Hindawi
Journal of Food Quality
Volume 2024, Article ID 7318407, 17 pages
https://doi.org/10.1155/2024/7318407

Research Article

Chemical Profiling and Biological Activities of *Ziziphus Mauritiana* var. *spontanea* (Edgew.) R.R. Stewart ex Qaiser & Nazim. and *Oenothera Biennis* L.

Ambrin Ambrin,[1] Muhammad Adil,[1,2] Faten Zubair Filimban,[3] and Muhammad Naseer[1]

[1]*Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology, Peshawar, Pakistan*
[2]*Centre for Plant Sciences and Biodiversity, University of Swat, Swat, Pakistan*
[3]*Division of Botany, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia*

Correspondence should be addressed to Muhammad Adil; adilbotany@qurtuba.edu.pk and Muhammad Naseer; 17630@qurtuba.edu.pk

Received 2 January 2024; Revised 29 February 2024; Accepted 9 March 2024; Published 28 March 2024

Academic Editor: Mohan Li

Bioactive compounds of medicinal plants, including polyphenols, flavonoids, terpenoids, and alkaloids, are essential sources for developing analgesic, anti-inflammatory, and antidiarrheal drugs. In the current study, secondary metabolites were assessed through phytochemical screening and GC-MS analysis whereas analgesic activity was carried out through hot plate (HP) and acetic acid-induced method (AAI), anti-inflammatory through paw edema model (PEM), and antispasmodic activity via charcoal meal test (CMT) using ethyl acetate and ethanolic extract of *Ziziphus mauritiana* var. *spontanea* and *Oenothera biennis*. The phytochemical screening revealed that the ethyl acetate and ethanolic extracts of *Z. mauritiana* and *O. biennis* were rich in alkaloids, flavonoids, tannins, steroids, triterpenoids, and saponins. GC-MS analysis of *Z. mauritiana* and *O. biennis* of ethyl acetate and ethanolic extract showed the existence of many bioactive substances at various retention durations (min). These included pharmacologically active compounds such as heptadecane, 2-methoxy-4-vinylphenol, hexadecanoic acid, and tetradecanoic acid. The results of the HP method revealed that ethanolic and ethyl acetate extracts of *Z. mauritiana* and *O. biennis* at 300 mg/kg increased basal reaction time significantly ($p < 0.001$) after 90 min. The results of the AAI method revealed that the ethanolic and ethyl acetate extract of *Z. mauritiana* and *O. biennis* showed significant ($p < 0.001$) peripheral analgesic activity at the dose of 200 and 300 mg/kg body weight. The dosage of 100, 200, and 300 mg/kg body weight of the ethyl acetate and ethanolic extracts of *Z. mauritiana* and *O. biennis* showed significant ($p < 0.001$) anti-inflammatory activity. According to PEM, the ethanolic extract of *O. biennis* showed the highest reduction in paw volume (73.3%) at 300 mg/kg. The results of CMT revealed that ethanolic and ethyl acetate extract of *Z. mauritiana* and *O. biennis* significantly ($p < 0.001$) inhibited charcoal movement at 300 mg/kg. The maximum percent inhibition (67.2%) was shown by ethyl acetate of *O. biennis* at 300 mg/kg. From the present study, it can be concluded that ethanolic and ethyl acetate extracts of *Z. mauritiana* and *O. biennis* have the potential to manage inflammation, pain, and diarrhea-related problems mainly at a higher dose, i.e., 300 mg/kg. The presence of alkaloids, flavonoids, tannins, steroids, triterpenoids, and saponins might be among the responsible bioactive constituents. These plants showed significant medicinal and therapeutic efficacy which are novel. However, further studies are required to investigate the mechanism responsible for the activity.

1. Introduction

Medicinal plants are naturally God-gifted with a lot of chemical compounds that are formed in different plant parts and used in the treatment of various ailments. These chemical compounds are also known as secondary metabolites. These secondary metabolites can be utilized in the formulations of novel medications and have been reported as very effective in curing serious health problems. The World Health Organization (WHO) reported that about 80% of the population in the world still depends on medicinal plants for their basic healthcare due to their effectiveness and easy availability [1]. Advancements in biotechnology have made it possible to manufacture therapeutic protein drugs in significant quantities [2]. Historically, fruits have served as a basis for various medicinal and liquor products [3]. Additionally, plant hydrocolloids have shown potential in mitigating cardiovascular disease risk, decreasing blood cholesterol levels, and enhancing immune function [4].

Aromatic medicinal plants are widely used by people for various purposes including the food industry, perfumery, textile industry, and pharmaceutical industry. The medicinal properties of plants are due to the presence of bioactive compounds such as saponins, glycosides, quinones, alkaloids, and flavonoids [5].

