alkaloids Flavonoids	Stimulation on β- cells; elevation in insulin sensitivity to glucose; protein tyrosine phosphatase activity Presence of antidiabetic properties	Srivastava et al. [75] Cheng et al. [62] Li et al. [68]
31.	*X. moluccensis*	Alkaloids steroids tannins triterpenes Proanthocyanidins	Insulin mimetic or insulin secretagogue activity insulin resistance reversal activity α-glucosidase inhibitory activity	Srivastava et al. [104]

Two species of genus *Heritiera,* namely, *H. fomes* and *H. littoralis,* are known from Indian mangroves, of which the former is reported only from Sundarbans and Odisha, but due to the reduction in freshwater input in both places, it has become rare. *H. littoralis* is known from Odisha, Maharashtra, and ANI. Dose-dependent reductions in the concentration of serum glucose in mice upon treatment with crude methanol extract of *H. fomes* bark are utilized for antidiabetics. At 60 minutes following glucose administration, *H. fomes* bark extract (250 mg/kg) significantly lowered blood glucose levels by 49.2% compared to 43.5% by glibenclamide (antidiabetic drug). It was further reported that the crude methanolic extract possesses long-term action in its glucose-lowering effect and is also better than glibenclamide drug [91]. In the future, there is an in-depth study to be needed on phytochemicals speciation of *H. littoralis* from Indian mangroves. In ANI, *H. fomes* and *H. littoralis* are common in both groups of islands [1]. There is a need for more in-depth study on the bioactive compounds from the two species of genus *Heritiera* from ANI as well as Indian mangrove.

2.2.7. Rhizophoraceae. The family Rhizophoraceae constitutes the four genera *Bruguiera, Ceriops, Kandelia* and *Rhizophora* representing Rhizophoraceae that are found in Indian mangroves.

2.2.8. Bruguiera. There are six species present in this genus as reported throughout the world. Four species of genus *Bruguiera,* namely, *B. gymnorrhiza, B. cylindrica, B. parviflora,* and *B. sexangula,* are reported from India. Of these, the first two species are commonly distributed in Indian mangroves, while *B. parviflora* is restricted to Sundarbans, Odisha, ANI, and Maharashtra. Bark part of *B. gymnorrhiza* was extracted with ethanol solvent which displayed antihyperglycemic effect in streptozotocin- (STZ-) induced diabetic rats. Treatment with ethanolic bark extracts (400 mg/kg) for 21 days reported significant reduction in blood glucose level in the STZ-induced diabetic rats, which was comparable to that of standard drug glibenclamide (0.5 mg/kg). Further, a decreased level of total cholesterol, triglycerides, very-low-density lipoprotein, and low-density lipoprotein along with increased high-density lipoprotein level in the diabetic rats was observed [121]. Oral administration of ethanolic extract of *B. gymnorrhiza* normalized the levels of blood glucose in rats. The potent antidiabetic effect of the plant extract suggests the presence of various potent antidiabetic active compounds, which produced an antihyperglycemic effect in diabetic rats. The present literature survey observed that different compounds such as bruguierol, β-sitosterol, α-amyrin, β-amyrin, lupeol, oleanolic acid, ursolic acid, taraxerol, gymnorhizol, and ellagic acid were isolated from *B. gymnorrhiza* plant and its potent antidiabetic activity.

2.2.9. Ceriops sp. Rhizophoraceae includes the genus *Ceriops* that constitutes two species; *C. decandra* and *C. tagal* are common in Indian mangroves. Both species have been reported from ANI. A significant serum glucose level lowering capacity was marked in alloxan-induced diabetic mice (animal experiment model) upon oral administration of ethanolic leaf extract taken from *C. decandra* (120 mg/kg) developed by Nabeel et al. [32]. This capacity was comparable to that of glibenclamide (0.1 mg/kg bw). The increase in insulin secretion, body weight, and hemoglobin (Hb A1c) levels and decrease in HbA1c levels in diabetes-induced rats were due to ethanolic leaf extract of *C. decandra* treatments. The ethanolic *C. decandra* leaf extracts were found to be involved in the regulation of hexokinase, glucose-6-phosphatase, and fructose-1,6-bisphosphatase which play a key role in glucose metabolism in mitochondria organelles. Normal levels of glucose-6-phosphatase and fructose-1,6-bisphosphatase activities were observed in diabetic rats upon the administration of ethanolic *C. decandra* leaf extracts. Further, diabetic rats also showed increased hexokinase activity. Antihyperglycemic action of *C. decandra* can be attributed to its ability in stimulating surviving β-cells in pancreases to produce more insulin [32]. A study by Tiwari et al. concluded that treatment with ethanolic leaf extracts of *C. tagal* improved glucose tolerance of normoglycemic rats after sucrose load. The treatment also lowered blood glucose levels upon a 250 mg/kg oral dose in STZ-induced diabetic rats. The application of hexane subfraction of ethanolic extract of *C. tagal* (100 mg/kg) in normal healthy rats after sucrose load proved to be most effective for antiglycemic activity. This can be compared to the effect of metformin. Unique compounds isolated from *C. tagal* hexane fraction showed significant inhibition against protein tyrosine phosphatases (PTPase) enzyme activity which is involved in insulin action in Type 2 diabetes [85]. Tamrakar et al. [86] reported that n-hexane soluble fraction of ethanolic leaf extracts of *C. tagal* stimulates glucose uptake in L6 muscle cells in a dose-dependent manner, which is comparable to metformin standard. Similarly, Lawag et al. revealed that antihyperglycemic activity of hydroalcoholic bark of *C. tagal* is due to its α-glucosidase inhibition potential [87].

2.2.10. Rhizophora sp. Three out of the ten species of *Rhizophora* genus (*R. apiculata, R. annamalayana,* and *R. mucronata*) possess antidiabetic activity [56]. At 250 mg/kg doses, the ethanolic root extracts of *R. apiculata* showed promising antihyperglycemic activity in experimental rats. A large number of phytochemicals are found in the chloroform and aqueous subfractions of ethanolic root extract accounting for its antihyperglycemic activity. After purification, seven compounds were isolated: lupeol, oleanolic acid, β-sitosterol, palmitic acid, β-sitosterol-β-D-glucoside, inositol, and pinitol. Among these, inositol and pinitol showed promising activity in the STZ model with 100 mg/kg dose level [93]. The ethanolic leaf extracts of *R. apiculata* showed hypoglycemic and antihyperglycemic activities in normal, glucose-fed, and STZ diabetic rats [94]. The hypoglycemic action *of R. apiculata* is due to the presence of flavonoids along with other bioactive compounds. The antidiabetic properties of the hydromethanolic leaf extracts of *R. apiculate* are due to its radical scavenging and β-cell protection properties. Nabeel et al. have reported the antidiabetic potential of *R. mucronata, R. apiculata, and R. annamalayana* [56, 92]. 60 mg/kg *Rhizophora* aqueous extract was orally administered in alloxan-induced diabetic rats. The results revealed that it aided in modulation/acceptable normal levels of blood glucose level. The noticeable antidiabetic activity was observed from the extract of *R. apiculata* in comparison to other mangrove extracts [92]. SDS-PAGE analysis elucidated the presence of insulin-like protein in the mangrove extracts and confirmed that assertion was done by an enzyme-linked immunosorbent assay (ELISA) [56]. Therefore, the antidiabetic property in *R. apiculate* is clearly noted due to the secretion of insulin-like protein biomolecules and its action against reducing the blood glucose level [92].

Gaffar et al. reported the antidiabetic activity of *R. mucronata* and revealed that it is a plant's sole capacity to inhibit carbohydrate digestion and absorption of glucose biomolecules [95]. Haque et al. [96] reported that the bark extracts of *R. mucronata* aqueous layer have hypoglycemic and antihyperglycemic activities. The bark extracts showed dose-dependent antidiabetic effects which helped suppress postprandial hyperglycemia. The most probable mechanism behind the hyperglycemic effect is glucose absorption inhibition. A similar study by Lawag et al. revealed that antihyperglycemic activity of hydroalcoholic bark of *R. mucronata* is due to its α-glucosidase inhibition potential observed. Traditionally, *R. mucronata* was utilized to cure diabetes [87].

2.2.11. Kandelia candel. The genus *Kandelia* is represented by two species, namely, *K. candel* and *K. obovata* of the family Rhizophoraceae in mangrove communities, of which the former is known from both east and west coasts of India and ANI. Mangrove species *Kandelia* mostly occurs in a middle zone of the mangrove ecosystem. Bark and leaves are used in the treatment of DM in different coastal regions of India. The bark of *K. candel* is suitable for an industrial application like tanning heavy leather and for dyeing in red and brown colours production. Further, phytochemical analysis of the *K. candel* species shows flavonoid, glycosides, triterpenoids, tannins, saponins, and polyphenols compounds. However, there is no availability of comprehensive study on purified compounds of *K. candel* from Indian coast [97].

2.2.12. Lythraceae. Two genera, namely, *Pemphis* and *Sonneratia,* representing Lythraceae are found in Indian mangroves. In India, the genus *Sonneratia* is represented by seven species, namely, *S. alba, S. caseolaris, S. griffithii, S. ovata, S. lanceolata, S. urama,* and *S. gulngai*. Of these, *S. ovata, S. lanceolata, S. urama,* and *S. gulngai* are new records for India from the ANI. Out of the seven identified species of genus *Sonneratia*, antidiabetic activity has been reported in three species, namely, *S. alba*, *S. apetala,* and *S. caseolaris*.

Morada et al. reported the antidiabetic potential of methanolic leaf extracts of *S. alba* using *in vivo* mice model. The extreme blood-glucose-attenuating activity of the extract was related to complex polysaccharide molecule obtained from *S. alba* leaf extracts. Significant reduction in sugar level was observed during the first 6 (19.2%) and 12 h (66.9%) after the administration of the extracts to the diabetic mice [55]. Fruits of *S. caseolaris* have many therapeutic applications in folklore medicine [61]. Compounds such as oleanolic acid, β-sitosterol-β-D-glucopyranoside, and luteolin, which are isolated from the methanolic extract of its fruits, have shown inhibition of the α-glucosidase enzyme in a dose-dependent manner [98]. Further, the methanolic fruit extracts of this plant significantly reduced the serum glucose concentrations in mice loaded with glucose, in a dose-dependent manner [99, 100]. The antihyperglycemic activities of this plant can be due to a number of factors such as decreased intestinal glucose absorption, increase in pancreatic secretions, glucose uptake, insulin secretion, and better glycemic control. Similarly, the antihyperglycemic activity of seeds and pericarps of *S. apetala* fruits were reported in STZ-induced diabetic mice [101]. The antihyperglycemic activity of this plant may be due to insulin mimetic activity, better glucose utilization, regeneration of islets of Langerhans in the pancreas, and enhanced transport of blood glucose to the peripheral tissue.

2.2.13. Myrsinaceae. *A. corniculatum* belongs to the family Myrsinaceae in the mangrove communities and is commonly known from Indian mangroves. Several parts of the plant have been traditionally used for the treatment of inflammation, antioxidant, rheumatism, arthritis, free radical scavenging, and hepatoprotective activities [102]. The previous report revealed that ethanolic extract of *A. corniculatum* leaves regulates blood glucose level in alloxan-induced diabetic rats at a total of 100 mg/kg. Development in body weight in the diabetic-induced rat was observed along with a decrease in the activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase and glycosylated hemoglobin along with the increased activity of liver hexokinase [37, 56].

2.2.14. Meliaceae. Meliaceae is represented by two genera, namely, *Xylocarpus* and *Aglaia* in mangroves. The genus *Xylocarpus* is represented by three species, namely, *X. granatum, X. moluccensis,* and *X. rumphii* in India. Of these *X. granatum* and *X. moluccensis* are true mangrove species, whereas *X. rumphii* is a nonmangrove species [1]. In India, all three species are known to occur on the coast of ANI. Srivastava et al. reported that antidiabetic activities are exhibited by *X. granatum* and *X. moluccensis* [75]. Recently, Akter et al. revealed that methanolic extract showed antibacterial, antioxidant, and cytotoxicity potential with low concentration [103]. The ethyl acetate fraction of epicarp showed antidyslipidemic effects in diabetic-induced rats [104].

2.3. Diabetes Remedies from Traditional Knowledge on Mangrove Plants. Diabetes is one of the major public health concerns all over the world. Diabetes or hyperglycemia is considered to be one of the common public health hazards; optimal control of which is still not possible. Persistent hyperglycemia or uncontrolled diabetes has the potential to cause serious complications such as kidney disease, vision loss, cardiovascular disease, and lower-limb amputations which contributed towards morbidity and mortality in diabetes [105]. In India, traditional remedies have been used in the treatment of diabetes since the time of physicians Charaka and Sushruta. From the ethnobotanical recording, it is estimated that about 800 plants may possess antidiabetic potential [35]. Several plants have been used as a dietary adjuvant and in treating diseases even without any knowledge on their proper functions and constituents ref. This practice may be due to its fewer side effects compared to the synthetic hypoglycemic agents and because of their safety, effectiveness, and availability [6]. Ethnobotanical information reports a huge number of plants that may possess antidiabetic potential, of which *Momordica charantia*, *Pterocarpus marsupium*, and *Trigonella foenum-graecum* have been reported to be beneficial for the treatment of Type 2 diabetes. Throughout the world, terrestrial plants have been used in the treatments of diabetes. In contrast, very limited works have been carried out on the antidiabetic property of mangroves plants from India as well as globally.

Recently, ethnopharmacological records divulged the traditional usages of mangrove *A. corniculatum* (Linn.) Blanco distributed in coastal and estuarine areas of Southeast India [102]. Traditionally, more than 100 numbers of mangroves and mangrove-associated plant used for the treatment of diabetes have been reported, but only a very few numbers of plants are evaluated and reported scientifically [61]. The antidiabetic effect of leaves of mangrove plants *R. mucronata* and *C. decandra* had been documented and the gut perfusion studies on Long-Evans rats reported the mode of action of *R. mucronata* leaves' hypoglycemic conditions [32, 95]. Recently, the medicinal values of mangroves and associated plants persist to provide priceless therapeutic agents, both in modern medicines and in traditional systems [26].

3. Research Gaps in Indian Mangroves

In the past few decades, there is an increase in research works on mangrove plants in terms of conservation aspects especially from India. Mangrove species from several coasts all over the world have been studied for their medicinal values and for their bioactive potential to treat diseases like cancer, rheumatism, free radical scavenging, anti-inflammatory, antinociceptive, painful arthritis, inflammation, asthma, antioxidant, DM, and as hepatoprotective agents [102, 107].

However, research on developing drug derivatives from mangroves by Indian scientific communities is very limited. Based on our data compilation on antidiabetic research, we suggest that research on mangroves is very much important as there are many potential medically significant compounds that have been reported from different regions, but very

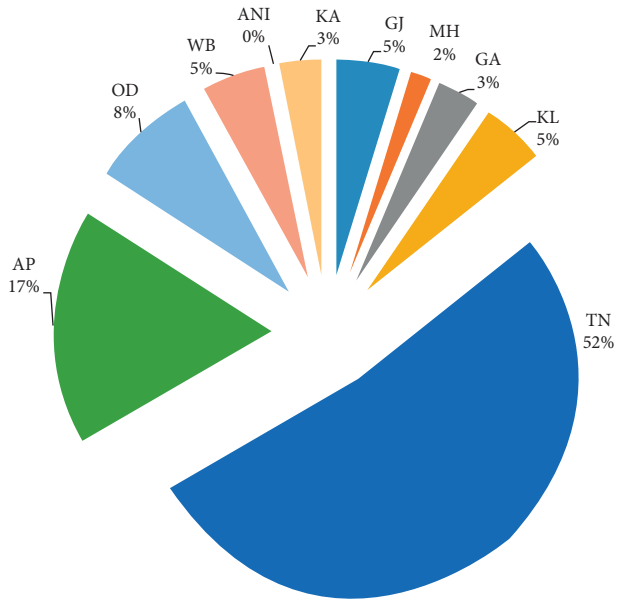


Figure 3

limited work has been carried out from the Indian coast, specifically on the phytochemical speciation. Further, there is a large gap in antidiabetic studies on ANI mangrove resources (Figure 3). Hence, antidiabetic studies in partnership with the indigenous communities of ANI based on their traditional knowledge are imperative.

4. Conclusions

In a nutshell, mangrove plants show high potential to address DM through their unique chemical structures. In recent years, most of the pharmaceutical industries are focusing mainly on the development of new drugs for DM on large-scale productions. But, there are promising potential from alternate sources like herbal medicines/traditional knowledge-based drugs which have multiple targets and potentially can be evolved as new drugs/complementary which needs serious attention. There is an important need to renew scientific research based on traditional knowledge of indigenous communities to be included in drug discovery programs. Medicinal valued plants are one of the chief components of our natural resource which was comprised of nearly 34 true mangrove species and 12 associated mangroves species from Indian coastal region. In order to enhance the anticipation, strategic selection of particular species and shortlisting of mangroves species is a necessary task for India. New research avenues on the traditional knowledge of medicinal plants may help to conserve time, money, and side effects (toxicity), which are three key major parameters that hurdle in any drug developmental program. Since TK is a community based knowledge, the historical laws of the indigenous community should be considered. Documented traditional knowledge on medicinal values of mangroves species might simplify issues associated with poor predictability and scientific research on such knowledge will create a new pathway for developing potential drugs against diabetes. Indian coastal communities have rich traditional knowledge on plant-based drug formulations that are protective and curative for many health ailments. However, there is a large research gap in antidiabetic studies on ANI mangrove resources with coastal communities based TK. In future, Indian tropical islands mangroves resources need to be studied in detail on antidiabetic compounds extraction for new drugs mining from unexplored pristine islands ecosystem.

Abbreviations

AGE:	Advanced glycation end-products
ANI:	Andaman and Nicobar Islands
Alk-CF:	Alkaline chloroform fraction
DOCK:	Molecular docking
DCM-F:	Dichloromethane fraction
DM:	Diabetes mellitus
DPP:	Dipeptidyl peptidase
ELISA:	Enzyme-linked immunosorbent assay
GC-MS:	Gas chromatography–mass spectrometry
HbA1c:	Glycated hemoglobin
Hb:	Hemoglobin
IUCN:	International Union for Conservation of Nature
kg:	kilogram
MCF-7:	Michigan Cancer Foundation-7 breast cancer cell line
mg:	milligram
NCI-H-292:	Nonsmall lung cancer
NIDDM:	Noninsulin-dependent diabetes mellitus
NIST:	National Institute of Standards and Technology
PTPase:	Protein tyrosine phosphatases
PAGE:	Polyacrylamide gel electrophoresis
TK:	Traditional knowledge
TM:	Traditional medicines
SDS:	Sodium dodecyl sulfate
STZ:	Streptozotocin
WHO:	World Health Organization
USA:	United States of America
US:	United States.

Disclosure

This study was undertaken as part of the in-house research study of NCSCM on TRaMP studies. Views expressed are of the authors only and not necessarily of the affiliated organizations.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors thank the Director of the National Centre for Sustainable Coastal Management for providing the facilities for this study. This study was financially and technically supported by the Ministry of Environment, Forest and

Climate Change, Government of India, and the World Bank under the India ICZM Project.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2019, Article ID 3476567, 11 pages
https://doi.org/10.1155/2019/3476567

Research Article

Studies on Antibacterial Activity and Diversity of Cultivable Actinobacteria Isolated from Mangrove Soil in Futian and Maoweihai of China

Feina Li,[1] Shaowei Liu,[1] Qinpei Lu,[1] Hongyun Zheng,[1,2] Ilya A. Osterman,[3,4] Dmitry A. Lukyanov,[3] Petr V. Sergiev,[3,4] Olga A. Dontsova,[3,4,5] Shuangshuang Liu,[6] Jingjing Ye,[1,2] Dalin Huang,[2] and Chenghang Sun[1]

[1]*Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*
[2]*College of Basic Medical Sciences, Guilin Medical University, Guilin 541004, China*
[3]*Center of Life Sciences, Skolkovo Institute of Science and Technology, Moscow 143025, Russia*
[4]*Department of Chemistry, A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119992, Russia*
[5]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, The Russian Academy of Sciences, Moscow 117997, Russia*
[6]*China Pharmaceutical University, Nanjing 210009, China*

Correspondence should be addressed to Dalin Huang; huangdalin@glmc.edu.cn and Chenghang Sun; chenghangsun@hotmail.com

Received 29 March 2019; Accepted 21 May 2019; Published 9 June 2019

Guest Editor: Jayanta Kumar Patra

Mangrove is a rich and underexploited ecosystem with great microbial diversity for discovery of novel and chemically diverse antimicrobial compounds. The goal of the study was to explore the pharmaceutical actinobacterial resources from mangrove soil and gain insight into the diversity and novelty of cultivable actinobacteria. Consequently, 10 mangrove soil samples were collected from Futian and Maoweihai of China, and the culture-dependent method was employed to obtain actinobacteria. A total of 539 cultivable actinobacteria were isolated and distributed in 39 genera affiliated to 18 families of 8 orders by comparison analysis of partial 16S rRNA gene sequences. The dominant genus was *Streptomyces* (16.0 %), followed by *Microbacterium* (14.5 %), *Agromyces* (14.3 %), and *Rhodococcus* (11.9 %). Other 35 rare actinobacterial genera accounted for minor proportions. Notably, 11 strains showed relatively low 16S rRNA gene sequence similarities (< 98.65 %) with validly described species. Based on genotypic analyses and phenotypic characteristics, 115 out of the 539 actinobacterial strains were chosen as representative strains to test their antibacterial activities against "ESKAPE" bacteria by agar well diffusion method and antibacterial mechanism by the double fluorescent protein reporter system. Fifty-four strains in 23 genera, including 2 potential new species, displayed antagonistic activity in antibacterial assay. Meanwhile, 5 strains in 3 genera exhibited inhibitory activity on protein biosynthesis due to ribosome stalling. These results demonstrate that cultivable actinobacteria from mangrove soil are potentially rich sources for discovery of new antibacterial metabolites and new actinobacterial taxa.

1. Introduction

Currently, antibiotic resistance is occurring more and more severely and already has become a global challenge to public health [1, 2]; however, new types of antibacterial drugs are so extremely limited that clinicians are forced to the situation as "Bad bugs, No drugs." In early 2017, a request was made to the World Health Organization (WHO) by member states to develop a global priority pathogen list (PPL) of antibiotic-resistant bacteria to help in prioritising the research and development of new and effective antibiotic treatments [3, 4].

Actinobacteria, especially, the genus *Streptomyces*, are major producers of bioactive secondary metabolites [5, 6]. After decades of screening, it has become increasingly difficult to discover new antibiotics from actinobacteria isolated from common soil environments. Nowadays, more and

Table 1: Information of soil samples.

Samples	Sampling sites	Location	The characteristic of soil	Sampling depth
Sample 1	Futian	22°31'45.82" N 114°00'09.04" E	Rhizosphere soil of *Aegiceras corniculatum*	5 cm under surface
Sample 2	Futian	22°31'45.79" N 114°00'09.00" E	Rhizosphere soil of *Aegiceras corniculatum*	5 cm under surface
Sample 3	Maoweihai	21°51'20.51" N 108°36'14.11" E	Muddy soil	10 cm under surface
Sample 4	Maoweihai	21°51'20.58" N 108°36'14.12" E	Rhizosphere soil of *Aegiceras corniculatum*	10 cm under surface
Sample 5	Maoweihai	21°44'35.73" N 108°35'40.85" E	Rhizosphere soil of *Aegiceras corniculatum*	10 cm under surface
Sample 6	Maoweihai	21°44'35.84" N 108°35'40.87" E	Muddy soil	10 cm under surface
Sample 7	Maoweihai	21°44'36.30" N 108°35'40.93" E	Muddy soil	10 cm under surface
Sample 8	Maoweihai	21°44'36.44" N 108°35'40.82" E	Muddy soil	10 cm under surface
Sample 9	Maoweihai	21°44'36.03" N 108°35'40.69" E	Muddy soil	10 cm under surface
Sample 10	Maoweihai	21°44'36.10" N 108°35'40.50" E	Muddy soil	10 cm under surface

more researches are focused on special habitats and extreme environments [7, 8], such as desert [9], marine [10], and mangrove [11], since microbes in special environments have to develop unique defense mechanism against the stress from their habitats and can evolve adaptive biosynthetic pathways for synthesizing novel biological compounds [12]. In fact, a large number of new bioactive compounds produced by actinobacterial strains residing in special environments have been discovered in recent years [13–15].

Mangrove is unique intertidal ecosystem with the condition of high moisture, high salinity, low oxygen, and high organic matter content [16, 17]. Because the mangrove soil conditions are extremely different from common terrestrial conditions, microorganisms especially actinobacteria in mangrove soil have distinctive adaptation characteristics and have the potential to produce novel bioactive metabolites [18]. Investigations in many countries indicated that the mangrove actinobacteria have rich diversity and various biological activities [6, 13, 16, 19, 20]. At the time of writing, at least 86 new actinobacterial species including 8 novel genera have been isolated from mangrove. In addition, more than 84 new compounds produced by mangrove actinobacteria including some attractive structures such as salinosporamides, xiamycins, and novel indolocarbazoles [21, 22] have been reported. From north to south, mangroves in China mainly distribute along the southeast coast including Zhejiang province, Fujian province, Guangdong province, and Guangxi Zhuang Autonomous Region. Among them, Guangdong and Guangxi possess most of the mangrove area [23, 24].

In order to explore the antibacterial resources and gain insight into the diversity of cultivable actinobacteria, mangrove soil samples from Futian, Guangdong, and Maoweihai, Guangxi, were collected and investigated. Due to the high prevalence of multidrug resistance among "ESKAPE" bacteria, defined by the Infectious Diseases Society of America as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* spp., these pathogens in the global PPL of antibiotic-resistant bacteria were selected as the indicator bacteria in this study. In addition, a high-efficiency pDualrep2 reporter system was combined to accelerate the discovery of actinobacterial strains with clearly antibacterial mechanism from mangrove soil.

2. Materials and Methods

2.1. Collection of Mangrove Soil Sample. A total of ten soil samples were collected from 2 mangrove reserves of China in August, 2017. Two samples were collected from Futian, Shenzhen, Guangdong province and 8 from Maoweihai, Qinzhou, Guangxi Zhuang Autonomous Region. The information for the samples is listed in Table 1. All the samples were packed in sterilized envelopes and brought to the laboratory at the earliest possible time. Prior to grinding with mortar and pestle, each sample was immediately air-dried in the laminar flow hood at room temperature for 2 days.

2.2. Cultivable Actinobacteria Isolation and Maintenance. Ten media were prepared to isolate the actinobacterial strains (Table S1). All the isolation media were added 3 % seawater. In addition, nalidixic acid (20 mg/L), cycloheximide (50 mg/L), and potassium dichromate (50 mg/L) were also added in the media to prevent the growth of Gram-negative bacteria and fungi.

Actinobacteria were isolated by using dilution plating technique as described by Li et al. [25]. 0.2 mL of 10^{-2} soil suspension was spread onto isolation agar plates. After incubation at 28°C for 2-4 weeks, colonies were picked up and streaked on the freshly prepared YIM 38 medium (1 L sterile water: 4.0 g glucose, 4.0 g yeast extract powder, 5.0 g malt extract powder, 15.0 g agar, pH 6.0) to obtain the pure isolates. The pure cultures were maintained on YIM 38 agar slants at 4°C for several weeks and also preserved in glycerol suspensions (20 %, v/v) at −80°C.

2.3. PCR Amplification and Sequencing of 16S rRNA Gene. Genomic DNA was extracted as described by Li et al. [26] and used as the template to amplify the 16S rRNA gene by PCR with the primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [27]. The reaction mixture (50 μL) contained 25 μL 2×supermix (TransGen, Beijing), 1 μL each of the primers (10 mM, Sangon Biotech, Beijing), 1.5 μL DNA, and 21.5 μL ddH_2O. The PCR amplification included the following parameters: (i) 95°C for 3 min (initial denaturation), (ii) 30 cycles of 94°C for 1 min (denaturation), 60°C for 1 min (annealing), and 72°C for 1 min (extension), and (iii) 72°C for 10 min (final extension). The amplicons were then visualized by gel electrophoresis using 5 μL of PCR product in a 1 % agarose gel. The PCR products were purified and then sequenced on the ABI PRISM™ 3730XL DNA Analyzer (Foster City, CA).

2.4. Sequence Analysis. The 16S rRNA gene sequences obtained were compared with those of the type strains available in NCBI (http://www.ncbi.nlm.nih.gov/) and the EzBioCloud (https://www.ezbiocloud.net/) [28] using the Basic Local Alignment Search Tool (BLAST) [29] to determine an approximate phylogenetic affiliation of each strain. The corresponding sequences of closely related type species were retrieved from GenBank database using the EzBioCloud server. Multiple alignments were made using CLUSTAL_X tool in MEGA version 7.0 [30]. Phylogenetic tree based on neighbour-joining method [31] was constructed using the MEGA version 7.0. Evolutionary distances were calculated using the Kimura's two-parameter model [32]. The topology of the phylogenetic tree was evaluated by bootstrap method with 1000 replications [33].

2.5. Nucleotide Sequence Accession Numbers. The sequences obtained in this study were deposited in GenBank with the 16S rRNA gene sequences under the accession numbers: MK589722 -MK589799 and MK685120.

2.6. Small-Scale Fermentation. To check the antibacterial potential of isolated actinobacterial strains, small-scale fermentation was performed. One hundred and fifteen representative strains were selected based on analyses of partial 16S rRNA gene sequences and phenotypic characteristics. Each strain was inoculated separately in six of 500 ml Erlenmeyer flasks containing 100 ml of YIM 38 broth medium. After being incubated for 7 days at 28°C with shaking (at 180 rpm), the 600 ml fermentation broth was centrifuged and its supernatant was extracted twice with ethyl acetate (EtOAc, 1:1, v/v). Organic layer was dried up by rotary evaporation, and residue was dissolved in 3 ml of methanol. Sixty milliliter of water layer was lyophilized, and then its residue was dissolved in 3 ml of 50 % methanol-water. The mycelium was soaked overnight in acetone and then filtered. The acetone extract was dried in vacuo and dissolved in 3 ml of 50 % methanol-water. Ultimately, each strain has three kinds of sample for antibacterial assay.

2.7. Antibacterial Screening. Six sets of indicator bacteria were used in antibacterial assay. Each set consisted of two strains, one was sensitive strain and another was drug-resistant strain. The indicator bacteria were *Enterococcus faecalis* (*E. faecalis*, ATCC 33186, 310682), *Staphylococcus aureus* (*S. aureus*, ATCC 29213, ATCC 33591), *Klebsiella pneumoniae* (*K. pneumoniae*, ATCC 10031, ATCC 700603), *Acinetobacter baumannii* (*A. baumannii*, 2799, ATCC 19606), *Pseudomonas aeruginosa* (*P. aeruginosa*, ATCC 27853, 2774), and *Escherichia coli* (*E. coli*, ATCC 25922, ATCC 35218). *E. faecalis* 310682, *A. baumannii* ATCC 19606, and *E. coli* ATCC 35218 are resistant to vancomycin, carbapenems, and ampicillin, respectively. *S. aureus* ATCC 33591 is resistant to both cefoxitin and oxacillin. Both *K. pneumoniae* ATCC 700603 and *P. aeruginosa* 2774 are resistant to aminoglycosides; meanwhile, *K. pneumoniae* ATCC 700603 is resistant to β-lactam antibiotics and *P. aeruginosa* 2774 is resistant to carbapenems. Indicator bacteria were obtained either from American Type Culture Collection (ATCC) or from the clinic and deposited in Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences.

Antibacterial assay was performed using agar well diffusion method [34]. After drying up, paper disk (diameter 6 mm with 60 μL prepared sample) was placed on Mueller-Hinton (MH) agar containing the indicator bacteria. Meanwhile, 60 μL methanol without sample and with 1 μg levofloxacin was used as the negative control and positive control, respectively. The plates were incubated at 37°C for 24 h, and the antibacterial activity was evaluated by measuring the inhibition zone.

2.8. Mechanism of Action Determination. Ribosome and DNA biosynthesis inhibitors were screened by the double fluorescent protein reporter system with reporter strain JW5503-pDualrep2 [35]. Briefly, 100 μL of ethyl acetate extract was dried up in laboratory hood and 100 μL DMSO was added as sample to be tested. 2 μL of sample was applied to agar plate containing a lawn of the reporter strain. After overnight incubation at 37°C, the plate was scanned by ChemiDoc (Bio-Rad) system with two channels including "Cy3-blot" (553/574 nm, green pseudocolor) for RFP fluorescence and "Cy5-blot" (588/633 nm, red pseudocolor) for Katushka2S fluorescence. Induction of Katushka2S expression is triggered by translation inhibitors, while RFP is upregulated by induction of DNA damage SOS response. Levofloxacin and erythromycin were used as positive controls for DNA biosynthesis and ribosome inhibitors, respectively.

Table 2: Information on genera distribution of actinobacterial strains.

Genera	No. of isolates	No. of strains for assay	No. of strains with antibacterial activity
Streptomyces	86	27	20
Microbacterium	78	6	0
Agromyces	77	5	2
Rhodococcus	64	6	4
Sinomonas	46	5	3
Mycobacterium	28	3	0
Curtobacterium	23	2	0
Nocardia	20	7	2
Arthrobacter	15	3	1
Leifsonia	13	4	1
Paenarthrobacter	11	1	1
Kocuria	10	5	1
Nocardiopsis	7	4	3
Brachybacterium	7	1	0
Agrococcus	6	2	0
Glutamicibacter	6	2	1
Kitasatospora	5	1	1
Isoptericola	4	1	1
Mycolicibacterium	4	4	2
Aeromicrobium	3	1	0
Brevibacterium	2	1	0
Schumannella	2	2	0
Micrococcus	2	2	1
Arsenicicoccus	2	2	0
Cellulosimicrobium	2	2	1
Gordonia	2	2	2
Micromonospora	2	2	2
Pseudarthrobacter	1	1	1
Homoserinibacter	1	1	1
Amnibacterium	1	1	1
Frigoribacterium	1	1	0
Oerskovia	1	1	0
Janibacter	1	1	0
Streptosporangium	1	1	0
Actinomadura	1	1	1
Modestobacter	1	1	0
Pseudonocardia	1	1	1
Nocardioides	1	1	0
Microlunatus	1	1	0
Total number	539	115	54

3. Result

3.1. Isolation and Diversity of Cultivable Actinobacteria from Mangrove Soil. Among 843 isolates obtained, 539 isolates were identified as actinobacterial strains by partial 16S rRNA gene sequence comparison analysis and further assigned to 39 genera in 18 families of 8 orders as follows: *Streptomyces*, *Microbacterium*, *Agromyces*, *Rhodococcus*, *Sinomonas*, *Mycobac-terium*, *Curtobacterium*, *Arthrobacter*, *Nocardia*, *Kocuria*, *Paenarthrobacter*, *Nocardiopsis*, *Glutamicibacter*, *Brachybacterium*, *Agrococcus*, *Isoptericola*, *Aeromicrobium*, *Kitasatospora*, *Mycolicibacterium*, *Micrococcus*, *Arsenicicoccus*, *Brevibacterium*, *Schumannella*, *Leifsonia*, *Cellulosimicrobium*, *Gordonia*, *Micromonospora*, *Homoserinibacter*, *Pseudarthrobacter*, *Amnibacterium*, *Frigoribacterium*, *Oerskovia*, *Janibacter*, *Streptosporangium*, *Actinomadura*, *Modestobacter*, *Pseudonocardia*, *Nocardioides*, and *Microlunatus* (Figure 1). The predominant genus was *Streptomyces* (16.0 %, 86 strains), followed by *Microbacterium* (14.5 %, 78 strains), *Agromyces* (14.3 %, 77 strains), and *Rhodococcus* (11.9 %, 64 strains) (Table 2).

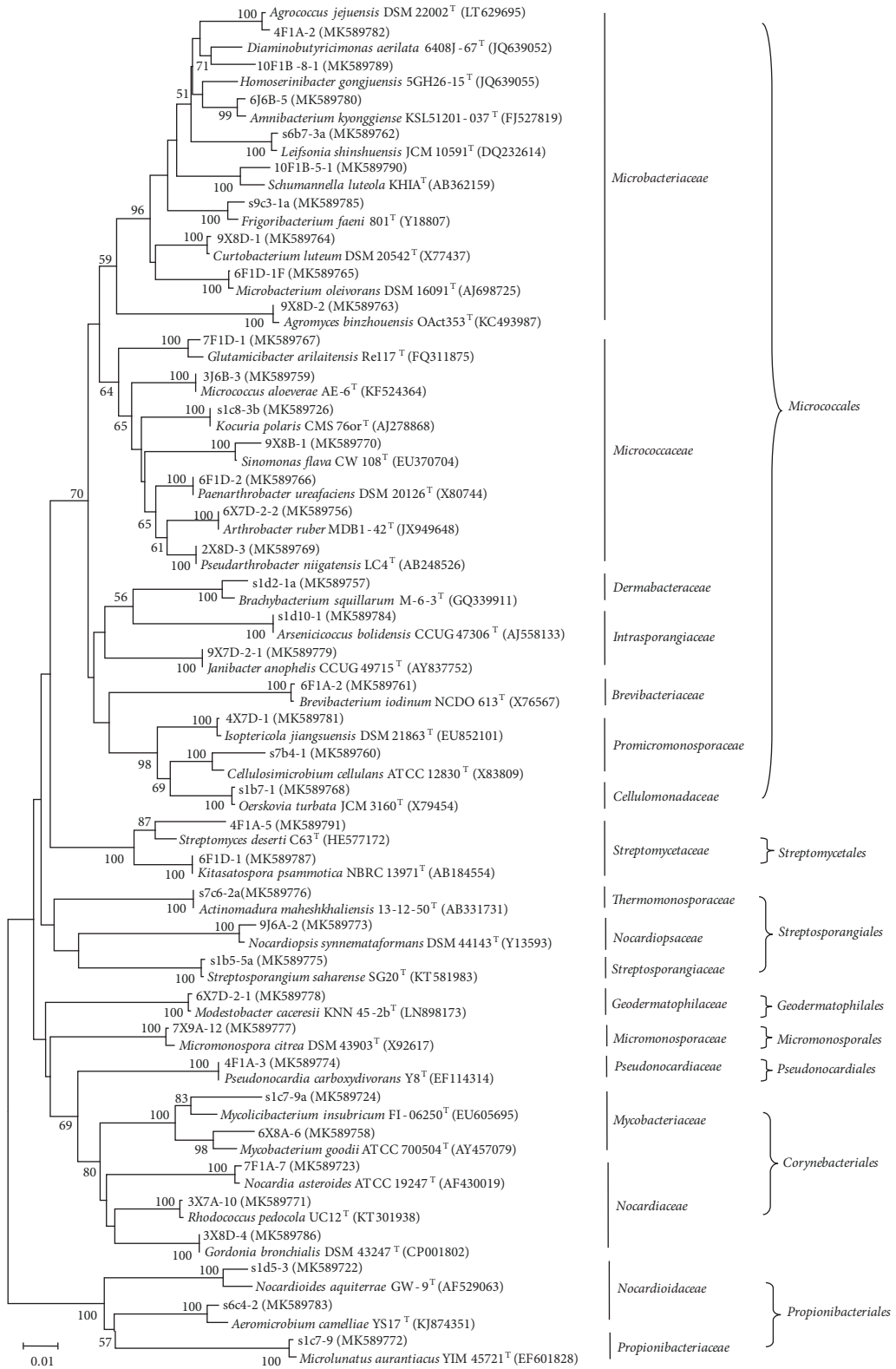

Figure 1: Phylogenetic tree based on the 16S rRNA gene sequences using neighbour-joining method for the representative actinobacterial strains and their closely related type strains. Numbers at nodes indicate the level of bootstrap support based on 1000 replications (only values > 50 % are shown). Bar, 1 nt substitutions per 100 nt.