Pain is associated with potential or actual tissue damage. Pain is not only a symptom used to diagnose several diseases and conditions but also has a protective function [6]. Analgesic drugs have been used for the elimination of pain without significantly altering consciousness. The use of synthetic analgesic drugs has several side effects including gastrointestinal disorders, bleeding, and ulcers [7]. A physiological response that protects a body from tissue injury is called inflammation. There are two types of inflammation, i.e., acute inflammation and chronic inflammation [8]. The nonsteroidal anti-inflammatory drugs are used to treat inflammation but it has a lot of side effects, including gastrointestinal and cardiovascular complications. Therefore, the development of new drugs is necessary for the management of pain and inflammation. Plant-based beverages typically contain advantageous bioactive compounds, including flavonoids, phenolic acids, lignans, and phytosterols, known for their exceptional antioxidant, analgesic, and anti-inflammatory properties, contributing significantly to health benefits [9, 10]. In the past twenty years, numerous research studies have underscored the pivotal role of the gut microbiome in influencing health and disease. Alterations in the composition of the intestinal microbiome have been linked to diverse intestinal and metabolic disorders, including inflammatory bowel disease, diabetes, and obesity [11].

An imbalance in the gut microbiota plays a crucial role in the pathogenesis of functional Dyspepsia (FD). This imbalance disrupts the intestinal environment, ultimately causing a decrease in beneficial probiotics and triggering a range of acute and chronic diseases [12]. The condition of increased stool frequency, liquidity, or volume is called diarrhea. It mainly affects neonates and babies and is a significant cause of illness and mortality in developing countries [13]. Medicinal plants are reservoirs of essential antidiarrheal bioactive constituents without any side effects and can be used to treat gastrointestinal disorders, for example, constipation and diarrhea [14].

Ziziphus mauritiana belonging to the family Rhamnaceae Juss., locally called Ber, is a fruit tree that grows worldwide in tropical and subtropical regions [15]. *Z. mauritiana* is a medicinal plant used to cure several disorders such as ulcers, asthma, allergies, depression, digestive problems, weakness, obesity [16, 17], diabetes, urinary issues, and skin infections [18].

Oenothera biennis L. also known as evening primrose belongs to Onagraceae Juss. It is distributed in Peshawar Pakistan and eastern and central North America. The seed oil of *O. biennis* is used for the treatment of several ailments such as asthma, eczema, breast problems, rheumatoid arthritis, menopausal syndrome [19] fistulas, and lung disease [20]. The present study will analyze the plant in terms of drug standardization and evaluating its pharmacological effect which can be used as a potential candidate for future drug developments.

2. Materials and Methods

2.1. Plant Collection. *Ziziphus mauritiana* was collected from the area of Palosai Peshawar, and *Oenothera biennis* was collected from the Department of Botany, University of Peshawar KPK Pakistan which is located at 34.0011°N and 71.4874°E in August 2022. The identification of the plant was carried out with the help of Flora of Pakistan and Ghulam Jelani (plant taxonomist) and kept in the herbarium for future reference with Voucher Number Ambrin Bot. 33 (PUP) and Ambrin Bot. 34 (PUP).

2.2. Extraction of Plant Material and Sample Preparation. Whole plants were washed with distilled water, shade dried in the air dryer, and ground into a fine powder, and 50 g was soaked in 250 mL each in ethanol and ethyl acetate, respectively, which were supplied by U.M. enterprises. After 48 hours, the extract was passed through muslin cloth followed by filtration through filter paper. The extract was concentrated using a rotary evaporator (RE-100D Phoenix) supplied by MED Lab Services. The derived ethyl extracts (10.1 g) and ethanol extract (9.5 g) of *Z. mauritiana* and ethyl extracts (10.4 g) and ethanol extract (10.12 g) each were kept at 4°C in capped bottles before use [21].

2.3. Phytochemical Screening. Phytochemical analysis of the extracts was carried out to detect flavonoids, alkaloids, tannins, steroids, triterpenoids, and tannins following protocols [22].

2.4. Gas Chromatography-Mass Spectrometry (GC/MS). A gas chromatography-mass spectrometry of the crude extract of *Z. mauritiana* and *O. biennis* was carried out by coupling Thermo GC-Trace Ultra version 5.0 with Thermo MS DSQ II which was supplied by MED Lab Services. The sample was prepared by adding 2 mg crude extract in 5 mL of respective

solvents. To purify the samples, the mixtures were divided on a ZB 5-MS capillary regular nonpolar column (30 m 0.25 mm ID 0.25 lm FILM). The temperature of the column was kept at 70°C with an increasing rate of 2°C/minute. Finally, the increase in temperature was raised to 260°C at 6°C/minute with a holding time of about ten minutes. The splitless mode was used to introduce the particle-free, diluted sample (10 mL/min split flow and 1 min spitless period). Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and 1 L of sample was injected. Peak area normalization was used to quantify the relative percentages of crude extract elements. In full scan mode, the mass spectral scan range was adjusted to 50 to 650 (m/z). By comparing the retention indices of the compounds with those of real samples stored on the Wiley and Main Lab computer library search software, the compounds were identified [23].

2.5. Analgesic Activity

2.5.1. Acetic Acid-Induced Writhing. Ziziphus mauritiana and *Oenothera biennis* ethanolic and ethyl acetate extracts (sample was prepared by adding 10 mg crude extract in 25 mL of respective solvents) were tested for their analgesic activity following the method of [24] with few modifications. Mice were divided into five groups containing five mice in each group. Group 1 served as control and administered only with normal saline (10 ml/kg i.p.) Group 2 was administered with standard diclofenac sodium (25 mg/kg), and groups 3–5 were administered with three doses *of Z. mauritiana* and *O. biennis* ethanolic and ethyl acetate extracts (100 mg/kg, 200 mg/kg, and 300 mg/kg), respectively, and these extracts were administered orally one hour before intraperitoneal injection of 0.6% v/v acetic acid and after five mins of postinjection; the number of writhing was counted for the next 20 min. The percent analgesic effect was calculated by the following formula:

$$\%\ \text{analgesic effect} = 100 - \text{no of writhing in tested animals no of writhing in control animals X } 100. \quad (1)$$

2.5.2. Eddy's Hot-Plate Method. Its requirements were similar to the previous method. To perform this activity, the method of [25] was followed, the albino mice (male) were divided randomly into 5 groups, and each group consisted of 5 mice. Group 1 served as control and administered only with normal saline (10 ml/kg ip), group 2 was treated with the standard drug diclofenac sodium (25 mg/kg), and groups 3–5 were orally administered with different concentrations (100, 200, and 300 mg body weight) of ethanolic and ethyl acetate extracts, respectively. The initial reaction time of control and test group animals was recorded by placing them on the hot plate (55 ± 0.5°c), and the licking of the paw or jumping was taken as the index of reaction of heat: the post-treatment reaction time of each animal after the administration of plant extracts recorded at 30 min, 60 min, and 90 min.

$$\text{elongation}\ (\%)\ \text{latency (test)} - \text{latency (control)} \times 100\ \text{latency (test) statistical analysis} \quad (2)$$

2.6. Anti-Inflammatory Activity. The anti-inflammatory activity of the *Z. mauritiana* and *O. biennis* ethanolic and ethyl acetate extracts was investigated on carrageenan-induced inflammation in mice paws following the procedure [26]. The sample was prepared by adding 10 mg crude extract in 25 mL of respective solvents. Animals were divided into 5 groups comprising five animals per group. In all groups, acute inflammation was produced by subplantar injection of 0.1 ml freshly prepared 1% suspension of carrageenan. The paw volume was measured plethysmometrically from 0 to 180 min after carrageenan injection. All the animals were orally premedicated with diclofenac sodium (10 mg/kg b.wt), two hours before the infection. The mean increase in paw volume was measured, and the percentage was calculated for all the extracts. Percentage inhibition of paw volume was calculated by the following formula:

$$\text{Vc} - \text{Vt}\ \%\ \text{inhibition of paw edema} = \text{x } 100\ \text{Vc}, \quad (3)$$

where Vt = increase in paw volume in mice treated with test compounds and Vc = increase in paw volume in the control group of mice.

2.7. Antispasmodic Activity. The antispasmodic activity of *Z. mauritiana* and *O. biennis* ethanolic and ethyl acetate extracts was carried out by adopting the method of [27]. The sample was prepared by adding 10 mg crude extract in 25 mL of respective solvents. The selected mice were divided into four groups of five mice each. At first, 1 ml of castor oil was given orally to every mouse in each group to produce diarrhea. After 1 hr, group I (control group) orally received saline (10 ml/kg). Group II received the standard drug (atropine sulfate 10 mg/kg b. wt in), and group it-V (the rest of the three groups) received ethanolic and ethyl acetate extracts of plants (100, 200, and 300 mg/kg b. wt ip, respectively). After 1 h, all animals orally revived of the charcoal meal (10 charcoal is a pension in 5% gum acacia). After one hour following the charcoal meal administration, all animals were sacrificed, and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum, was measured and expressed as a percentage of the distance moved intestinal transit.

$$(\%) = (\text{D/L}) \times 100, \quad (4)$$

where D = distance covered by charcoal (in meters) and L = intestinal length (in meters).

2.8. Ethical Approval. The animal study was reviewed and approved by the Ethical Committee Pharmacy Lab, Qurtuba University of Science and Information Technology Peshawar, Pakistan, under permit no (148/VIEC/VRIP).

2.9. Statistical Analysis. The data were analyzed by Dunnett's *t*-test statistical methods using SPSS Software 22.0. For the statistical tests, $p < 0.001$, 0.01, and 0.05 was considered as significant.

3. Results

3.1. Phytochemical Screening. The phytochemical screening of ethanolic and ethyl acetate extracts of *Z. mauritiana* and *O. biennis* revealed the existence of different bioactive compounds. The ethanolic extract of *Z. mauritiana* showed the presence of alkaloids, flavonoids, tannins, steroids, and triterpenoids whereas saponins were found absent. Similarly, the ethyl acetate extracts of *Z. mauritiana* detect alkaloids, flavonoids, saponins, tannins, and triterpenoids while steroids were absent. Likewise, the ethanolic extract of *O. biennis* revealed the presence of alkaloids, flavonoids, saponins, and steroids and the absence of tannins and triterpenoids. The ethyl acetate extract of *O. biennis* unveiled the presence of alkaloids, flavonoids, tannins, steroids, and triterpenoids and the absence of tannins (Table 1).