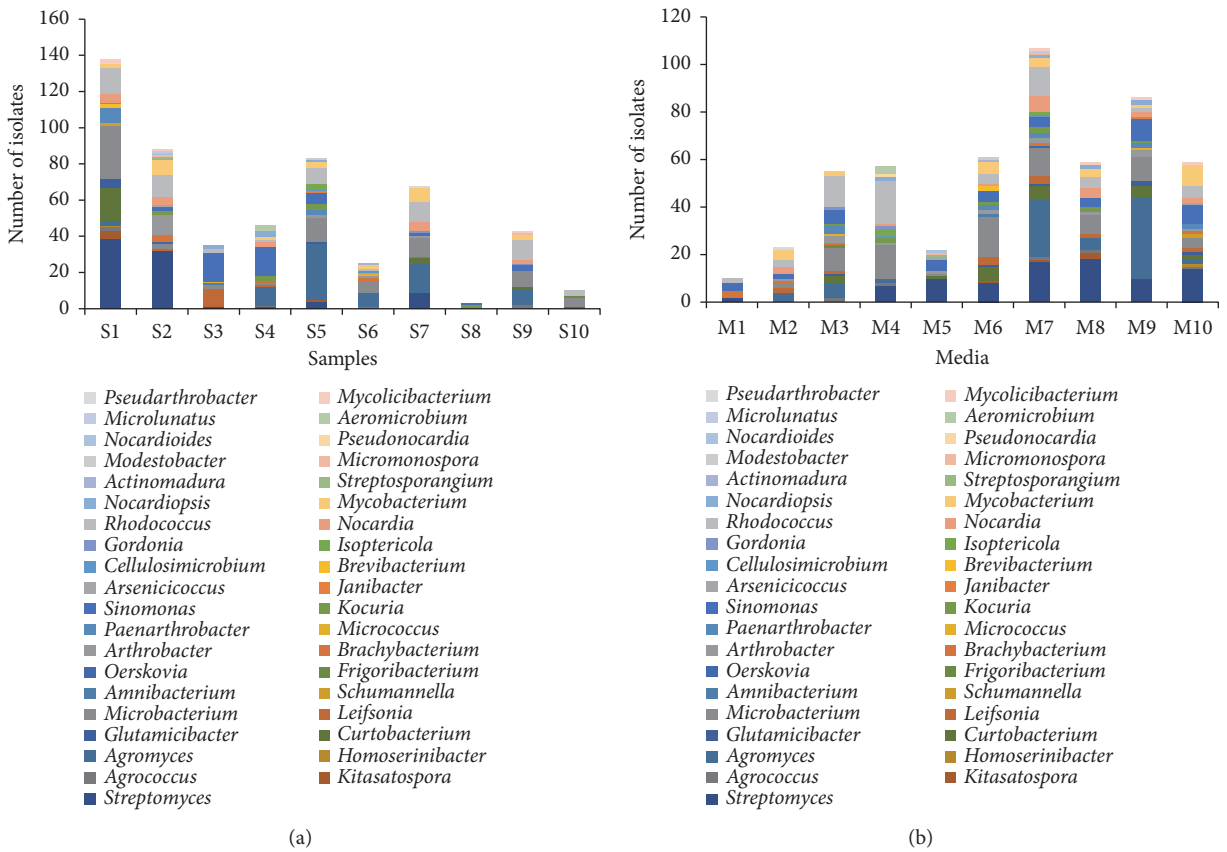

Figure 2: Diversity of cultivable actinobacteria from mangrove soil in Futian and Maoweihai. (a) Number of actinobacterial isolates from different samples. (b) Number of actinobacterial isolates recovered from the different culture media.

Table 3: The sequence analyses based on almost full-length 16S rRNA gene (> 1321 bp) of 11 potential new species.

Strain	Accession number	Closest type species	Similarity of 16S rRNA gene sequence
9X7D-4	MK589797	*Agromyces brachium* IFO 16238^T	98.1 %
2X8D-4	MK589796	*Agromyces neolithicus* 23-23^T	98.1 %
9X9A-10	MK589795	*Agromyces luteolus* IFO 16235^T	98.4 %
s7b8-3	MK589792	*Agromyces italicus* DSM 16388^T	98.2 %
s6c9-2a	MK589794	*Agromyces binzhouensis* OAct353^T	98.3 %
s6c8-3a	MK589793	*Agromyces binzhouensis* OAct353^T	98.4 %,
7X8A-10	MK589799	*Agromyces tropicus* CM9-9^T	98.3 %
7X7D-2	MK589798	*Agromyces tropicus* CM9-9^T	97.2 %
10F1B-8-1	MK589789	*Homoserinibacter gongjuensis* 5GH26-15^T	97.7 %
10F1B-5-1	MK589790	*Schumannella luteola* KHIA^T	98.2 %
4F1A-5	MK589791	*Streptomyces deserti* C63^T	98.0 %

The distribution of the 539 actinobacterial strains from 10 samples is displayed in Figure 2(a) and Table S2. Sample 2 gave the highest diversity (18 genera), followed closely by sample 1 (17 genera), sample 5 (16 genera), sample 4 (13 genera), sample 7 (12 genera), both sample 6 and sample 9 (11 genera), sample 3 (8 genera), sample 10 (4 genera), and sample 8 (2 genera). Among the 10 different media used for isolation of actinobacteria, M7 generated the most successful isolation according to the number and diversity of obtained isolates as shown in Figure 2(b) and Table S3. Totally, 107 actionbacterial strains distributed in 23 genera were obtained from M7. M10 produced the second-highest diversity of isolates (18 genera), and M9 generated the second-highest number of isolates (86 strains). Meanwhile, M1 yielded the lowest number and diversity of isolates (10 strains in 4 genera).

3.2. Novelty of Cultivable Actinobacteria. Among the 539 actinobacterial strains, 11 strains exhibited low 16S rRNA gene sequence similarities (< 98.65 %, the threshold for differentiating two species) [36] with validly described species based on the results of BLAST search in EzBiocloud (Table 3), which indicated that these isolates could represent novel taxa. The

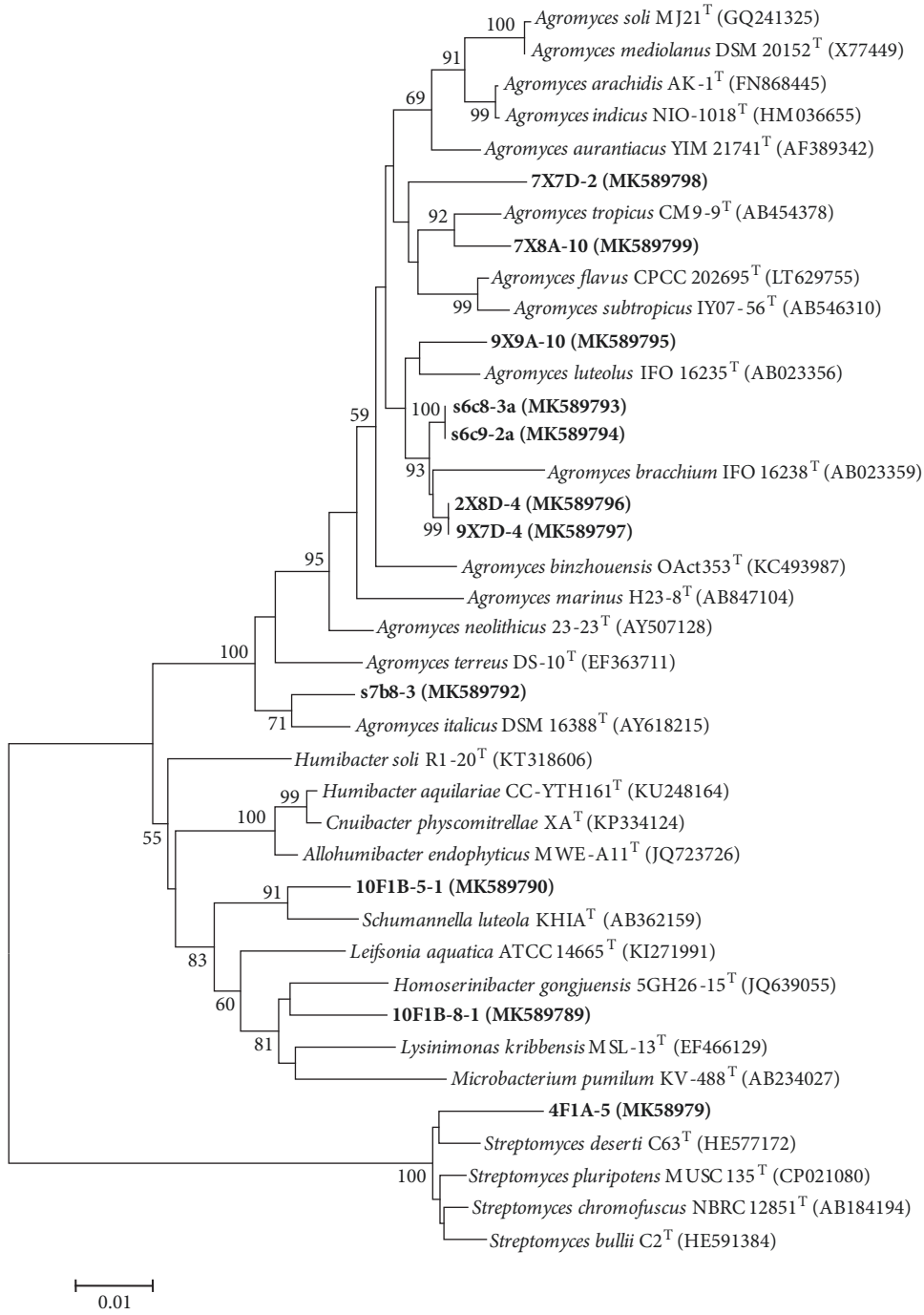

Figure 3: Neighbor-joining phylogenetic tree based on almost full-length 16S rRNA gene sequences of 11 potential novel strains and their closely related type strains. Numbers at nodes indicate the level of bootstrap support based on 1000 replications (only values > 50 % are shown). Bar, 1 nt substitutions per 100 nt.

phylogenetic tree based on almost full-length 16S rRNA gene sequences (Figure 3) showed these potential novel strains were assigned to 4 genera including *Agromyces* (8 strains), *Homoserinibacter* (1 strain), *Schumannella* (1 strain), and *Streptomyces* (1 strain). These strains will be further identified with a polyphasic approach to determine their taxonomic positions.

3.3. Antibacterial Activity of Actinobacterial Isolates. Among the 115 strains selected for antibacterial assay, 54 strains, affiliated to 23 different genera, showed antagonistic activity against at least one of the indicator bacteria (Table 2). The antibacterial profiles of the 54 strains against "ESKAPE" bacteria are shown in Figure 4. Among them, 37 strains were active against at least one of Gram-positive bacteria and 32

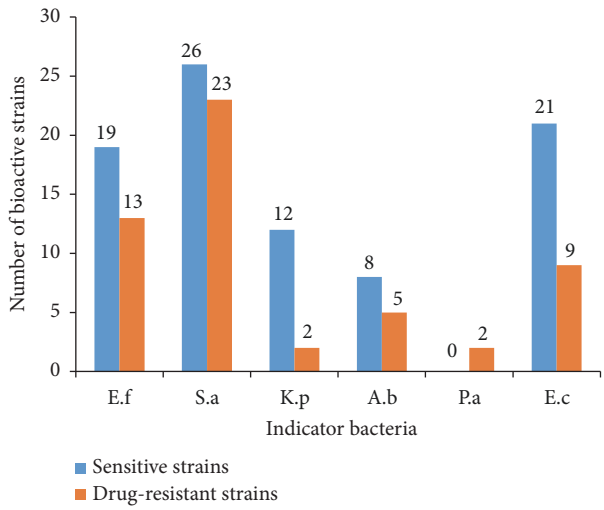

FIGURE 4: The antibacterial profiles of the actinobacteria against "ESKAPE" bacteria (E.f: *Enterococcus faecalis*; S.a: *Staphylococcus aureus*; K.p: *Klebsiella pneumoniae*; A.b: *Acinetobacter baumannii*; P.a: *Pseudomonas aeruginosa*; E.c: *Escherichia coli*).

strains were active against at least one of Gram-negative bacteria; meanwhile, 16 strains exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria.

3.4. Mechanism of Action Determination. Ethyl acetate extracts from the culture broths of 115 strains were screened by the double fluorescent protein reporter system. Five strains, including 3 strains (strains 10X7D-1-3, 7X8A-5, and s1b9-3) in genus *Streptomyces*, 1 strain (strain s1d5-4) in genus *Micromonospora*, and 1 strain (strain s7b4-1) in genus *Cellulosimicrobium*, induced Katushka2S expression as erythromycin did. Meanwhile, no strain induced SOS-response as levofloxacin did (Figure 5).

4. Discussion

Actinobacteria are widely dispersed throughout the mangrove environments [21, 37]. Previous studies exhibited 34 actinobacterial genera have ever been isolated from mangrove soil in Futian and Maoweihai [38–47]. In this study, 226 actinobacteria in 29 genera and 313 actinobacteria in 31 genera were isolated from samples collected from Futian and Maoweihai, respectively. Twenty-one genera recovered were shared by both Futian and Maoweihai. The combination of 10 culture media and 10 mangrove soil samples led to the discovery of 39 actinobacterial genera and 11 potential new species, which not only provided more diverse strains for assay, but also demonstrated that it is necessary to use various types of isolation media to increase in the number and diversity of actinobacteria. Mangrove microorganisms especially actinobacteria have been reported to have the ability to produce structurally unique and bioactive natural products [16, 21, 48]. According to the report of Xu et al. [21], about 73 novel compounds have been reported from mangroves originated actinobacteria and among these, 40 new compounds were reported from actinobacteria isolated from the mangrove soil samples only, which shows that actinobacteria from mangrove soil have great advantage to produce new bioactive metabolites.

In the antibacterial assay, 54 strains affiliated to 23 genera, including 26 strains in 11 genera from Futian samples and 28 strains in 15 genera from Maoweihai samples, exhibited inhibitory activities against at least one of "ESKAPE" bacteria as shown in Table S4. These active strains consisted of 20 strains in genus *Streptomyces* and 34 strains in 22 rare genera. The predominant active strains belong to genus *Streptomyces*, which is in line with the previous reports [19, 39, 49]. Twenty *Streptomycete* strains, including a potential new species designated as strain 4F1A-5, showed inhibitory activity against at least one of Gram-positive bacteria, and 8 of them also showed activity against at least one of Gram-negative bacteria. As the biggest genus in actinobacteria, *Streptomyces* contains 848 species and 38 subspecies (http://www.bacterio.net/streptomyces.html), members of genus *Streptomyces* are well-known as the main sources of antibiotics with diverse biological activities and chemical structures [50], since they usually harbor the large genome size and possess a number of biosynthetic gene clusters that encode multifunctional biosynthetic enzymes [51, 52].

Rare actinobacteria also are important sources in the discovery of novel antibiotics [53]. Recently, mangromicins, a group of new secondary metabolites with unique chemical structures, were found from *Lechevalieria aerocolonigenes* K10-0216 isolated from a mangrove sediment sample by Omura's group [54–56], which further indicated the rare actinobacteria deserve to be studied extensively to find new antibiotics. In the present study, several active strains in 22 rare genera such as *Sinomonas*, *Pseudarthrobacter*, *Leifsonia,* and *Gordonia* have been rarely studied. Notably, strain 10F1B-8-1, as a potential new species in rare genus *Homoserinibacter*, showed broad-spectrum antibacterial activity (Table S4) and is definitely worth studying in priority.

pDualrep2 reporter system is a very sensitive screening model for sorting out antibiotic's mechanisms of action, which can distinguish simultaneously between antibiotics that induce the SOS response due to DNA damage and cause the Katushka2S expression due to ribosome stalling. The existence of ribosome inhibitors such as erythromycin will lead Katushka2S expression, and the existence of inhibitors of DNA biosynthesis such as levofloxacin will lead RFP expression. In this study, screening results indicated 5 strains produced inhibitors of ribosome, but none produced inhibitors of DNA biosynthesis. Taking results of antibacterial activities of the 5 strains into consideration, strains s1b9-3, 10X7D-1-3, and s7b4-1 should be studied by order of importance to find potential antibacterial compounds.