3.2. Gas Chromatography-Mass Spectrometry. Gas chromatography-mass spectrometry profiling identified the probable phytochemicals in the ethanolic and ethyl acetate extract of *Z. mauritiana* and *O. biennis*. In ethanolic and ethyl acetate extract of *Z. mauritiana*, fourteen phytoconstituents were detected by GC–MS (Tables 2 and 3; Figures 1 and 2). Similarly, eight phytoconstituents were detected in the ethanolic extract of *O. biennis*, and eight phytoconstituents were detected in its ethyl acetate extract (Tables 4 and 5; Figures 3 and 4). The results showed that phytoconstituents found in maximum concentration in ethanolic extract of *Z. mauritiana* was heptadecane (9.06%) followed by 2-methoxy-4-vinylphenol (6.54%) and dodecanoic acid (4.50%) (Table 2; Figure 1). In the ethyl acetate extract of *Z. mauritiana*, tetracosane (11.52%) was detected in the highest concentration followed by dodecane, 1,1-dimethoxy- (4.06%), and 2-methoxy-4-vinylphenol (6.36%) (Table 3; Figure 2). In an ethanolic extract of *O. biennis*, bioactive constituents detected in the maximum amount were phytol (7.45%), followed by furfural (6.53%) and 4-vinyl-2-methoxy-phenol (2.36%) (Table 4; Figure 3). Likewise, the phytoconstituents detected with maximum concentration in ethyl acetate extract of *O. biennis* include 9,12-octadecadienoic acid (Z, Z)- (7.33%), followed by hexathiane (1.06%) and ethyl hexadecane (4.37%) (Table 5; Figure 4).

3.3. Analgesic Activity

3.3.1. Writhing Method. The ethanolic and ethyl acetate extract of *Z. mauritiana* and *O. biennis* exhibited significant ($p < 0.001$) analgesic activity when measured by acetic acid-induced writhing inhibition method at a dose of 300 mg/kg body (Table 6). The ethyl acetate and ethanolic extract of *Z. mauritiana* showed percent inhibition of 22.0%, 57.5%, 25.0%, and 72.6% at a dose of 100 mg/kg and 200 mg/kg body weight whereas the ethyl acetate and ethanolic extract of *O. biennis* showed percent inhibition of 24.0% and 64.1% and 29.1% and 59.4%, respectively, at a dose of 100 mg/kg and 200 mg/kg body weight which was comparable with the positive control diclofenac sodium (68.5%). The ethanolic extract of *Z. mauritiana* was more effective than *O. biennis* extracts.

3.3.2. Hot-Plate Method. The ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* showed a significant ($p < 0.001$) increase in latency time after 90 min at a dose of 200 mg/kg and 300 mg/kg body weight (Tables 7 and 8). Both the plants showed dose-dependent effects, and a significant result was obtained after 90 min at 300 mg/kg comparable with diclofenac sodium. The inhibition percentage at 200 and 300 mg/kg of ethyl acetate and ethanolic extract of *Z. mauritiana* was 46.7%, 56.0% and 38.1%, 52.3% while that of *O. biennis* 40.3%, 53.7% and 48.5%, 59.5% after 90 min, respectively. The highest central analgesic effect was shown by the ethanolic extract of *O. biennis* which was higher than diclofenac sodium.

3.4. Anti-Inflammatory Activity

3.4.1. Carrageenan-Induced Paw Edema. A significant reduction ($p < 0.001$) in the paw volume was revealed by ethanolic and ethyl acetate extract (100, 200, and 300 mg/kg) of *Z. mauritiana* and *O. biennis* at the 5th hour dose-dependently. The diclofenac sodium caused a significant ($p < 0.001$) reduction at the 4th and 5th hours in the volume of the paw (Tables 8 and 9). The inhibitory effect of the ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* at 100 mg/kg, 200 mg/kg, and 300 mg/kg was 64.6%, 68.6%, 70.0%, 57.3%, 60.6%, and 66.0% at 5th hour while the inhibitory effect of the ethyl acetate and ethanolic extract of *O. biennis* at 100 mg/kg, 200 mg/kg, and 300 mg/kg was 53.3%, 68.0%,71.3%, 66.6%, 69.3%, and 73.3%, respectively, at 5th hour. The percent inhibition of ethanolic extract of *O. biennis* was more than the *Z. mauritiana* but less than diclofenac sodium (10 mg/kg) which showed the highest 75.4% inhibition in paw volume at the 5th hour.

3.5. Antispasmodic Activity

3.5.1. Charcoal Meal Test. The ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* significantly ($p \leq 0.001$) decreased the distance travelled by a charcoal

Table 1: Phytochemical screening of *Ziziphus mauritiana* var. *spontanea* and *Oenothera biennis*.

Chemical constituents	*Ziziphus mauritiana* var. *spontanea.*		*Oenothera biennis*	
	Ethyl acetate extract	Ethanol extract	Ethyl acetate extract	Ethanol extract
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	−	+	−	+
Tannins	+	+	+	−
Steroids	+	−	+	+
Triterpenoids	+	+	+	−

meal at a dose of 300 mg/kg body weight which was comparable to atropine sulfate (10 mg/kg) (Tables 10 and 11). The 300 mg/kg dose of ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* presented 64.4%, 56.8%, 67.2%, and 50.7% percent inhibition in the charcoal meal movement whereas the ethyl acetate extract of *Z. mauritiana* and *O. biennis* showed maximum percent inhibition of 64.4% and 67.2% in charcoal meal movement which was comparatively higher inhibition than the standard drug atropine sulfate. However, the ethyl acetate extract of *O. biennis* showed the highest percent inhibition (67.2%) than the ethyl acetate extract (64.4%) of *Z. mauritiana*.