5. Conclusion

In our study, the diversity, novelty, and antibacterial activity of cultivable actinobacteria from mangrove soil in Futian and Maoweihai of China were investigated. A total of 539 cultivable actinobacterial strains were identified and affiliated

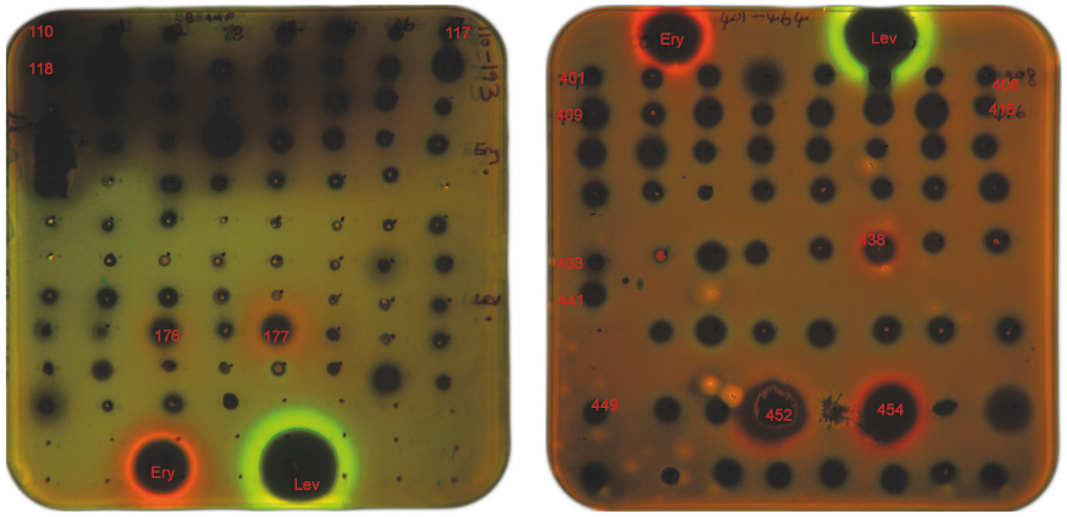

Figure 5: Induction of a two-color dual reporter system sensitive to inhibitors of the ribosome progression or inhibitors of DNA replication, respectively. Spots of erythromycin (Ery), levofloxacin (Lev), and tested samples were placed on the surface of an agar plate containing *E. coli* tolC cells transformed with the pDualrep2 reporter plasmid. Shown is the fluorescence of the lawn of *E. coli* cells scanned at 553/574 nm (green pseudocolor) for RFP fluorescence and 588/633 nm (red pseudocolor) for Katushka2S fluorescence. Induction of Katushka2S expression is triggered by translation inhibitors, while RFP is upregulated by induction of DNA damage SOS response. 176: 10X7D-1-3; 177: 7X8A-5; 438: s1d5-4; 452: s7b4-1; 454: s1b9-3.

to 39 genera in 18 families of 8 orders. Eleven strains were considered as potential new taxa. The antibacterial assays showed 54 strains in 23 genera had antagonistic activities against at least one of "ESKAPE" bacteria, and the screening results based on pDualrep2 reporter system indicated the cultural broth of 5 strains could cause ribosome stalling as erythromycin did. Comprehensive analyses of all results in present study reveal that *streptomycetes* and rare actinobacteria isolated from mangrove soil are valuable sources to find new antibiotics. Notably, it seems that culture broths of *streptomyces* more frequently exhibit inhibitory activities against Gram-positive bacteria such as *E. faecium* and *S. aureus* than against Gram-negative bacteria such as *P. aeruginosa*. Sensitive and reliable screening model based on mechanism of action can accelerate the selection of target strains for further chemical studies.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This research was partly supported by the Drug Innovation Major Project of China (Grant No. 2018ZX09711001-007-001), PUMC Youth Fund (Grant No. 2017350022), CAMS Innovation Fund for Medical Sciences (Grant No. CAMS 2017-I2M-1-012), Russian Science Foundation (Grant No. 18-44-04005), and National Natural Sciences Foundation of China (Grant No. 81621064; 81361138020; 81460537).

Supplementary Materials

Table S1: Composition of ten different media used for isolation of actinobacteria from mangrove soil in Futian and Maoweihai. Table S2: Information on genera distribution of actinobacterial isolates from different samples. Table S3: Information on genera distribution of actinobacterial isolates recovered from the different culture media. Table S4: Antibacterial activity of 54 actinobacterial strains from mangrove soil in Futian and Maoweihai. *(Supplementary Materials)*

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2019, Article ID 1263260, 8 pages
https://doi.org/10.1155/2019/1263260

Research Article

Antioxidant, Hypoglycemic, and Neurobehavioral Effects of a Leaf Extract of *Avicennia marina* on Autoimmune Diabetic Mice

Mohammad K. Okla,[1] Saud A. Alamri,[1] Abdulrahman A. Alatar,[1] Ahmed K. Hegazy,[2] Abdullah A. Al-Ghamdi,[1] Jamaan S. Ajarem,[3] Mohammad Faisal,[1] Eslam M. Abdel-Salam,[1] Hayssam M. Ali,[1,4] Mohamed Z. M. Salem,[5] and Mostafa A. Abdel-Maksoud[3,6]

[1]*Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
[2]*Department of Botany, Faculty of Science, Cairo University, Cairo, Egypt*
[3]*Department of Zoology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia*
[4]*Timber Trees Research Department, Sabahia Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt*
[5]*Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt*
[6]*Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt*

Correspondence should be addressed to Mohamed Z. M. Salem; zidan_forest@yahoo.com

Received 21 February 2019; Revised 24 April 2019; Accepted 2 May 2019; Published 21 May 2019

Guest Editor: Jayanta Kumar Patra

Diabetes mellitus (DM) is a metabolic disease that can affect the central nervous system and behavioral traits in animals. Streptozotocin-induced diabetes is considered an autoimmune disease. The aim of the current study was to determine whether supplementation with the alcoholic extract of *Avicennia marina* leaves could improve diabetes-associated pathological changes. The animals were divided into four groups: a control group (A), an *A. marina* receiving nondiabetic group (B), a diabetic group (C), and a DM group orally supplemented with *A. marina* alcoholic leaf extract (D). The DM group of animals receiving the alcoholic extract of *A. marina* leaves had reduced blood glucose levels, improved blood picture, and organ functions. This group also showed improvement in locomotory behavior. The results of this study showed that supplementation with the alcoholic extract of *A. marina* leaves reduced oxidative stress and blood sugar levels, protected the liver, and improved the neurobehavioral changes associated with diabetes in mice. Introducing alcoholic leaf extract of *A. marina* to diabetic mice decreased inflammatory cells aggregation, vacuolation, and hemorrhage. Additionally, a positive effect of the alcoholic leaf extract on the histopathological changes was observed in the testicular tissue of treated mice.

1. Introduction

Medicinal plants have recently gained much attention from research groups worldwide. The need for new, safer, and effective therapeutic agents represents the main targets for clinical investigators [1]. Owing to the fluctuations in temperature, salinity, and oxygen availability mangrove forests can undergo metabolic pathway adaptations and consequently produce valuable metabolites [2].

Avicennia marina is one of the most important mangrove plants that have gained more attention because of its medical importance [3, 4]. Indeed, the study of the medical importance of *A. marina* started early when Bell and Duewel [5] isolated triterpenoids from the bark of *A. marina*. These terpenoids were later identified as lupeol, taraxerol, and betulinic acid [6]. When the antibacterial activity against bacterial specified pathogens was assessed for some mangrove plants, maximum antibacterial activity was observed with the

leaf extract of *A. marina* [7]. Additionally, *A. marina* leaf extract has shown antimicrobial activity against some clinical pathogens isolated from urinary tract infections including *Klebsiella pneumoniae* [8]. The antiviral activity of *A. marina* leaf extract was also elucidated [4]. Moreover, the methanolic crude extracts of *A. marina* have inhibited the growth of *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Aspergillus niger,* and *Candida albicans* [9].

Diabetes mellitus (DM) is a worldwide disease with a rapidly growing incidence and severe complications, especially in older individuals. Saudi Arabia ranks as having the second highest incidence of diabetes in the Middle East. It is estimated that around 7 million of the Saudi population are diabetic [10]. "In fact, diabetes has approximately registered a tenfold increase during the last three years in Saudi Arabia" and "DM has been found to be related to high mortality, morbidity, and vascular complications, accompanied by poor general health and lower quality of life" [11]. However, there is a severe lack of studies dealing with herbal therapy for diabetes in Saudi Arabia. Almost all the available data are perspective rather than curative studies [12–14]. Besides, the mangrove ecosystem of the Saudi Arabian Red Sea coast has not yet been investigated enough.

For these reasons, the current study was conducted to investigate the possible therapeutic effects of the alcoholic extract of *A. marina* leaves on streptozotocin (STZ)-induced diabetes in mice.

2. Materials and Methods

2.1. Animals and Housing. Forty male Swiss Webster (SW) mice were purchased from the animal house (College of Pharmacy, King Saud University). Their average weight was 25-30 gm and they were maintained and monitored in a specific pathogen-free environment. All animal procedures were performed as described elsewhere [15]. The animals had free access to food and water and blood samples were collected at equivalent times relative to feeding.

2.2. Diabetes Induction and Experimental Groups. To induce diabetes, groups of animals were intraperitoneally injected with STZ (70 $mg{\cdot}kg^{-1}$). STZ-injected animals exhibited massive glycosuria and hyperglycemia (200-250 $mg{\cdot}dL^{-1}$) unlike the control (50-100 $mg{\cdot}dL^{-1}$) animals within 5 days of STZ administration. Animals were divided into four groups (10 mice/group) as follows: group (A), negative control (administered phosphate buffered saline), neither diabetic nor receiving the extract; group (B), Positive control not diabetic group, receiving the alcoholic extract of *A. marina* leaves; group (C), diabetic; and group (D), diabetic receiving the alcoholic extract of *A. marina* leaves.

2.3. Samples Collection and Preparation of Plant Extract. Fresh older leaf samples of *A. marina* were collected from the Jazan district (southwest), Kingdom of Saudi Arabia (KSA). Two hundred grams of dried *A. marina* leaves was chopped into small pieces and soaked in 500 ml of ethanol for 7 days. The colored ethanol solvent was subjected to filtration and kept under a rotary flash evaporator (Buchi, Japan) to obtain the solid extract. The extract was dissolved in 80% ethanol and sterile distilled water was added to prepare a final volume of 100 mg/ml and sterilized by filtration. The animals were administered 2 mg/gm of the extract for four weeks and the dose was calculated according to the average weight of the animals. The percentage of extraction was calculated using the following formula:

$$\text{Percentage of extraction } (\%) = \frac{\text{Weight of the extract } (\text{g})}{\text{Weight of the dried plant material } (\text{g})} \times 100. \quad (1)$$

2.4. Sample Preparation for Cell Blood Count (CBC) and Histological Analysis. After four weeks of *A. marina* supplementation, mice were prepared for sampling and blood was collected from the heart in heparinized tubes and divided into two parts: one part for the determination of hematological parameters and the other to obtain plasma.

Both liver and testis were removed and cut into small pieces in sterile saline and then fixed in neutral buffered formalin (10%) for histological sections or Tris buffer for biochemical analyses. Sections were cut and stained with hematoxylin and eosin (H/E) and then analyzed under a light microscope (Labomed, Laboamerica, Inc., USA). A pathologist blinded for the experimental regimen performed the pathological evaluation of the H/E stained tissue sections.

2.5. Liver and Testis Function Testing. Analysis of plasma samples was performed using commercial kits (Biomerieux, Marcy I'Etoil, France) for alanine aminotransferase (ALT) and creatinine (Creat.) according to the manufacturer's instructions. Absorbance was measured using the Ultrospec 2000 U/V spectrophotometer (Amersham, Pharmacia Biotech, Cambridge, England).

2.6. Oxidative Stress Assessment in Hepatic Tissue. Oxidative stress markers were determined in the liver homogenate using commercial kits (Biodiagnostic, Dokki, Giza, Egypt) for nitric oxide (NO), hydrogen peroxide (H_2O_2), reduced glutathione (GSH), and malondialdehyde (MDA) according to the manufacturer's instructions.

2.7. Antioxidant Activity Assessment in Hepatic Tissue. Antioxidant activity in hepatic tissue was assessed in the liver homogenate using commercial kits (Biodiagnostic, Dokki, Giza, Egypt) for the determination of catalase (CAT) activity according to the manufacturer's instructions.

2.8. Locomotory Behavior in the Open-Field Area. After 4 weeks from the start of the experiment, the four animal groups were tested for locomotory behavior using the Ugo Basile 47420-Activity Cage (Italy) that can record the spontaneous coordinate activity in mice and correlate variation of this activity with time.

2.9. Statistical Analysis. First, for the normality check of the data, the Anderson-Darling test was applied. The data

were normally distributed and are expressed as the mean ± standard error of the mean (SEM). Second, significant differences among groups were analyzed using a one- or two-way analysis of variance followed by Bonferroni's test for multiple comparisons using PRISM (GraphPad Software). Differences were considered statistically significant at $P < 0.05$.

3. Results and Discussion

3.1. Improved CBC in Diabetic Mice Receiving the Alcoholic Leaf Extract of A. marina. CBC is usually used as a biological indicator of the physiological status and pathological consequences of diseases. As described in Tables 1 and 2, aberrant CBC was exhibited by group C as compared with group A. This abnormal CBC was represented as decreased levels of red blood corpuscles (RBCs), white blood corpuscles (WBCs), and hemoglobin (Hb) with a concomitant increase in the levels of hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets. Group D showed an improvement in this altered CBC and a restoration to near normal levels was observed. Group B showed a nonsignificant change from the normal values of group A.

3.2. Hypoglycemic Effect of the Alcoholic Extract of A. marina Leaves on Diabetic Mice. Blood glucose level was determined in all groups of mice. Blood glucose levels were higher in group C (131.4 ± 2.97 mg/dl) ($P < 0.05$) than in group A (77.91 ± 3.76 mg/dl) (Table 3). Oral supplementation with the alcoholic extract of *A. marina* leaves was associated with a significant decrease in the diabetes-associated hyperglycemia. Group D, unlike group A, showed a significant decrease in blood glucose levels (75.5 ± 4.1 mg/dl) ($P < 0.05$). Group B showed blood glucose levels (79.9 ± 3.4 mg/dl) that were not significantly different ($P > 0.05$) from the normal values of the control group (Table 3).

3.3. Improved Liver and Testis Functions in Diabetic Mice Receiving the Alcoholic Leaf Extract of A. marina. Liver and testis functions were investigated to evaluate the effect of oral supplementation with the alcoholic leaf extract of *A. marina*. As described in Table 4, blood levels of the liver functions indicator, ALT, and the testis functions indicator, Creat., were significantly increased (55.6 ± 4.4 and 3.7 ± 0.1 U/l, respectively) ($P < 0.05$) in group C compared to those in group A. Meanwhile, oral supplementation with the alcoholic leaf extract of *A. marina* had an ameliorating effect on the blood levels of both ALT and Creat. as observed in group D in comparison to that in the control group. Group B showed near normal values of both ALT and Creat.

When investigating the blood level of testosterone hormone in the experimental groups, we found that there was no significant change between the diabetic and the control groups of mice (Table 5). However, when using only the leaf extract, there was a highly significant increase in the testosterone level in the blood samples from the group of mice receiving *Rhizophora* leaf extract [16, 17]. Surprisingly, the concomitant effect of the leaf extract was not significant in the diabetic group of mice compared to that in the control group. This dampening effect on diabetes may be altered when using another part of the plant or another type of solvent.

3.4. Hepatoprotective and Antioxidant Effects of the Alcoholic Leaf Extract of A. marina in Hepatic Tissue of Diabetic Mice. Oxidative stress is a major pathological sign of many diseases including diabetes. Here, group C, unlike group A, showed increased oxidative stress in hepatic tissues, characterized by increased levels of nitrate, MDA, and H_2O_2 with a decrease in the levels of the antioxidant enzymes GSH and CAT (Table 5). Oral supplementation with the alcoholic leaf extract of *A. marina* had a positive effect on diabetes-associated hepatic tissue oxidative stress, whereas it decreased the levels of oxidative stress indicators, nitrate (4.088 ± 0.226 mg/gm), MDA (401.50 ± 33.97 nmol/gm), and H_2O_2 (2.620 ± 0.760 mMol/gm) and increased the levels of the antioxidant enzymes GSH (4.389 ± 0.421 μg/g) and CAT (11.872 ± 0.318 nmol/sec/gm). When orally supplemented with the alcoholic leaf extract of *A. marina*, unlike the negative control group mice, the positive control group mice showed a slight increase in the levels of the antioxidant enzymes, GSH and CAT (Table 6).

3.5. Ameliorating Effects of the Alcoholic Leaf Extract of A. marina on the Diabetes-Associated Behavioral Changes in Diabetic Mice. Locomotory behavior in the open-field area was recorded after 4 weeks from the start of the experiment. In the activity cage, the DM group of mice appeared anxious and attained higher scores in the horizontal and vertical activities than the normal and extract-receiving animals (Figure 1). After receiving the alcoholic extract of *A. marina* leaves, group D mice exhibited a mild amelioration in both the horizontal and vertical activities compared to that by group C mice. Surprisingly, group B, receiving only the alcoholic extract of *A. marina* leaves, exhibited the best profile for locomotory behavior.

3.6. Effect of the Alcoholic Leaf Extract A. marina on the Tissue Sections of Liver. Alcoholic leaf extract of *A. marina* ameliorated the diabetes-associated pathological signs in the liver sections. In the H/E stained liver sections of mice, both group A (Figure 2(a)) and the *A. marina* receiving (Figure 2(b)) groups of mice showed the typical normal structure of hepatic tissue with the strands of hepatocytes arranged around the central vein and normal vascularity. In contrast, tissue sections of group C (Figure 2(c)) exhibited pathological features such as inflammatory cells aggregation, hepatocytic vacuolation, hemorrhage, and edema. Interestingly, the introduction of *A. marina* leaf extract to diabetic mice ameliorated this diabetic-associated tissue pathology as illustrated from the decreased inflammatory cells aggregation, decreased vacuolation, and hemorrhage (Figure 2(d)). These data augment the observation of decreased oxidative stress in liver tissue samples seen in this group of mice.

Table 1: Effect of the alcoholic leaf extract of *Avicennia marina* on the cell blood count (CBC) in mice.

Groups of mice	WBC ($\times 10^9$/L)	RBC ($\times 10^6$/mm^3)	Hb (g/dl)	HCT (%)	MCV (μm^3)	MCH (pg)
Group (A)	7.9 ± 0.5	8.50 ± 0.73	15.6 ± 1.8	37.9 ± 3.7	46.8 ± 3.2	17.3 ± 0.4
Group (B)	8.3 ± 0.6	8.6 ± 0.63	15.9 ± 1.99	41.2 ± 5.2	47.3 ± 3.7	17.8 ± 0.8
Group (C)	6.9 ± 0.8	6.84 ± 0.46∗	11.8 ± 1.5∗	43.3 ± 2.8	52.8 ± 2.2	17.6 ± 0.8
Group (D)	7.4 ± 0.9	7.88 ± 0.09	14.1 ± 2.1	38.6 ± 4.5	48.2 ± 3.1	17.2 ± 0.6

∗$P < 0.05$ for diabetic group of mice vs. negative control; AM: *Avicennia marina;* Hb: hemoglobin; HCT: hematocrit; MCH: mean corpuscular hemoglobin; MCV: mean corpuscular volume; RBC: red blood corpuscles; STZ: streptozotocin; WBC: white blood cells.

Table 2: Effect of the alcoholic leaf extract of *Avicennia marina* on the mean corpuscular hemoglobin concentration, platelets, neutrophils, lymphocytes, and monocytes in mice.

Groups of mice	MCHC (%)	Platelets (10^3/mm^3)	Neutrophils %	Lymphocytes %	Monocytes %
Group (A)	33.6 ± 1.1	998 ± 33	24 ± 2.1	57 ± 4.4	3 ± 0.54
Group (B)	33.5 ± 0.7	890 ± 70	27 ± 2.2	68 ± 3.1	4 ± 0.66
Group (C)	33.8 ± 1.3	1015 ± 31	29 ± 3.15	78 ± 1.6	4 ± 0.9
Group (D)	32.9 ± 0.9	950 ± 60	22 ± 2.6	52 ± 2.3	4 ± 0.25

MCHC: mean corpuscular hemoglobin concentration.

Table 3: Effect of the alcoholic leaf extract of *Avicennia marina* on blood glucose level (mg/dl) in the experimental groups of mice.

Experimental group	Glucose (mg/dl)
Group (A)	(77.91 ± 3.76)
Group (B)	(75.5 ± 3.4)
Group (C)	(131.4 ± 2.97)∗
Group (D)	(79.9 ± 4.1)#

∗$P < 0.05$ for diabetic group of mice vs. control; #$P < 0.05$ for diabetic + AM group of mice vs. control. AM: *Avicennia marina*; STZ: streptozotocin.

Table 4: Effect of the alcoholic leaf extract of *Avicennia marina* on liver functions in diabetic mice.

Groups of mice	ALT (U/l)	Creatinine (mg/dl)
Group (A)	25 ± 5.1	0.6 ± 0.9
Group (B)	27 ± 6.4	0.66 ± 0.09
Group (C)	55.6 ± 4.4∗	3.7 ± 0.1∗
Group (D)	30 ± 5.3	0.7 ± 0.05

∗$P < 0.05$ for diabetic group of mice vs. control. ALT: alanine aminotransferase.

Table 5: Effect of the alcoholic leaf extract of *Avicennia marina* on blood testosterone level in mice.

Experimental group	Testosterone (ng/ml)
Group (A)	0.020 ± 0.002
Group (B)	0.072 ± 0.005∗
Group (C)	0.026 ± 0.001
Group (D)	0.027 ± 0.003

∗$P < 0.05$ for diabetic group of mice vs. control.

3.7. Effect of the Alcoholic Leaf Extract of A. marina on Tissue Sections of the Testis. In the H/E stained testis sections of mice, both the control (Figure 3(a)) and the *A. marina* receiving (Figure 3(b)) groups of mice showed the normal structure of testicular tissue with the characteristic arrangement of seminiferous tubules and the different sperm-forming layers (spermatogonia-primary spermatocyte-secondary spermatocyte). In group C (Figure 3(c)), the situation changed such that the normal structure was disturbed. Vacuolation between tubes, decrease in the sperm number with increase in the number of immature sperms, interstitial edema, and necrosis were the major signs. In the H/E stained testis sections of mice, both of the control and the *A. marina* receiving groups of mice have showed the normal structure of testicular tissue with the characteristic arrangement of seminiferous tubules and the different sperm-forming layers (Spermatogonia- primary spermatocyte-secondary spermatocyte). In the diabetic group of mice, the situation was changed in the way that the normal structure is disturbed. Vacuolation between tubes, decrease in the sperms number with increase in the number of immature sperms, interstitial edema, and necrosis were the major signs. Alcoholic leaf extract showed a positive effect on the histopathological changes in the testicular tissue of treated mice with STZ (Figure 3(d)).

4. Discussion

DM is a metabolic disorder that is considered a major health problem and affects millions of people worldwide. The adjunctive use of standardized pharmaceutical-grade nutrients, known as nutraceuticals, has recently gained the increased interest of many research groups [18] and many nutraceuticals are now being used for treating several diseases. *A. marina* is a mangrove plant that could be introduced as a nutraceutical for diabetes. *A. marina* has previously been shown to have an ameliorating effect on experimental

Table 6: Effect of the alcoholic leaf extract of *Avicennia marina* on oxidative stress parameters in hepatic tissue of mice.

Groups of mice	Nitrate (mg/gm)	MDA (nmol/gm)	H_2O_2 (mMol/gm)	GSH (μgram/g)	CAT (nmol/sec/gm)
Group (A)	3.148 ± 0.258	363.61 ± 37.21	2.179 ± 0.096	5.793 ± 0.748	9.082 ± 0.990
Group (B)	3.367 ± 0.256	347.15 ± 16.81	2.384 ± 0.088	4.198 ± 0.691	3.537 ± 0.146
Group (C)	5.399 ± 0.196∗	597.54 ± 43.16∗	5.376 ± 0.226∗	2.443 ± 0.424∗	3.719 ± 0.071∗
Group (D)	4.088 ± 0.226	401.50 ± 33.97	2.620 ± 0.760	4.389 ± 0.421	11.872 ± 0.318

∗$P < 0.05$ for diabetic group of mice vs. control. CAT: catalase; GSH: reduced glutathione; H_2O_2: hydrogen peroxide; MDA: malondialdehyde.

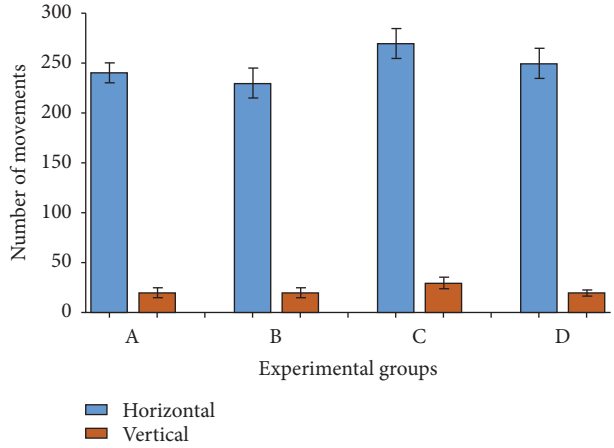

Figure 1: Effect of the alcoholic extract of *Avicennia marina* leaves on the locomotory behavior of the diabetic mice in the activity cage. (A) Negative control group; (B) positive control group; (C) diabetic group; (D) diabetic + AM group (STZ + AM leaf extract). Number of movements per second was plotted for the four experimental groups. AM: *Avicennia marina;* STZ: streptozotocin.

diabetic animals. The current study aimed to investigate the possible effects of oral supplementation with ethanolic extracts from *A. marina* on hematological parameters, liver and kidney functions, oxidative stress, and antioxidant parameters in diabetic mice. Our data revealed a disturbed CBC in diabetic mice. Previous studies have reported an altered red cell turn over in diabetic mice [19]. Additionally, in humans, it has also been reported that monocyte counts in the blood of patients with type-1 diabetes being lower than that in patients without diabetes and this was considered as a side effect of diabetes-associated ketosis [20]. In the current study, oral supplementation with the alcoholic extracts from *A. marina* leaves exerted a hypoglycemic effect on the diabetic mice. This is in accordance with previous studies that reported a significant decrease in the blood glucose level in STZ-induced diabetic rats receiving the aqueous- and hydroalcoholic extracts of *A. marina* leaves [21]. The protective effect of *A. marina* against kidney, liver, and cardiac toxicities was recently elucidated [3]. In the present study, we observed an ameliorating effect of the alcoholic extract of *A. marina* leaves on both kidney and liver functions.

The observed amelioration in liver functions of the diabetic mice that were orally supplemented with the alcoholic extracts from *A. marina* leaves may be attributed to the concomitant oxidative stress-lowering effect that was observed in the same group unlike in the control group. It was reported that diabetes is associated with many pathological signs among which is the increased production of free radicals concomitantly with the decreased antioxidant potential [22].

Indeed, persistent hyperglycemia can induce reactive oxygen species (ROS) generation and consequently diabetic-associated pathological complications appear [23, 24]. For example, nitrite generates an oxidant stress and increases NO in EA.hy926 endothelial cells. Nitrite is a breakdown product of NO that in turn is oxidized to nitrate in cells [25] to attenuate intracellular oxidative stress [26]. It has been reported that NO and ROS are associated with several pathophysiological events in hepatic tissue leading to fibrosis and cirrhosis [27]. Our data revealed improved liver functions along with ameliorating effects of diabetes-associated oxidative stress in mice that were orally supplemented with the alcoholic extracts from *A. marina* leaves compared to those in the control group. These findings augment previous reports on the gastroprotective effect of *Avicennia sp.* leaves [28]. At the neurological level, alcoholic leaf extract of *A. marina* has a mild effect on the locomotory activity of SW mice, either diabetic or not. The open-field activity monitoring system used in the current study is a globally accepted method used to measure locomotor and anxiety-like behavior in mice [29] and for monitoring skeletal muscle diseases [16]. Our results indicating no overt behavioral changes in *A. marina* extract-receiving mice are consistent with previous reports [30]. Taken together, our data revealed an ameliorating effect of the alcoholic leaf extract of *A. marina* on diabetes-associated

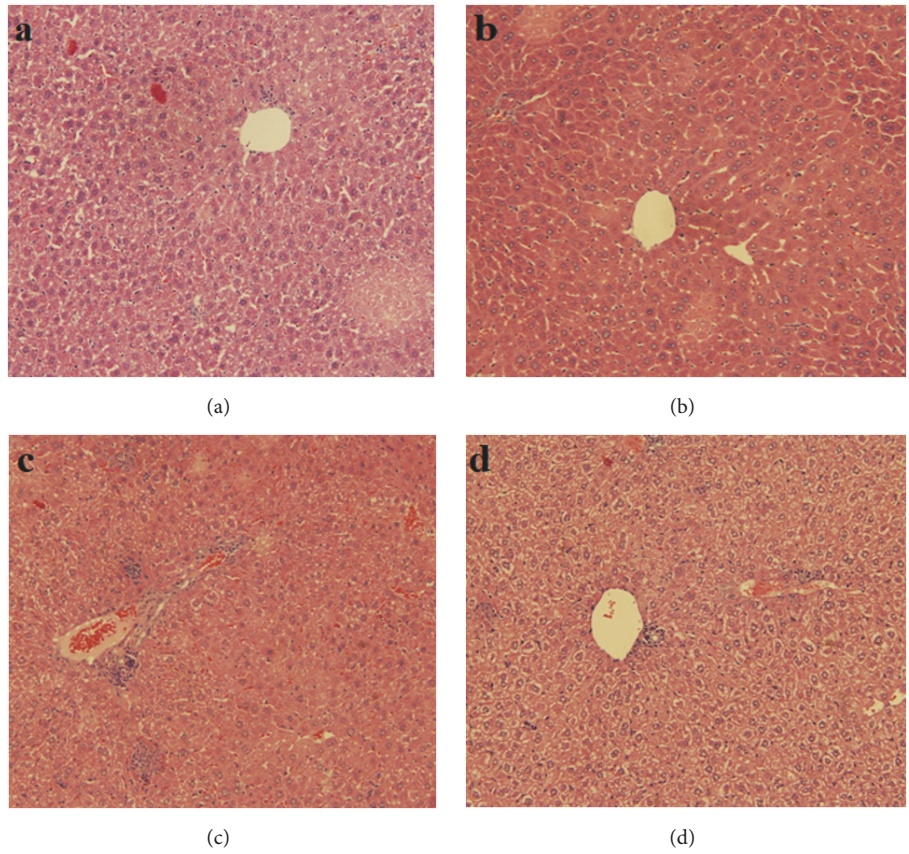

Figure 2: Effect of alcoholic leaf extract of *Avicennia marina* on the tissue sections of liver. (a) Negative control (did not receive any treatment); (b) positive control group receiving the leaf extract; (c) diabetic group (injected with STZ); (d) group receiving STZ + leaf extract. STZ: streptozotocin.

pathology. These effects varied from mild effects to significant ones.

In the current study, the alcoholic leaf extract of *A. marina* showed positive effects on the hepatic tissue pathology of diabetic mice. The ameliorating effect on the histological level was augmented by the biochemical effect of the extract, whereas it exerted oxidative stress-lowering activity in the hepatic tissue as represented by decreased levels of H_2O_2, MDA, and NO concomitantly with increased levels of the antioxidants, CAT and GSH. These results are consistent with previous reports [16, 31, 32]. Moreover, the positive effects of the alcoholic leaf extract of *A. marina* on the testicular tissue were numerous. These effects may be partially attributed to the decreased oxidative stress observed in the testicular tissue concomitantly with the increased level of testosterone hormone in blood. The observed effect of the alcoholic leaf extract of *A. marina* on the testicular tissue may be considered as an extension to previous reports [33, 34].

5. Conclusion

The alcoholic leaf extract of *A. marina* has antioxidant, hypoglycemic, and neurobehavioral effects on diabetic mice.

Data Availability

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Mohammad K. Okla, Saud A. Alamri, Abdulrahman A. Alatar, Ahmed K. Hegazy, Abdullah A. Al-Ghamdi, Mostafa A. Abdel-Maksoud, Jamaan S. Ajarem, Mohamed Faisal, and Eslam M. Abdel-Salam designed the experiments and carried out experiments. Hayssam M. Ali and Mohamed Z.M. Salem analyzed experimental results. All coauthors wrote and revised the manuscript.

Acknowledgments

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center. The authors also thank the Deanship of Scientific

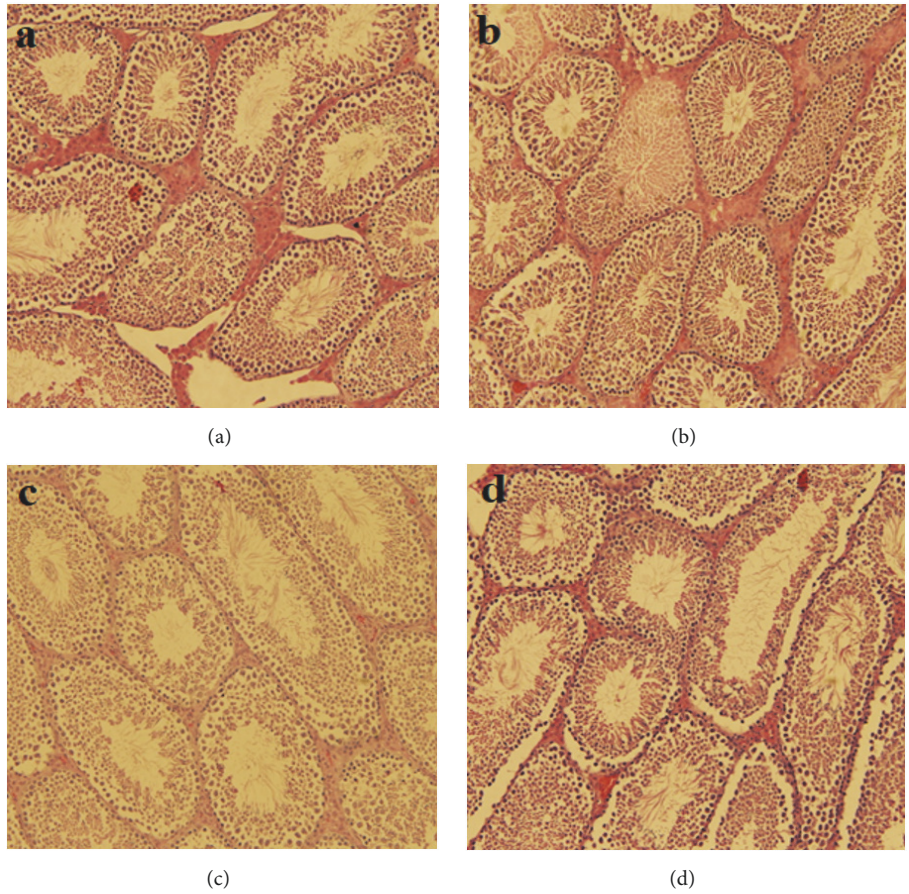

Figure 3: Effect of alcoholic leaf extract of *Avicennia marina* on the tissue sections of testis in mice. (a) Negative control (did not receive any treatment); (b) group receiving the leaf extract; (c) positive control (injected with STZ); (d) group receiving STZ + leaf extract. STZ: streptozotocin.

Research and RSSU at King Saud University for their technical support.

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Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2019, Article ID 8493190, 13 pages
https://doi.org/10.1155/2019/8493190

Research Article

Effect of *Xylocarpus granatum* Bark Extract on Amelioration of Hyperglycaemia and Oxidative Stress Associated Complications in STZ-Induced Diabetic Mice

Swagat Kumar Das,[1,2] **Arpita Prusty**,[2] **Dibyajyoti Samantaray**,[1] **Mojeer Hasan**,[3] **Srikanta Jena**,[2] **Jayanta Kumar Patra**,[4] **Luna Samanta**,[2] **and Hrudayanath Thatoi**[5]

[1]*Department of Biotechnology, College of Engineering and Technology, Biju Patnaik University of Technology, Bhubaneswar, Odisha-751003, India*
[2]*Redox Biology Laboratory, Department of Zoology, School of Life Sciences, Ravenshaw University, Cuttack, Odisha 753003, India*
[3]*Microbial & Pharmaceutical Biotechnology Laboratory, Faculty of Pharmacy, Jamia Hamdard University, Hamdard Nagar, New Delhi 110062, India*
[4]*Research Institute of Biotechnology & Medical Converged Science, Dongguk University-Seoul, Goyang-si, Republic of Korea*
[5]*Department of Biotechnology, North Orissa University, Sriram Chandra Vihar, Takatpur, Baripada, Odisha 757003, India*

Correspondence should be addressed to Jayanta Kumar Patra; jkpatra@dongguk.edu
and Hrudayanath Thatoi; hn_thatoi@rediffmail.com

Received 5 November 2018; Revised 11 February 2019; Accepted 25 March 2019; Published 2 May 2019

Academic Editor: Sakthivel Muniyan

Xylocarpus granatum is a medicinal mangrove plant, traditionally used for the treatment of diarrhoea, cholera, fever, dyslipidaemia, inflammation, etc. The present study was aimed to evaluate the *in vitro* antidiabetic (α-glucosidase inhibition assay) and antioxidant (ABTS scavenging and metal chelating assay) activities of ethanol, methanol, and aqueous extracts of leaves and barks of *X. granatum* followed by *in vivo* antidiabetic and antioxidant evaluation of ethanol bark extracts in streptozotocin- (STZ-) induced diabetic mice. The *in vitro* evaluation revealed higher α-amylase inhibition and ABTS scavenging activities in ethanol bark extracts of *X. granatum* (XGEB). Administration of XGEB at 100 and 200 mg/kg BW doses to STZ-induced diabetic mice resulted in significant decrease ($P < 0.05$) in blood glucose, triglyceride (TG), total cholesterol (TC), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transminase (SGPT), and urea levels in the serum of the extract administered groups as compared to diabetic control group. The levels of SOD, CAT, GPx, GR, and GST in liver along with LPx, SOD, GST, and GR activities in brain tissues were found to be ameliorated in XGEB treated diabetic mice. Histopathological alternations of liver tissues were also found to be restored in XGEB treated diabetic groups. The HPLC fingerprint analysis of XGEB revealed the presence of simple polyphenols, isoflavone, and flavonol-like compounds. The DSC and UV-VIS analysis also confirmed the presence of phenolic compounds in XGEB. The GC-MS analysis of XGEB showed the presence of a number of bioactive compounds. These results demonstrated the beneficial effect of XGEB in controlling hyperglycaemia and ameliorating oxidative stress associated complications associated with diabetes.