4. Discussion

For peripherally acting drugs, the acetic acid-induced abdominal constriction test is used. The pain induction occurs by releasing endogenous substances and other pain mediators such as prostaglandins [28]. The dosage of 300 mg/kg of ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* significantly ($p < 0.001$) inhibited the writhing's paralleled to diclofenac sodium ($p < 0.001$). The ethanolic extract of *Z. mauritiana* was more effective in inhibiting the writhing response which showed comparatively higher percent inhibition 72.6% in writhing than the standard drug diclofenac sodium (68.5%) (Table 6). The results indicated that the reduction in pain was dose-dependent; hence, the 300 mg/kg dose proved to be most effective. These results are in line with [29] who reported effective analgesic results from methanolic extracts of *Phyllanthus seeds*. Pain inhibitory activity of plant extracts may be interrelated to the inhibition of prostaglandin synthesis [30]. The analgesic activity of *Z. mauritiana* and *O. biennis* might be due to several bioactive constituents such as terpenoids, flavonoids, tannins, alkaloids, and steroids which were detected in plant extracts. Flavonoids showed analgesic action by increasing the endogenous serotonin level or its interaction with 5-HT2A and 5-HT3 receptors. Acetic acid-induced writhing has been connected with upgraded levels of prostaglandin (PGE2 and PGF2α) in the peritoneal liquids and the lipoxygenase items [31]. The ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* possibly showed analgesic action by inhibiting the synthesis of the arachidonic acid metabolite. The hot-plate test has been used for the assessment of centrally mediated analgesic responses, which emphasizes mostly changes above the spinal cord level. The findings revealed that after 90 min at 200 mg/kg and 300 mg/kg, the ethyl acetate and ethanolic extract of *Z. mauritiana* and *O. biennis* significantly ($p < 0.001$) increased in the reaction time (Tables 7 and 8). The effect increased with an increase in time and dose, and a greater effect was attained after 90 min at a higher dose. The ethanolic extract of *O. biennis* was more effective which showed a maximum percent increase (59.5%) in reaction time which was comparatively higher than the standard drug diclofenac sodium which showed a 57.2% percent increase in reaction time. These results are in line with [32] who reported similar results from the Moroccan medicinal plants. The *Z. mauritiana* and *O. biennis* showed antinociceptive activity in the hot-plate test by increasing the latency to discomfort. This action could be stimulating the periaqueductal gray matter to release endogenous peptides (endorphins or encephalins) [33]. These endogenous peptides run down the spinal cord and at the synapse in the dorsal horn and function as inhibitors of pain impulse transmission. *Z. mauritiana* and *O. biennis* showed central analgesic activity due to their action on the central opioid receptors or promoted release of endogenous opioid peptides. The analgesic activity of *Z. mauritiana* and *O. biennis* might be due to several secondary metabolites such as tannins, flavonoids, steroids, alkaloids, and terpenoids which were detected in plant extracts (Table 1). Flavonoids are involved in the management of pain by increasing the quantity of endogenous serotonin or by interacting with various receptors. Alkaloids have also been associated with the ability to inhibit pain perception [34, 35].

Anti-inflammatory drugs can be tested by the carrageenan-induced inflammation model [36]. Acute inflammation is produced in the rat paw by the subcutaneous injection of carrageenan, and certain mediators including histamine, prostaglandin, and serotonin are released causing fever and pain. The findings indicated that ethyl acetate and ethanolic extract (100, 200, and 300 mg/kg) of *Z. mauritiana* and *O. biennis* at the 5th hour exhibited significant inhibition ($p < 0.001$) in the volume of paw but less than the diclofenac sodium ($p < 0.001$) (Tables 9 and 10). The high percent inhibition (73.3%) in paw edema was caused by ethanolic extract of *O. biennis* at the 5th hour at 300 mg/kg when compared to *Z. mauritiana* but less than the standard drug diclofenac sodium (75.4%). The ethanolic extract significantly suppressed edema in 1st phase, which might primarily be credited to the drop in the release and synthesis of serotonin and histamine. The anti-inflammatory activity shown by both plants was dose-dependent and time-dependent revealing similar dose-dependent and time-dependent anti-inflammatory activity from the ethanolic

Table 2: GCMS analysis of ethanol extract of *Ziziphus mauritiana* var. *spontanea*.