1. Introduction

Diabetes mellitus is a multifactorial metabolic syndrome characterized by defect in the secretion of insulin associated with deregulation in carbohydrate, protein, and lipid metabolism. It is one of the most prevalent diseases affecting all ages of people across the globe. It affected an estimated 415 million people in 2015 and is expected to increase up to 642 million by 2040 [1]. Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays an important role in the pathogenesis of diabetes mellitus. In diabetic condition, free radicals are formed disproportionately mainly due to glucose oxidation and nonenzymatic glycation of proteins leading to depletion of endogenous antioxidant components resulting in increased oxidative stress and development of insulin resistance [2]. Although

several hypoglycaemic drugs are available for treatment of diabetes, they have side effects and usually fail to alleviate oxidative stress and its associated complications. Therefore, management of diabetes without side effects remains a challenge. On the other hand medicinal plants with antidiabetic and antioxidant properties can serve as ideal phytotherapy for treatment of diabetes and oxidative stress associated complications. In this context, mangrove plants growing in the stressful environment at the interface of sea and land can be an important source of drug for treatment of diabetes due to its antidiabetic as well as antioxidant properties because of possession of rich secondary metabolites.

Xylocarpus granatum J. Koenig (Meliaceae) is an important medicinal mangrove plant and well distributed in a number of countries of south-east Asia, Australia, and east Africa [3]. Different parts of this plant have been used traditionally as astringent and febrifuge along with treatment of fever, malaria, thrush, cholera, dysentery, and diarrhoea in many countries including India [4]. Earlier studies have reported the free radical scavenging properties of leaves and barks extracts of *X. granatum* [5]. The epicarp of fruit extracts of this plant has also reported for antidiabetic and antidyslipidaemic properties [6]. Secondary metabolites of different classes have been reported in *X. granatum* such as limonoids (gedunin, xyloccensins, xylograntins, xylocarpins, and xylomexicanins), catechin, epicatechin, 6-dehydroxyxylocarpin D, kaempferol 3-*O*-β-D-glucoside, ergosterol peroxide, β-sitosterol, daucosterol, 4-hydroxybenzoic acid, ethyl 3,4-dihydroxybenzoate, carapolide-A,B, alkaloids, harzianone, trichoacorenol, and trichodimerol [7, 8].

The preliminary study by the authors revealed the higher *in vitro* antidiabetic and antioxidant activities of ethanol bark extract of *X. granatum* as compared to leaves extracts [9]. However, no reports are available on the protective effect of the ethanol bark extract of *X. granatum* (XGEB) on hyperglycaemia mediated oxidative stress in STZ-induced diabetic mice. Therefore, the present study is aimed to undertake a detailed investigation on *in vivo* evaluation of ethanol bark extracts of *X. granatum* (XGEB) on hyperglycaemia and its oxidative stress associated complications in STZ-induced insulin-dependent diabetic mice model. Further, the gas chromatography mass spectroscopy (GC-MS) analysis of ethanol bark extract of *X. granatum* was conducted to identify the major bioactive compounds.

2. Materials and Methods

2.1. Chemicals. p-Nitrophenyl-α-D-glucopyranoside (pNPG), Folin-Ciocalteu's phenol reagent (FCP), catechol, catechin, potassium persulfate, ferrous sulphate, sodium potassium tartrate, 3,5-dinitrosalicylic acid (DNS), sodium hydroxide, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Butylated hydroxy toluene (BHT) and α-amylase, streptozotocin (STZ), and ferrozine were purchased from SRL India, Ltd. Acarbose was purchased from Sigma Aldrich India. All the chemicals and reagents used in the study were of analytical grade.

2.2. Collection of Plant Material. Leaves and barks of *X. granatum* (family-Meliaceae) were collected from the mangrove forest of Mahanadi delta area of Odisha coast (India). The specimen was authenticated by Prasanna Kumar Nayak, Herbarium keeper, Integrated Coastal Zone Management Project (ICZMP), Forest Department, Govt. of Odisha, India. The specimens were identified at Department of Natural Products, Institute of Minerals and Materials Technology, Bhubaneswar (RRL-B), Odisha, India, and voucher specimen (VS No. RRL-B 12567) was deposited.

2.3. Extraction of X. granatum. Successive Soxhlet extraction method was followed to prepare crude extracts from leaves and barks of *X. granatum*. The leaves and barks plant materials were dried under shade and pulverized. The pulverized plant materials (20g) were then extracted successively with 200 ml of 90% ethanol, methanol, and water in Soxhlet apparatus [10]. After extraction, the extracts were concentrated under reduced pressure in rotary evaporator (IKA- RV10).

2.4. In Vitro Antioxidant and Antidiabetic Activities. The *in vitro* antioxidant activities of the ethanol, methanol, and aqueous leaves and barks extracts were evaluated by ABTS scavenging [11] and metal chelating assay [12] and *in vitro* antidiabetic activities by α-amylase inhibition assay [13]. The antioxidant assay was carried out using standard antioxidant compound Butylated hydroxy toluene (BHT). Similarly, the *in vitro* α-amylase inhibition assay was carried out using standard antidiabetic compound Acarbose.

2.5. Experimental Animals. A total of 30 healthy, adult male Balb/c mice of 2 months of age and average body weight of 30 g were maintained under controlled conditions of temperature (23±2°C) and humidity and a 12 h light-dark cycle and were used for the experiment. They were housed in sanitised polypropylene cages and had free access to standard mice pellet diet and water *ad libitum*. All the experimental procedures were performed in IMGENEX India Pvt. Ltd. (No. 526/CPCSEA; 21-01-2002) in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.6. Induction of Diabetes. STZ was dissolved in 0.05 M citrate buffer (pH 4.5) and injected intraperitoneally (*i.p.*) to overnight fasted mice at a single dose of 125 mg/kg body weight. The animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia. After 72 h of STZ administration, blood samples were collected from tail and glucose levels were estimated by glucostrips (One Touch Glucometer, Life Scan, Europe). Mice having fasting blood glucose levels above 200 mg/dl were considered diabetic and subsequently used in the present study.

2.7. Acute Toxicity Study. The toxicity of the extract was assessed as per the previously described method. [14]. Healthy Balb/c mice were randomly assigned into three groups and were given the XGEB extracts at the doses of 100, 300, and 1000 mg/kg body weight orally daily for 4 days by dissolving in 0.05 M citrate buffer (pH 4.5) as vehicle. The

animals were then observed for 96 h. Behaviour signs were recorded.

2.8. Experimental Design. After acclimatization mice were divided into six groups of five animals each.

NC group: normal control mice supplemented with vehicle (0.05 M citrate buffer, pH 4.5);

NCT: normal mice treated with ethanol bark extracts of *X. granatum* (200 mg/kg);

DMC: diabetic control mice supplemented with vehicle;

DMD: diabetic mice treated with glibenclamide drug (3 mg/kg);

DML: diabetic mice treated with ethanol bark extracts of *X. granatum* (100 mg/kg);

DMH: diabetic mice treated with ethanol bark extracts of *X. granatum* (200 mg/kg).

The glibenclamide and *X. granatum* ethanol bark extracts were dissolved in 0.05 M citrate buffer (pH 4.5) and administered orally daily for 30 days. On 0, 7^{th}, 14^{th}, 21^{st}, and 30^{th} day, the body weights of mice were recorded and blood samples were collected from each animal by puncturing the tail veins. At the end of the experiment, blood was collected from the mice by retroorbital bleeding. Liver and brain tissues (cerebral cortex) were excised immediately after sacrificing mice and stored at -20°C till further use.

2.9. Biochemical Analysis. Different parameters like total cholesterol (TC), triglycerides (TG), urea, serum glutamate oxaloacetate transminase (SGOT), and serum glutamate pyruvate transminase (SGPT) levels were determined using the commercial kits (Tulip Diagnostics, India). The liver and brain tissues were assayed for activities of different parameters like lipid peroxidation (LPx), reduced glutathione content (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione-s-transferase (GST) employing established experimental methods [15–20].

2.10. Histopathology of Liver. The liver tissues from each animal were collected and small pieces of each tissue were fixed in sublimate formol and processed by the paraffin technique. Thin sections (7 μm) were cut and stained with hematoxylin-eosin as per the routine staining method. The tissue samples were then examined and photographed under a light microscope [21]. Different changes in the liver tissue such as degeneration of hepatocytes, fatty changes of hepatocytes, inflammatory cell infiltrations, and sinusoidal dilation were evaluated. Scoring of the histopathological changes was done as either present (+) or absent (-).

2.11. Phytochemical Analysis. A quantitative phytochemical test for determination of flavonoid and tannin content of the leaf and bark extracts of *X. granatum* were carried out using standard procedures [22].

2.12. UV-VIS Spectral Analysis. The UV–VIS spectrophotometer UV–117 (Systronics™) was used to measure UV–VIS absorbance spectra of different extracts of *X. granatum* [23]. The absorbance measurements were measured in 200 –700 nm range with a 1 nm step and the characteristic peaks were detected.

2.13. HPLC Analysis. For phytochemical fingerprinting, the ethanol bark extract of *X. granatum* was analyzed by high performance liquid chromatography (HPLC) (Analytical Technologies, Baroda, India) technique following the method described by Kumar et al. (2008) [24]. Simultaneous rapid separation of polyphenols was performed by using RP-C18 Lichrocart 250-4, 5 μm (250 x 4.6 mm) as stationary phase and a linear gradient elution is carried out by mobile phase (water and acetonitrile acidified with 0.02% trifluoroacetic acid) starting with 80% and ending with 20% water in total 22 min run time. The flow rate of mobile phase was kept at 1 ml min^{-1}. The detection was carried out at 280 nm using an UV detector.

2.14. Differential Scanning Calorimetric Analysis. The differential scanning calorimetry (DSC) curves were obtained on a TA Instruments Calorimeter, model DSC 4000 Perkin Elmer, Singapore. The analysis was performed using aluminium crucibles with about 2 ± 0.1mg of samples under nitrogen atmosphere, at a flow rate of 20 ml/min. DSC thermogram was recorded constantly and continuously by increasing the temperature from 20 to 300°C at a heating rate of 20°C/min. Indium (m.p. 156.6°C) was used as standard for equipment calibration [25].

2.15. GC-MS Analysis. The ethanolic bark extract of *X. granatum* was analyzed using GC-MS [26]. The analysis was conducted with an Agilent Technologies 7890B GC ION TRAP MS (Agilent Technologies, Santa Clara, California, USA). ADB-5SILMS capillary column was used (30 m x 0.25 mm internal diameter, 0.25 μm film thickness). The ultrapure helium was used as a carrier gas at a flow rate of 0.7 mL/min and a linear velocity of 37 cm/s. The injector temperature was set at 250°C. The initial oven temperature was 60°C, which was ramped up to 280°C at a rate of 10°C/min with a hold time of 3 minutes. The MS operating conditions were electron ionization mode at 70 eV and scan range 50-700 amu. Compounds were identified by comparing the retention times and mass fragmentation with the National Institute of Standards and Technology (NIST) library.

2.16. Statistical Analysis. The data were analyzed using the SPSS for Windows, version 20, IBM Corporation. Statistical analysis was done by two-way ANOVA for blood glucose level, whereas other biochemical parameters were analysed by one-way ANOVA, followed by LSD. Experimental data were expressed as mean ± standard deviation (SD). A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. In Vitro Antioxidant and Antidiabetic Activities. Table 1 demonstrates the *in vitro* antioxidant and antidiabetic activities of leaf and bark extracts of *X. granatum* in terms

Table 1: ABTS radical scavenging (expressed as IC_{50} value in μg/mL) and α-amylase inhibitory activities (expressed as IC_{50} value in mg/mL) of *X. granatum* extracts. EL = Ethanol leaf extracts; ML = Methanol leaf extracts; AL = Aqueous leaf extracts; EB = Ethanol Bark extracts; MB = Methanol Bark extracts; AB = Aqueous Bark extracts of *X. granatum*. The values are expressed as mean ± SD (n=3).

Sample	ABTS	α-Amylase
EL	42.02 ± 0.41	1.04 ± 0.05
ML	127.54 ± 1.34	0.95 ± 0.12
AL	43.30 ± 1.16	2.22 ± 0.03
EB	41.50 ± 0.9	0.36 ± 0.01
MB	43.29 ± 0.84	0.42 ± 0.03
AB	59.89 ± 0.89	0.99 ± 0.13
Standard	76.34 ± 0.66	0.15 ± 0.02

ABTS radical scavenging and α-amylase inhibitory activities, respectively. The ethanol bark extracts of *X. granatum* (XGEB) exhibited higher antioxidant and antidiabetic activities (expressed in terms of IC_{50} values) as compared to other solvent extracts of leaf and bark. The ethanol bark extract showed highest ABTS scavenging activity with IC_{50} value of 41.50 μg/ml. Under the similar condition the standard antioxidant compound Butylated hydroxyl toluene (BHT) showed antioxidant activity with 76.34 μg/ml. The highest α-amylase inhibitory activity was also observed in ethanol bark extract with IC_{50} value of 0.36 mg/ml. Under the similar condition, the standard drug Acarbose could inhibit the α-amylase enzyme with IC_{50} value of 0.15 mg/ml (Table 1). However, none of the extracts showed any metal chelating activity at 50, 100, or 150 μg/ml concentration.

3.2. Acute Toxicity Study. Oral administration of XGEB extracts at a dose of 1000 mg/kg body weight/day did not produce any signs of toxicity and no animals died up to 4 days. It showed that XGEB was nontoxic in mice up to an oral dose of 1000 mg/kg body weight. However, further investigation of antidiabetic and antioxidant activities was carried out using 100 and 200 mg/kg body weight dose levels where a significant lowering of blood glucose level in comparison to diabetic control was observed.

3.3. Changes in Body Weight. Changes in initial and final body weight in control and experimental groups were shown in Table 2. The body weight of DMC group was decreased significantly ($p<0.05$) as compared to NC group. The body weight of DMC at the end of 30^{th} day declined by 19.64% as compared to day 1. The body weight of DMD and DMH increased significantly ($p<0.05$) as compared to DMC group whereas the body weight of DML group decreased (9.06%) during 30-day experiment period. The increase in body weight for NC, NCT, DMD, and DMH groups at the end of experiment (30^{th} day) was 17.82%, 25%, 13.67%, and 4.59%, respectively, as compared to day 1.

3.4. Blood Glucose Level. The blood glucose level of DMC mice was increased significantly ($p<0.05$) after STZ-induction as compared with normal control mice. The DMC group displayed 311.4 ± 37.87 mg/dl glucose on day 1 which was increased to 393.3 ± 32.07 mg/dl on day 30 accounting an increase in 27.19% (Table 3). However, oral administration of glibenclamide and XGEB to diabetic mice significantly reduced ($P<0.05$) the blood glucose level in as compared with diabetic control mice. As shown in Table 3, after 30 days of treatment, the STZ-induced hyperglycaemia was significantly ameliorated by XGEB extracts which was related to dose and duration of treatment. The XGEB extract at 100 mg/kg and 200 mg/kg body weight reduced the blood glucose level by 19.58% in DML and 31.21% in DMH groups, respectively. No significant deviation was observed in normal mice treated with XGEB.

3.5. Biochemical Parameters of Blood. The TG and TC levels were increased significantly ($p< 0.05$) in DMC group mice as compared to NC group mice (Table 4). On the other hand, the administration of glibenclamide or XGEB (bothat 100 mg/kg and 200 mg/kg body weight) showed significant reduction ($p< 0.05$) in TG and TC levels in DMD, DML, and DMH groups as compared to DMC group. The TG levels were decreased by 32%, 16%, and 28%; while the TC levels were decreased by 36%, 35%, and 39% in DMD, DML, and DMH groups compared to DMC group.

The SGOT, SGPT, and urea levels were increased significantly ($p< 0.05$) in DMC group as compared to NC group (Table 4). Upon oral administration of glibenclamide and XGEB a significant reduction ($p< 0.05$) in SGOT, SGPT, and urea levels was observed in DMD, DML, and DMH groups in comparison to DMC group. The SGOT levels were decreased by 35%, 25%, and 39%; the SGPT levels were decreased by 28%, 16%, and 35% while the urea levels were decreased by 34%, 14%, and 32% in DMD, DML, and DMH groups as compared to diabetic control mice.

3.6. LPx and GSH Content in Liver and Brain. Significantly higher ($p<0.05$) liver LPx level was found in diabetic control mice as compared to normal mice (Table 5). However, the treatment with XGEB or glibenclamide did not show any change in liver LPx levels. The LPx level in brain tissue remained unchanged in DMC mice as compared to NC mice. However, treatment with XGEB and glibenclamide resulted in marked decrease ($p<0.05$) in the brain LPx levels compared to DMC group. The LPx levels in brain tissues were decreased by 62%, 23%, 49% DMD, DML, and DMH groups as compared with DMC mice.

The activities of nonenzymatic antioxidants such as NP-SH and P-SH in liver and brain tissues of the normal and diabetic mice are summarized in Table 5. The P-SH content of liver was significantly decreased ($p< 0.05$) in diabetic control mice as compared to normal mice. The NP-SH content of brain was significantly increased in XGEB treated (200 mg/kg bw) groups as compared to diabetic control mice. However, NP-SH content in liver along with P-SH and NP-SH contents in brain remained unchanged in diabetic control mice as compared to control mice. Administration of neither XGEB nor glibenclamide could ameliorate the NP-SH and P-SH levels in liver and brain tissues of diabetic mice except for

Table 2: Effect of *X. granatum* ethanol bark extracts on body weight. Data are expressed as mean ± SD (n= 5). Data in parentheses indicate percent gain (+) or loss (-) in weight. NC, Normal Control; NCT, Normal Control Toxicological (high) dose; DMC, Diabetic Control; DMD, Diabetic Drug (Glibenclamide); DML, Diabetic *Xylocarpus granatum* Low dose (100 mg/kg).; DMH, Diabetic *Xylocarpus granatum* High dose (200 mg/kg). [a]p<0.05 with respect to initial body weight in the same group. [b]p<0.05 with respect to final body weight of NC, [c]p<0.05 with respect to final body weight of DMC.

Groups	Mean body weight (g)	
	Initial (1st Day)	*Final (30th Day)*
NC	37.2 ± 2.38	43.83 ± 1.92[a] (+ 17.82%)
NCT	33.2 ± 2.94	41.5 ± 4.38[a,c] (+ 25%)
DMC	28 ± 1.87	22.5 ± 1.11[a,b] (-19.64%)
DMD	32.9 ± 2.01	37.4 ± 1.71[a,b,c] (+ 13.67%)
DML	34.2 ± 6.30	31.1 ± 6.93[a,b,c] (-9.06%)
DMH	33.94 ± 3.26	35.5 ± 2.69[a,b,c] (+ 4.59%)

Table 3: Effect of *X. granatum* ethanol bark extracts on blood glucose level. Data are expressed as mean ± S.D. (n=5). Data in parentheses indicate % increase. NC, Control mice; NCT, Normal Control mice +Toxicological (high) dose *Xylocarpus granatum*; DMC, Diabetic Control mice; DMD, Diabetic mice+ Drug (Glibenclamide); DML, Diabetic mice + *Xylocarpus granatum* Low dose (100 mg/kg); DMH, Diabetic mice + *Xylocarpus granatum* High dose (200 mg/kg). [a]p <0.05 compared with NC group. [b]p<0.05 compared with DMC group.

Groups	Blood glucose level (mg/dl)				
	1st Day	7th Day	14th Day	21st Day	30th day
NC	128.0 ± 6.32	131.6 ± 5.54	143.0 ± 9.13	151.2 ± 9.65	157.0 ± 9.77
NCT	125.4 ± 2.96	131.6 ± 3.91	144.4 ±13.59	149.0 ±14.86	154.0 ±15.76
DMC	311.4 ± 37.87[a]	346.2 ±30.93[a] (+11.17%)	358.0 ±31.45[a] (+14.96%)	380.6 ±37.36[a] (+22.22%)	393.2 ±32.07[a] (+26.26%)
DMD	343.0 ± 56.43	316.6 ±56.93 (-7.69%)	285.6 ± 61.47 (-16.73%)	257.4± 42.67[b] (-24.95%)	232.8±38.17[b] (-32.12%)
DML	322.2 ± 67.76	351.2 ± 72.59 (+9.0%)	338.6 ± 55.87 (+5.09%)	291.0 ± 61.15 (-9.68%)	261.2 ± 67.08 (-18.93%)
DMH	324.0 ± 79.68	335.2 ± 64.37 (+3.45%)	308.4 ± 61.06 (-4.81%)	268.2± 40.73[b] (-17.22%)	218.4±36.42[b] (-32.59%)

NP-SH level in brain tissue of DMH mice where a significant (p<0.05) increase is observed as compared to diabetic control mice.

3.7. In Vivo Antioxidant Levels. The activities of enzymatic antioxidants such as SOD, CAT, GPx, GST, and GR in liver and brain tissues of the normal and diabetic mice are summarized in Table 6. The SOD (liver and brain), GR (liver and brain), and GST (liver and brain) levels were significantly decreased (P< 0.05) in diabetic control mice as compared to normal control mice. But the decrease in CAT level in liver tissue was nonsignificant (P>0.05) as compared to normal control. Upon administration of glibenclamide and XGEB to diabetic mice, the activities of SOD and GR levels in liver tissues increased significantly (P<0.05) in treated mice as compared to diabetic control mice. Similarly, significant (P<0.05) increase in CAT level was observed in liver tissue of XGEB treated (200 mg/kg bw) mice as compared to diabetic control mice. But the increase in CAT level was nonsignificant in liver tissues of XGEB treated (100 mg/kg bw) and glibenclamide treated mice as compared to diabetic control mice. The GR and GST level in brain tissues were increased significantly (P<0.05) in both glibenclamide treated and XGEB (200 mg/kg bw) treated mice as compared to diabetic control mice whereas the increase was found to be nonsignificant (P>0.05) in XGEB (100 mg/kg bw) in treated mice. However, nonsignificant (P>0.05) increase in SOD level in brain tissues of both XGEB and glibenclamide groups mice was recorded as compared to diabetic control mice group.

3.8. Histopathological Analysis of Liver. The histopathological analysis of the liver in NC and NCT groups showed normal cell morphology with hexagonal lobular architecture. However, the liver sections in diabetic control mice showed progressive disruption of structural architecture characterized by an apparent decrease in number of intracytoplasmic

Table 4: Effect of *X. granatum* on serum biochemical parameters in STZ-induced diabetic mice. Data are expressed as mean ± SD, n=5. NC, Control mice; NCT, Normal Control mice +Toxicological (high) dose *Xylocarpus granatum*; DMC, Diabetic Control mice; DMD, Diabetic mice+ Drug (Glibenclamide); DML, Diabetic mice + *Xylocarpus granatum* Low dose (100 mg/kg); DMH, Diabetic mice + *Xylocarpus granatum* High dose (200 mg/kg). [a]$p< 0.05$ compared with the control mice (NC); [b]$p< 0.05$ compared with the diabetic control mice (DMC).

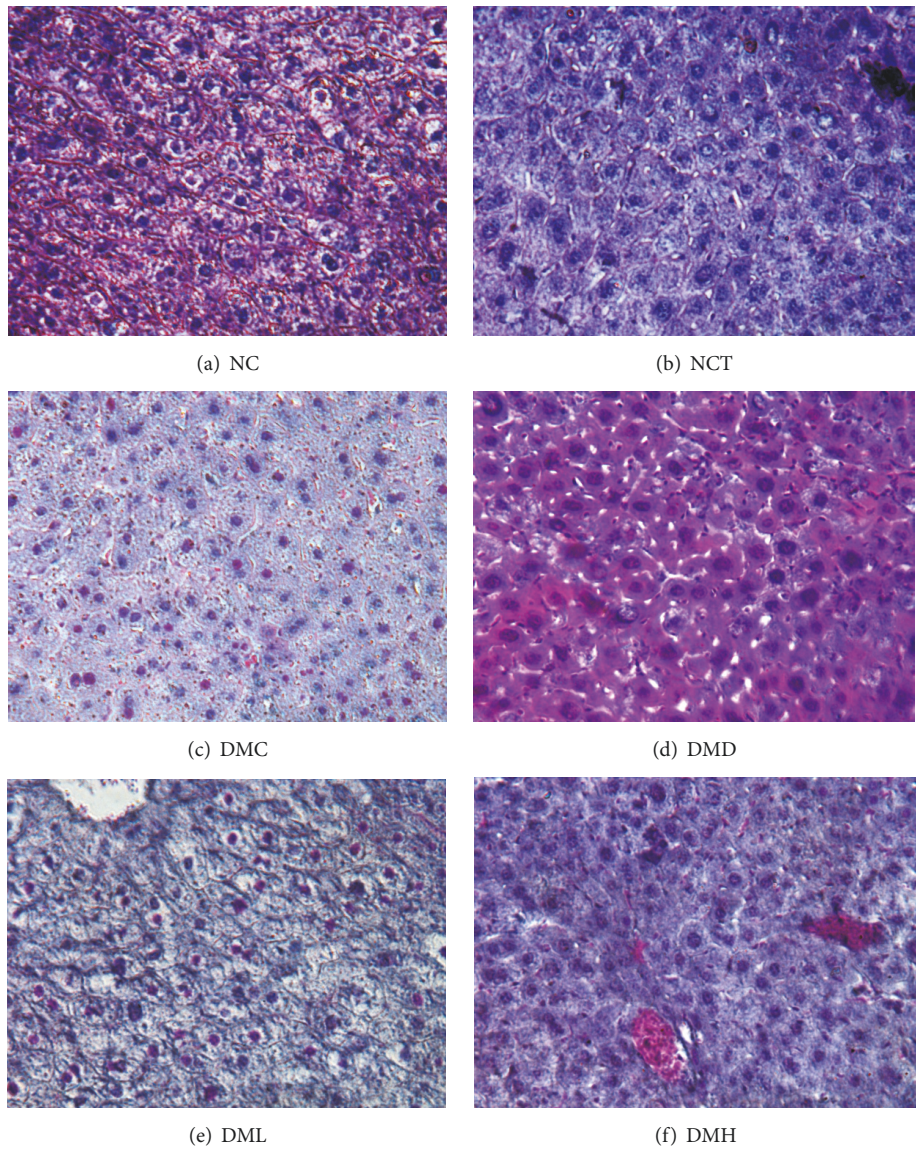
Groups	TG (mg/dL)	TC (mg/dL)	SGOT (U/L)	SGPT (U/L)	Urea(mg/dL)
NC	138.8 ± 5.89	102.6 ± 6.91	197.6 ± 13.84	89.6 ± 7.3	27 ± 1.58
NCT	152.6 ± 7.06[a]	87.8 ± 15.23	261 ± 19.72[a]	116.8 ± 8.95[a]	30.0 ± 1.0[a]
DMC	261.4 ± 11.43[a]	194.8 ± 12.3[a]	326.0 ± 9.38[a]	181.8 ± 8.01[a]	41.6 ± 2.60[a]
DMD	177.6 ± 6.23[b]	110.0 ± 17.42[b]	209.4 ± 25.48[b]	130.2 ± 5.21[b]	27.4 ± 2.07[b]
DML	219.4 ± 16.26[b]	131.6 ± 8.32[b]	242.6 ± 14.53[b]	152.4 ± 6.84[b]	35.2 ± 2.04[b]
DMH	187.2 ± 10.06[b]	104.6 ± 12.93[b]	198.6 ± 9.60[b]	118.6 ± 19.09[b]	28.4 ± 1.51[b]

Table 5: Effect of *X. granatum* on lipid peroxidation (LPx), non-protein-SH (NP-SH) and protein-SH (P-SH) in liver and brain tissues. Data are expressed as mean ± S.D. (n=5). NC, Control mice; NCT, Normal Control mice +Toxicological (high) dose *Xylocarpus granatum*; DMC, Diabetic Control mice; DMD, Diabetic mice+ Drug (Glibenclamide); DML, Diabetic mice + *Xylocarpus granatum* Low dose (100 mg/kg); DMH, Diabetic mice + *Xylocarpus granatum* High dose (200 mg/kg). [a]$p< 0.05$ compared with the control mice (NC); [b]$p< 0.05$ compared with the diabetic control mice (DMC).

Organs	Group	LPx (nmolesTBARS/mg)	NP-SH (μM/g tissue)	P-SH (μM/g tissue)
Liver	NC	0.60 ± 0.07	2.07 ± 0.28	4.91 ± 0.38
	NCT	0.54 ± 0.13	2.17 ± 0.20	4.38 ± 0.23
	DMC	0.75 ± 0.02[a]	2.18 ± 0.22	4.07 ± 0.23[a]
	DMD	0.77 ± 0.11	1.67 ± 0.37[b]	2.99 ± 0.15[b]
	DML	0.72 ± 0.19	1.81 ± 0.40	2.22 ± 0.33[b]
	DMH	0.73 ± 0.12	1.24 ± 0.19[b]	2.96 ± 0.48[b]
Brain	NC	1.68 ± 0.24	0.60 ± 0.07	4.34 ± 0.29
	NCT	0.66 ± 0.09[a]	0.56 ± 0.03	4.05 ± 0.21
	DMC	1.58 ± 0.27	0.61± 0.04	4.31 ± 0.43
	DMD	0.60 ±0.1[b]	0.59 ± 0.03	4.59 ± 0.29
	DML	1.22 ± 0.18[b]	0.55± 0.11	4.47 ± 0.20
	DMH	0.81 ± 0.17[b]	0.76 ± 0.07[b]	4.46± 0.77

Table 6: Effect of *X. granatum* on antioxidant enzymes in liver and brain tissues. Data are expressed as mean ± S.D., n=5. NC, Control mice; NCT, Normal Control mice +Toxicological (high) dose *Xylocarpus granatum*; DMC, Diabetic Control mice; DMD, Diabetic mice+ Drug (Glibenclamide); DML, Diabetic mice + *Xylocarpus granatum* Low dose (100 mg/kg); DMH, Diabetic mice + *Xylocarpus granatum* High dose (200 mg/kg). [a]$p< 0.05$ compared with the control mice (NC); [b]$p< 0.05$ compared with the diabetic control mice (DMC).

Organs	Group	SOD (U/mg)	CAT (nKatal/mg)	GPx (nmoles/min/mg protein)	GR (nmoles/min/mg protein)	GST (nmoles/min/mg protein)
Liver	NC	15.77 ± 2.80	3418.06 ± 375.19	26.11 ± 2.29	20.09 ± 4.92	2949.81 ± 116.54
	NCT	17.59 ± 4.20	2960.5 ± 761.71	30.62 ± 2.80[a]	20.69 ± 2.07	2416.51 ± 314.9[a]
	DMC	8.10 ± 0.40[a]	3297.6 ± 591.15	33.05 ± 1.31[a]	12.55 ± 1.48[a]	2083.95 ± 336.6[a]
	DMD	14.72 ± 1.42[b]	3792.69 ± 489.08	26.81 ± 0.82[b]	24.0 ± 1.84[b]	2093.61 ± 310.56
	DML	18.58 ± 4.13[b]	3892.74 ± 216.75	22.02 ± 2.71[b]	21.05 ± 2.23[b]	2416.51 ± 630.83
	DMH	18.35 ± 2.72[b]	4344.26 ± 779.38[b]	25.39 ± 1.11[b]	25.39 ± 3.28[b]	2016.54 ± 407.26
Brain	NC	12.87 ± 2.71	45.87 ± 13.44	26.42 ± 1.19	8.48 ± 0.43	261.64 ± 9.94
	NCT	12.02 ± 1.23	47.31 ± 11.01	30.22 ± 0.39[a]	9.8 ± 1.17[a]	306.22 ± 10.82[a]
	DMC	10.08 ± 0.39[a]	55.91 ± 7.44	33.19 ± 2.62[a]	6.32 ± 0.57[a]	222.89 ± 4.65[a]
	DMD	11.32 ± 2.4	37.27 ± 2.86[b]	18.08 ± 1.48[b]	11.81 ± 1.49[b]	270.81 ± 10.62[b]
	DML	10.56 ± 1.11	45.87 ± 9.93	23.27 ± 2.06[b]	7.32 ± 0.69	226.21 ± 6.84
	DMH	10.65 ± 1.92	41.57 ± 6.24	26.58 ± 1.14[b]	8.93 ± 0.69[b]	299.14 ± 8.0[b]



(a) NC (b) NCT

(c) DMC (d) DMD

(e) DML (f) DMH

Figure 1: Representative photomicrograph showing histopathology of liver. (a) normal control mice (NC); (b) normal control toxicological dose (NCT); (c) diabetic control mice (DMC); (d) diabetic + glibenclamide (DMD); (e) diabetic + *X. granatum* low dose (DML); (f) diabetic + *X. granatum* high dose (DMH). Magnification x 40.

organelles, inflammatory damage, sinusoidal dilation, and fatty changes. The sections of glibenclamide treated diabetic mice showed restoration of architecture of hepatocytes. However, moderate sinusoidal dilation and inflammatory damage were observed. On the other hand, liver sections from XGEB treated diabetic mice showed reduced histopathological damages as compared to diabetic control group (Figure 1). Inflammatory damages were not observed. The fatty changes of liver and sinusoidal dilation were not observed. The semiquantitative histological scoring of liver damage is presented in Table 7.

3.9. Phytochemical Analysis. Quantitative phytochemical screening showed that the aqueous leaf extract possessed highest amount of total flavonoid content (10 mg QE/g DW) amongst the different extracts of *X. granatum*. Similarly, amongst the different extracts, the ethanol bark extract, was found to possess highest total tannin content, i.e., 9.76 mg GAE/g DW (Table 8).

The ethanol bark extracts of *X. granatum* demonstrated highest antidiabetic and antioxidant activities amongst all the extracts studied, hence chosen for further phytochemical analysis to obtain some information on the active components present in extract. The ethanol bark extract was subjected to UV-VIS, HPLC, DSC, and GC-MS analysis. The UV-Visible absorbance profile of the ethanol bark extracts of *X. granatum* was studied for detection of phenolic compounds at a wave length range of 200 to 700 nm (Figure 2). The spectrum showed an absorbance maximum at 274 nm for ethanol bark extracts with the absorption values of 1.563 indicating the presence of phenolic acid derivatives.

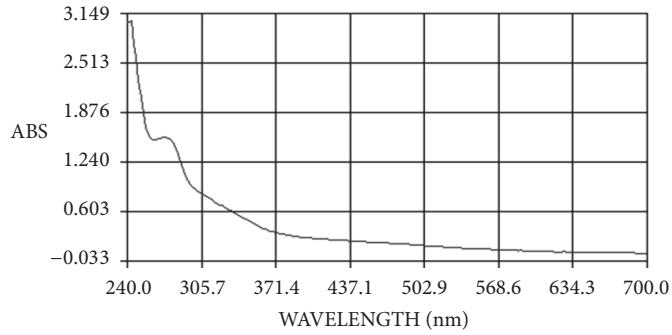


Figure 2: UV-VIS Spectra of ethanol bark extract of *X. granatum*.

Table 7: Semi-quantitative scoring of histopathological examination of liver. (-): No change, (+): Positive for the parameter studied.

Tissue damage	NC	NCT	DMC	DMD	DML	DMH
Degeneration of hepatocytes	-	-	+	-	-	-
Fatty change in hepatocytes	-	-	+	+	-	-
Inflammatory cell infiltrations	-	-	+	+	-	-
Sinusoidal dilation	-	-	+	+	-	-

Further, the purity and thermal behaviour of the sample was studied by differential scanning calorimetric method. The DSC thermogram of XGEB showed a broad peak at 119.9°C with onset and end at 63.99°C and 145.7°C (Figure 3). The heat of fusion for the XGEB was found to be 366 J/g. An additional peak at 100°C along with the peak at 119.9°C was also observed due to the loss of hydroxyl functional group as water which may be due to the presence of phenolic compounds in the ethanol bark extract.

For phytochemical fingerprint, the ethanol extracted bark sample of *X. granatum* was analyzed by high performance liquid chromatography (HPLC) which gave five major peaks at Rt 3.02, 3.58, 6.21, 8.19, and 13.01 min indicating the presence of simple polyphenolic compounds, isoflavone and flavonol (Figure 4).

The GC-MS analysis of the ethanol bark extract of *X. granatum* indicated the presence of 13 peaks out of which 8 peaks were characterized on the basis of their retention time and five peaks such as 2, 5, 6, 7, and 12 were not characterized (Figure 5). The compounds identified were phenol, 2,4-bis (1,1-dimethylethyl) [peak 1], tetracosamethyl cyclododeca siloxane [peak 3], bis(p-(phenylethynyl)phenyl) butadiyne [peak 4], 6,6'-diacetyl-7,7'-dihydroxy-2,2',4,4',5 [peak 8], 6,6'-diacetyl-7,7'-dihydroxy-2,2',4,4',5 [peak 9], bis(heptamethyl cyclotetrasiloxy) hexameth [peak 10], 3-phorbinepropanoic acid, 9-acetyl-14-et [peak 11], and phenol, 4,4'-methylene bis [2,6-bis (1,1-di) [peak 13].

4. Discussion

The present study has made a novel attempt to evaluate the antidiabetic and antioxidant properties ethanol bark extract of *X. granatum*, a mangrove plant by both *in vitro* and *in vivo* studies. Results from the present *in vitro* antidiabetic investigation have demonstrated that the ethanol, methanol and aqueous extracts of leaves and bark of *X. granatum* possess α-amylase inhibition activity with the highest potency noted in the ethanol bark extracts. Previously, the leaf and bark extracts of this plant have also been reported for their glucose uptake capacity and α-glucosidase inhibition property [6]. Inhibition of α-amylase and α-glucosidase is an effective strategy for prevention of diabetes as they play important roles in controlling postprandial blood glucose level by delaying carbohydrate digestion and consequently blunting the postprandial plasma glucose rise [27]. Inhibition of α-amylase and α-glucosidase enzymes have also been reported in several other mangrove plants like *Barringtonia racemosa*, *Rhizophora mucronata*, *Ceriops tagal*, *Sonneratia caseolaris* suggesting the presence of antidiabetic bioactive principles in mangrove plants which have therapeutic implications [27–30].