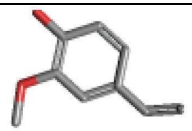
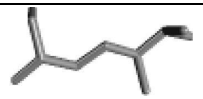
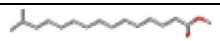
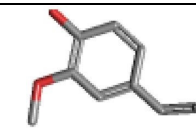
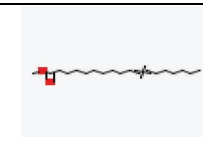
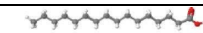
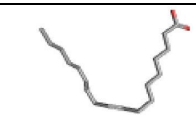
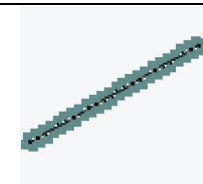
S. No	Name of the compound	Compound formula	Retention time (min)	Peak area (%)	Compound structure	Molecular weight
1	Heptadecane	$C_{17}H_{36}$	7.2	9.06		240.475 g·mol^{-1}
2	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	10.5	6.54		150.17 g/mol
3	Dodecanoic acid	$C_{12}H_{24}O_2$	17.4	4.50		201.31 g/mol
4	2,3,6-Trimethyl decane	$C_{11}H_{24}$	25.3	8.43		156.31 g/mol
5	Octadecanoic acid	$C_{18}H_{34}O_2$	30.2	5.36		282.5 g/mol
6	Methyl 14-methyl pentadecanoate	$C_{17}H_{34}O_2$	39.5	3.40		270.5 g/mol
7	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	42.6	2.53		330.5 g/mol

Table 3: GCMS analysis of ethyl acetate extract of *Ziziphus mauritiana* var. *spontanea*.

S. no	Name of the compound	Compound formula	Retention time (min)	Peak area (%)	Compound structure	Molecular weight
1	Dodecane, 1,1-dimethoxy-	$C_{12}H_{26}O_2$	6.3	4.06		202.33 g/mol
2	2-Methoxy-4-vinyl phenol	$C_9H_{10}O_2$	10.0	6.36		150.17 g/mol
3	9-Octadecene	$C_{18}H_{36}$	14.5	3.45		252.5 g/mol
4	Methyl 11-octadecenoate	$C_{19}H_{36}O_2$	19.4	10.65		296.5 g/mol
5	Hexadecanoic acid	$C_{16}H_{32}O_2$	24.4	7.06		287.61 g/mol
6	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{18}H_{32}O_2$	27.6	5.43		280.4 g/mol
7	Tetracosane	$C_{24}H_{50}$	30.6	11.52		389.0 g/mol

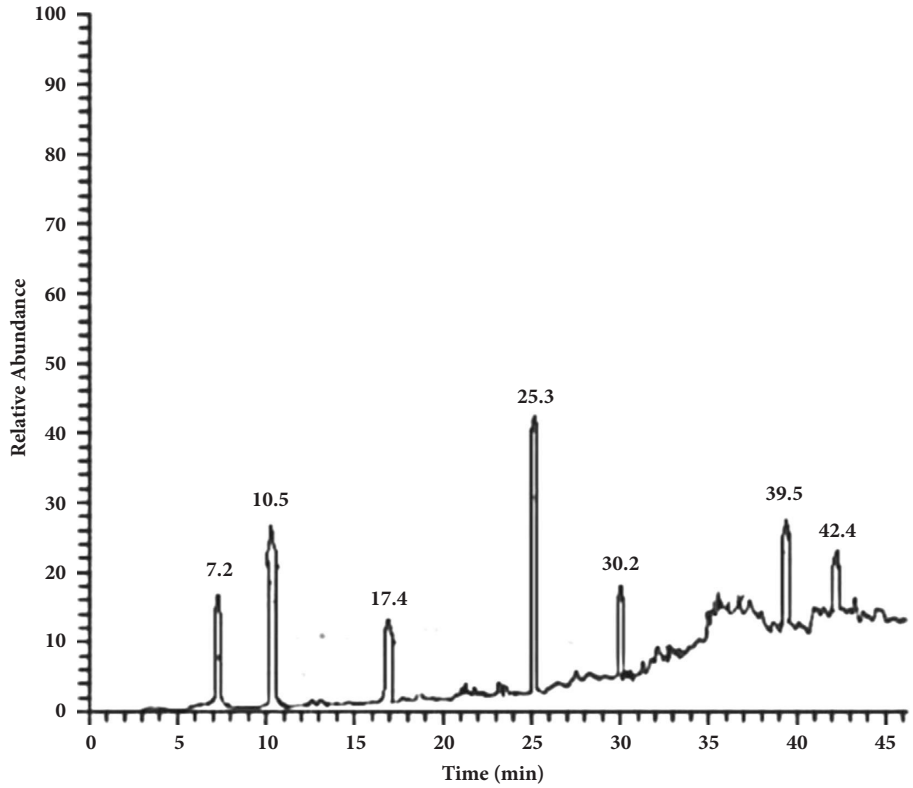


Figure 1: GCMS chromatogram of ethanolic extract *of Ziziphus mauririana* var. *sponatanea*.

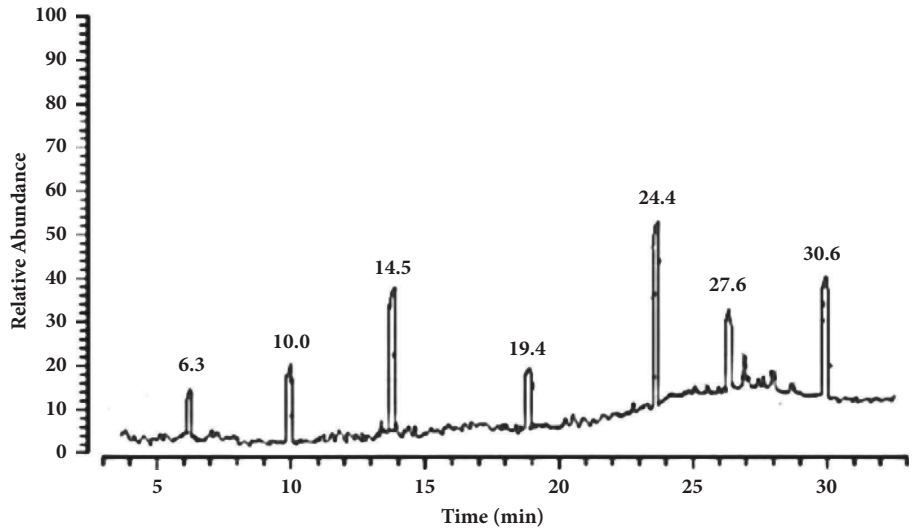


Figure 2: GCMS chromatogram of ethyl acetate extract *of Ziziphus mauririana* var. *sponatanea*.

Table 4: GCMS analysis of ethanol extract of *Oenothera biennis*.

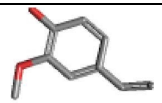
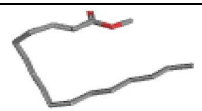
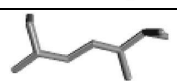
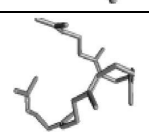
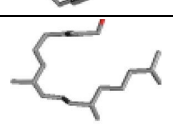
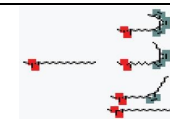
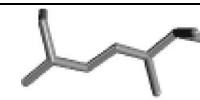
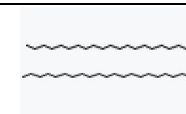
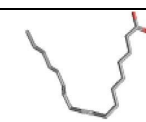
S. No	Name of the compound	Compound formula	Retention time (min)	Peak area (%)	Compound structure	Molecular weight
1	Furfural	$C_4H_5O_2$	9.3	6.53		96.08 g/mol
2	4-Vinyl-2-methoxy-phenol	$C_9H_{10}O_2$	14.2	2.36		150.17 g/mol
3	Hexadecanoic acid and methyl ester	$C_{17}H_{32}O_2$	16.5	1.42		268.4 g/mol
4	7-Methyl hexadecane	$C_{17}H_{36}$	20.2	4.06		240.5 g/mol
5	2,3,6-Trimethyl decane	$C_{11}H_{24}$	22.4	3.40		156.31 g/mol
6	Squalene	$C_{30}H_{50}$	26.5	2.53		410.7 g/mol
7	Phytol	$C_{20}H_{40}O$	30.3	7.45		296.5 g/mol

Table 5: GCMS analysis of ethyl acetate extract of *Oenothera biennis*.

S. no	Name of the compound	Compound formula	Retention time (min)	Peak area (%)	Compound structure	Molecular weight
1	Hexathiane	C_6H_8	15.3	1.06		80.13 g/mol
2	Ethyl hexadecanoic	$C_{98}H_{184}O_{10}$	18.6	4.37		1522.5 g/mol
3	2,3,6-Trimethyl decane	$C_{11}H_{24}$	22.4	3.16		156.31 g/mol
4	n-Eicosane	$C_{41}H_{86}$	25.3	6.05		579.1 g/mol
5	1,19-Eicosadiene	C_8H_{14}	28.5	5.32		110.20 g/mol
6	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	33.4	7.33		280.4 g/mol
7	Tetradecanoic acid	$C_{14}H_{28}O_2$	45.5	1.56		228.37

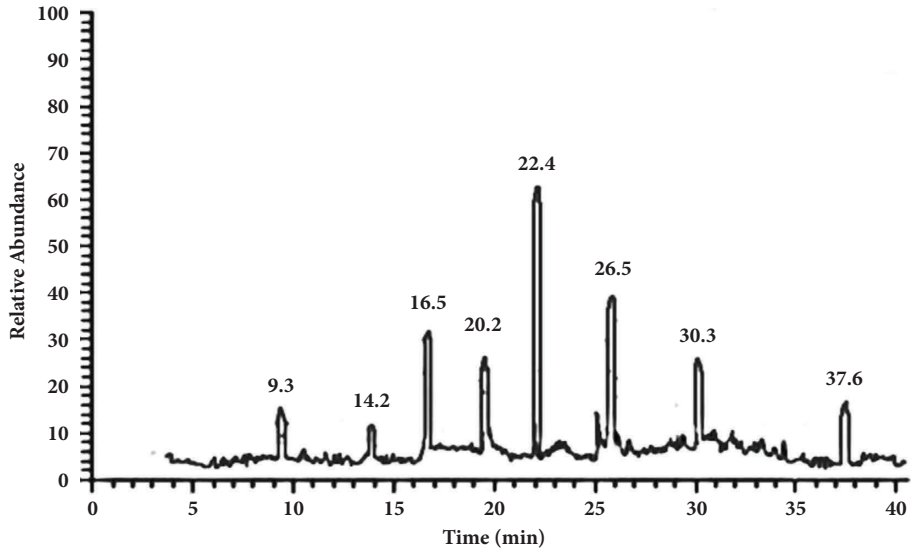


Figure 3: GCMS chromatogram of ethanolic extract *of Oenothera biennis*.