Postprandial hyperglycaemia is a common pathogenesis in diabetes incurred due to insulin resistance and β-pancreatic destruction [31]. In the present study, treatment with XGEB, at both 100 and 200 mg/kg, resulted in a significant ($P < 0.05$), consistent, and dose dependent decrease in blood glucose level throughout the experimental period indicating its potent antidiabetic activity. The result could be linked to the potent α-amylase and α-glucosidase inhibitory activity of XGEB that could cause decrease in the digestion of carbohydrates. Decrease in body weight is often found to be associated with diabetic conditions because of increase in muscle wastage, decrease in tissue proteins, and breakdown fat [32]. In the present study, XGEB at the dose level of 200 mg/kg showed an improvement in body weight gain as compared to diabetic control group suggesting the restorative effect of XGEB extract which may be due to the reversal of gluconeogenesis and glycogenolysis.

Uninhibited actions of lipolytic hormones on fat cells due to impairment of insulin secretions result in hypertriglyceridemia and hypercholesteromia in diabetes that further increase the risk of cardiovascular diseases [33]. In the present study, administration of XGEB at 100 and 200 mg/kg to the diabetic mice significantly ($P < 0.05$) improved the TG and TC levels towards normalcy which may be due to the decreased cholesterogenesis and enhanced glucose utilization. These results imply that XGEB administration could effectively

Table 8: Total flavonoid content and total tannin content of various extracts of *X. granatum*. The values are expressed as mean ± SD (n=3). EL = Ethanol leaf extracts; ML = Methanol leaf extracts; AL = Aqueous leaf extracts; EB = Ethanol Bark extracts; MB = Methanol Bark extracts; AB = Aqueous Bark extracts of *X. granatum*.

Sample	EL	ML	AL	EB	MB	AB
Total flavonoids	8.0 ± 0.20	8.0 ± 0.11	10 ± 0.09	7.0 ± 0.16	9.0 ± 0.15	8.0 ± 0.10
Total tannin	4.1 ± 0.03	5.54 ± 0.07	3.91 ± 0.09	9.76 ± 0.03	6.48 ± 0.04	5.28 ± 0.02

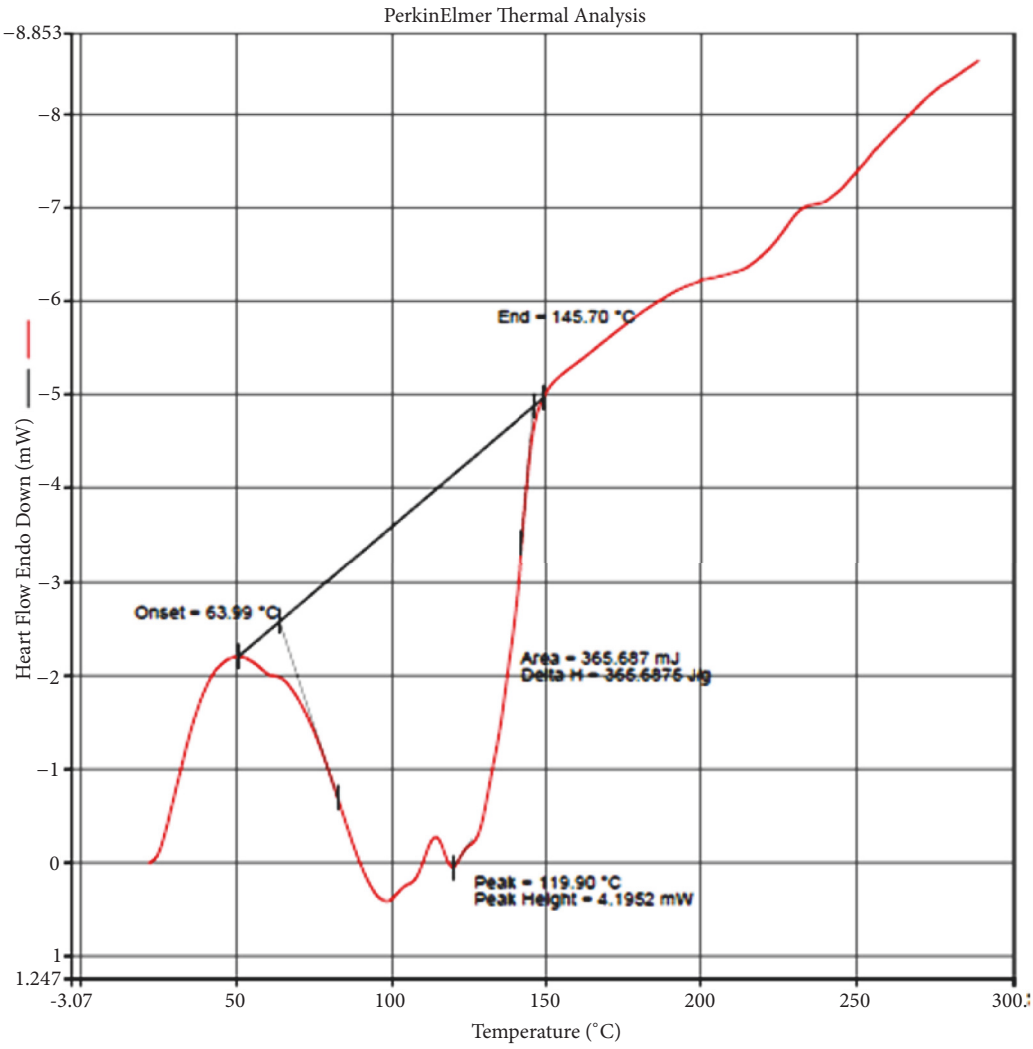


Figure 3: DSC curve for ethanol bark extract of *X. granatum*.

improve the metabolism of carbohydrates, lipids in diabetic patients. The urea and creatinine levels in the blood are considered as one of the noticeable indices for renal function under diabetic condition [34]. Significant ($P< 0.05$) decrease in the blood urea level in diabetic mice treated with XGEB (at 100 and 200 mg/kg) indicated that XGEB extract prevents the progression of renal damage in STZ-induced insulin-dependent diabetic mice.

The liver is the vital organ for metabolism and detoxification of xenobiotics. During diabetes, the liver cells are necrotized and released the liver enzymes like SGOT, SGPT, and alkaline phosphatase (ALP) into blood stream leading to increase in their concentration [35]. In the current study, reduction in SGOT and SGPT levels in blood of XGEB treated diabetic groups signified the hepatoprotective of *X. granatum* ethanol extracts. Therefore, restoration of these enzyme biomarker enzymes towards normal level indicate decreased diabetic complications in XGEB treated diabetic mice. Histopathological examination of liver also showed a similar effect. As per the histopathological results, XGEB extract (at both 100 and 200 mg/kg dose) could decrease sinusoidal dilation and inflammatory cell infiltration along with amelioration of degeneration and fatty changes of hepatocytes as compared to the diabetic control group. These results revealed

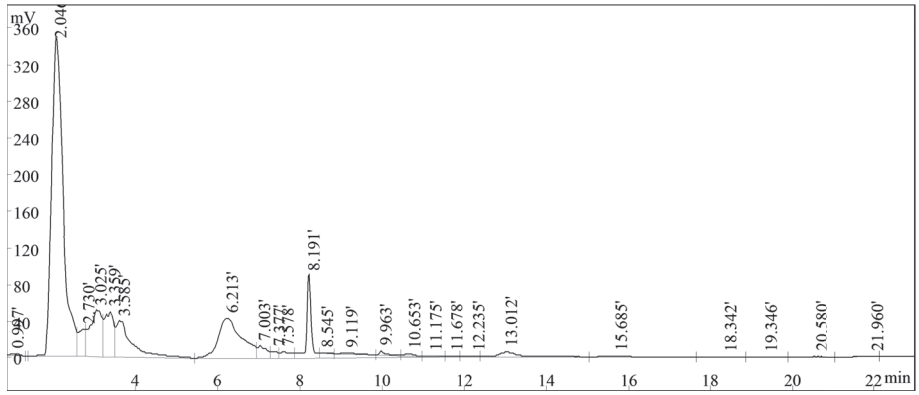


Figure 4: HPLC chromatogram of ethanol bark extracts of *X. granatum*.

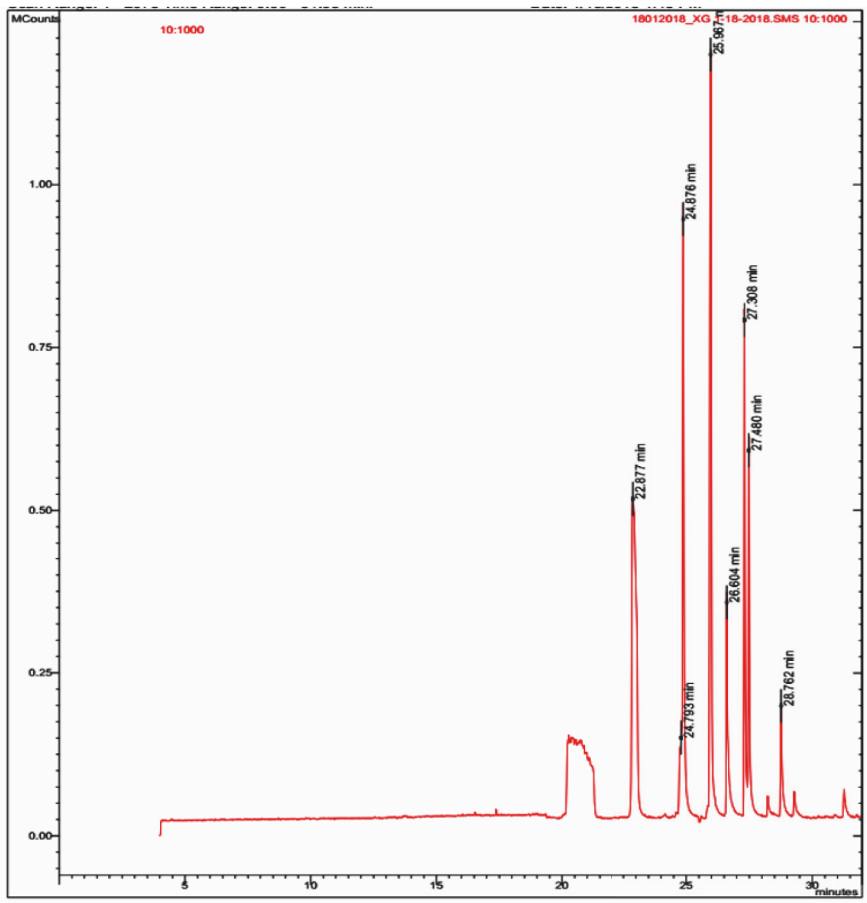


Figure 5: GC-MS analysis of ethanol bark extract of *X. granatum*.

that active components of XGEB extract could diminish oxidative stress, which was induced by STZ in the diabetic mice. Further, supplementation of XGEB to normal mice did not show much alteration in SGOT, SGPT enzyme activities indicating that XGEB administration was safe and possessed no significant toxicity. The ability to maintain the renal and hepatic factors close to the normal conditions supports the ability of the extract to protect them from nephropathy and hepatopathy.

During diabetes, persistent hyperglycaemia impairs prooxidant and antioxidant balance that reduces antioxidant level and increases production of ROS. Oxidative stress due to free radical generation and reduction of endogenous antioxidant is considered as one of the underlying factors in the development of diabetes complications. Hyperglycaemia induced oxidative stress leads to the activation of stress pathways which ultimately lead to tissue damage increasing lipid peroxidation that further impairs glucose metabolism

in biological systems [36]. In agreement with the above study, a significant increase in the LPx level was observed in the liver of diabetic mice in the current study. However, neither glibenclamide nor the XGEB extract was able to bring back the elevated LPx level to control. At the same time, it is noticed that both the drug and the plant extract were able to maintain the LPx level without further increase during the treatment period. Though brain is rich in fatty acids; the level of Lpx remained unaltered in diabetic mice but reduced significantly in drug and extract treated groups. Therefore, it will not be out of context to mention that both the drug and the plant extract might be exerting their tissue specific differential effects by modulating the activities of cellular antioxidant enzymes or by scavenging ROS generated due to diabetes after treatment or by maintaining a stable glucose level. In fact we have noticed marked alterations in the activities of antioxidant enzymes in the liver and brain of mice in response to drug and XGEB extract treatment.

Antioxidant enzymes act in a cascade. SOD dismutates $O_2^{\cdot-}$ to H_2O_2. H_2O_2 which is not very favorable to the cell is neutralized by CAT and GPx into O_2 and H_2O. The Km for H_2O_2 is > 10 mM in mammalian cells. Therefore, at low intracellular concentrations GPx is the pivotal enzyme for degradation of H_2O_2 [37]. In the present study, a significant decrease in the activity of SOD along with an augmentation in GPx activity is noticed in both liver and brain tissue of diabetic control mice. A decreased SOD activity in the tissues will lead to increase in $O_2^{\cdot-}$ content of the cell which is capable of generating $^{\cdot}OH$ in presence of transition metal ions such as iron or peroxynitrite in presence of NO. The highly reactive $^{\cdot}OH$ radical has the ability to attack any biomolecule within 1 atomic radius and thus oxidizing lipids and proteins and inducing strand breaks in DNA leading to cellular dysfunction. To corroborate the fact, enhanced LPx level was noticed in the liver in diabetic mice.

In case of liver, administration of XGEB at 200 mg/kg to diabetic mice showed a marked increase in SOD and CAT activity and brought back the level of GPx and GR to normal level, thus showing an enhancement of antioxidant defense in response to diabetic induction. This fact is further corroborated by decline in GST activity which is known to be induced by toxic xenobiotics and oxidative stress [38]. Glibenclamide and the lower dose of XGEB extract showed less impact on antioxidant defense. The decrease in NP-SH in extract treated diabetic mice may be due to direct scavenging of ROS by the antioxidants present in the extract thus circumventing the production of NP-SH more precisely GSH which is further corroborated by the decline in principal GSH metabolizing enzymes GPx and GST. Similarly, the lowered P-SH level indicates an adaptive response to protect proteins in response to diabetes induced ROS generation. In case of brain, the altered activities of SOD, GPx, and GST enzymes in diabetic mice brought back to the normal control level after administration of *X. granatum* at 200 mg/kg except GR which was elevated. This might be an adaptive response of brain to protect itself from ROS mediated cell damage [39].

As discussed earlier, this property of XGEB highlights that the activity could be due to either some potential antioxidant compounds or other biomolecules which could alone or synergistically act with the antioxidants present in the extract. The antioxidant action of XGEB may be attributed to the presence of antioxidant components or due to other biomolecules which could alone or synergistically act with the antioxidants present in the extract. In fact results from the present study on free radical scavenging activity using ABTS scavenging assay demonstrated that amongst all the extracts of leaves and barks of *X. granatum*, the ethanol bark extracts exhibited highest ABTS scavenging potential. This action could be beneficial for eliminating ROS and in turn attenuate the complications of diabetes.

The quantitative phytochemical assays also showed the presence of flavonoids and tannins in leaf and bark extracts of *X. granatum*. The HPLC fingerprinting is one of the simplest ways for chemical characterization of bioactive compounds from plants and their extracted fractions. According to fingerprint developed by Kumar et al. (2008) [24] simple polyphenols like gallic acid elutes first at Rt 3.63 min. After gallic acid, catechin (Rt 4.57) and epicatechin (Rt 5.24) group compounds are eluted in the mobile phase. Rutin belongs to isoflavone group eluted after catechins at 6.94 min, compounds of flavonol groups eluted in between Rt 9 to 16 min. Phytochemicals fingerprint of ethanol bark extract of *X. granatum* plant was compared with above-mentioned fingerprint under similar chromatographic conditions. Ethanolic extract of *X. granatum* bark shows well developed chromatographic peak at Rt 3.02, 3.58 min which represent the presence of simple polyphenolic compounds. This is followed by peaks at Rt 6.21 and Rt 8.19 min which represents isoflavone. Chromatographic peak at R t 13.01 min was also observed which represents the presence of flavonol in the extract. The present study indicated the presence of simple polyphenols, isoflavone, and flavonol in the ethanol bark extract of *X. granatum*. The DSC thermogram of *X. granatum* also indicated the presence of phenolic compounds in the ethanol bark extract [25]. The UV-VIS spectral analysis also confirmed the presence of phenolic derivatives in the ethanol bark extract [40]. The GC-MS analysis of the ethanol bark extract of *X. granatum* indicated the presence of different bioactive compounds such as phenol, 2,4-bis (1,1-dimethylethyl); bis(p-(phenylethynyl)phenyl) butadiyne; 6,6'-diacetyl-7,7'-dihydroxy-2,2',4,4',5; 6,6'-diacetyl-7,7'-dihydroxy-2,2',4,4',5; bis(heptamethyl-cyclotetrasiloxy) hexameth; 3-phorbinepropanoic acid, 9-acetyl-14-et; and phenol, 4,4'-methylene bis [2,6-bis (1,1-di) out of which 2,4-bis (1,1-dimethylethyl) phenol, tetracosamethylcyclododecasiloxane have been reported for their antioxidant properties in some previous studies [41, 42]. The antidiabetic potential of plant species has been tested in nicotinamide induced Type-2 diabetic rats [43] and the present studies also prove that plants are the rich source of natural antidiabetic compounds.

5. Conclusion

The results of the present study clearly indicated that the ethanol bark extracts of *X. granatum* possess antioxidant and antidiabetic potentials. The XGEB exerts its antioxidant effect by scavenging the free radicals and thereby regulates

the antioxidant status in STZ-induced diabetic mice. The antidiabetic potentials of XGEB were also comparable with the antidiabetic drug glibenclamide. The presence of different phenolic derivatives in XGEB may act as potential candidates in counteracting the oxidative damage and inhibiting the progression of diabetes and its associated complications. Therefore, the *X. granatum* bark supplementation may be helpful in management of diabetes complications. However, in-depth study is warranted to isolate the bioactive components and to elucidate their exact mechanism of action by which this plant regulates glucose homeostasis.

Data Availability

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

Conflicts of Interest

The authors confirm that there are no conflicts of interests.

Authors' Contributions

H. N. Thatoi and L. Samanta had conceptualized and designed the work. S. K. Das had conducted all the experiments. D. Samantaray helped in the *in vitro* experiments and A. Prusty helped in the biochemical study and they have equally contributed to this work. M Hasan helped in carrying out the HPLC study. S. Jena has helped in data analysis. J. K. Patra, H. N. Thatoi, and L. Samanta have edited the manuscript. All authors read and approved the manuscript.

Acknowledgments

The authors are thankful to PCCF (Wildlife), Govt. of Odisha, for giving the necessary permission for the research work. The authors are also thankful to the DFO, Rajnagar, Odisha and their field staff for their kind help and cooperation during the field study. The authors are thankful to Dr. P. Maiti, Senior Scientist and staffs of IMGENEX India Pvt. Ltd. for providing assistance during animal study. The authors also acknowledge the KIIT-TBI, Bhubaneswar, for carrying out GC-MS analysis.

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