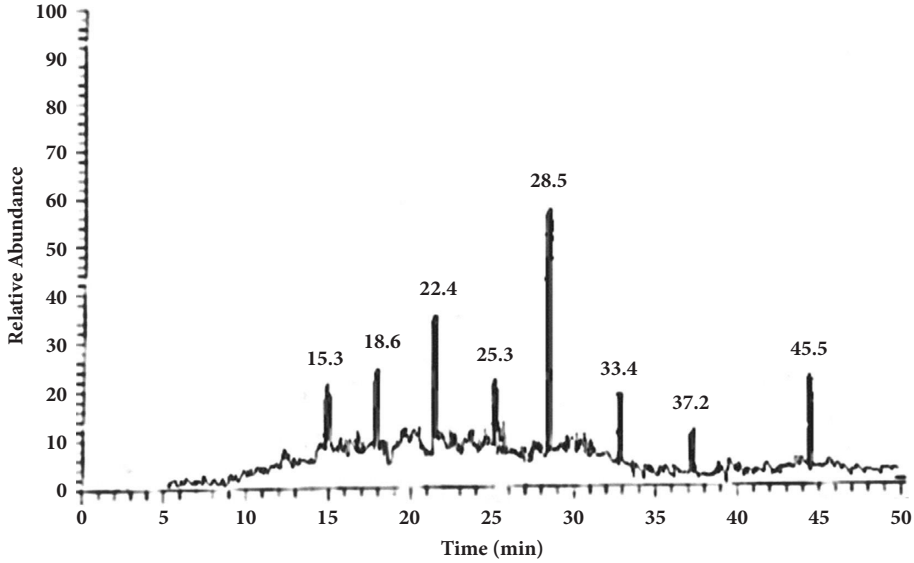


Figure 4: GCMS chromatogram of ethyl acetate extract *of Oenothera biennis*.

Table 6: Analgesic activity of *Ziziphus mauritiana.* var *spontanea* and *Oenothera biennis* L. by writhing method.

Treatment	Dose (mg/kg)	*Ziziphus mauritiana*		*Oenothera biennis*	
		Average no. of writhing	% Inhibition	Average no. of writhing	Inhibition %
Normal saline	5 ml/kg	70.6 ± 5.03	—	70.6 ± 5.03	—
Diclofenac sodium	10 mg/kg	22.3 ± 2.51***	68.5	22.3 ± 2.51***	68.5
Ethyl acetate	100 mg/kg	60.66 ± 9.45	14.1	58.66 ± 0.57	17.0
	200 mg/kg	55.00 ± 4.00**	22.0	53.66 ± 3.21**	24.0
	300 mg/kg	30.00 ± 5.00***	57.5	25.33 ± 5.50***	64.1
Ethanol	100 mg/kg	57.66 ± 6.65*	18.4	61.66 ± 6.65	12.7
	200 mg/kg	53.00 ± 4.35**	25.0	50.00 ± 1.73***	29.1
	300 mg/kg	19.33 ± 1.15***	72.6	28.66 ± 7.02***	59.4

Values are expressed as mean ± standard deviation. Significance is shown as *significant, **more significant, and ***highly significant.

Table 7: Analgesic activity of *Ziziphus mauritiana* by hot-plate method.

Treatment	Dose (mg/kg)	After 0 min	After 30 min	After 60 min	After 90 min	Percent decrease in latency time
Normal saline	10 ml/kg	3.30 ± 0.62	4.20 ± 0.36	3.73 ± 0.41	4.53 ± 0.80	...
Diclofenac sodium	10 mg/kg	3.70 ± 0.17	8.10 ± 0.45***	9.23 ± 0.37***	10.60 ± 0.10***	57.2
Ethyl acetate	100 mg/kg	3.50 ± 0.30	4.56 ± 0.15	4.13 ± 0.55	6.40 ± 0.52**	29.2
	200 mg/kg	3.53 ± 0.87	4.63 ± 0.45	5.23 ± 0.95*	8.50 ± 0.17***	46.7
	300 mg/kg	3.63 ± 0.20	4.73 ± 0.45	6.13 ± 0.50***	10.30 ± 0.45***	56.0
Ethanol	100 mg/kg	3.40 ± 0.79	4.26 ± 0.40	3.80 ± 0.36	5.56 ± 0.15	18.5
	200 mg/kg	3.30 ± 0.45	4.56 ± 0.11	4.70 ± 0.62	7.33 ± 1.05***	38.1
	300 mg/kg	3.46 ± 0.75	4.70 ± 0.10	5.80 ± 0.70***	9.50 ± 0.70***	52.3

Values are expressed as mean ± standard deviation. Significance is shown as *significant, **more significant, and ***highly significant.

Table 8: Analgesic activity of *Oenothera biennis* L. by hot-plate method.

Treatment	Dose (mg/kg)	After 0 min	After 30 min	After 60 min	After 90 min	Percent decrease in latency time
Normal saline		3.30 ± 0.62	4.20 ± 0.36	3.73 ± 0.41	4.53 ± 0.80	...
Diclofenac sodium	10	3.70 ± 0.17	8.10 ± 0.45	9.23 ± 0.37	10.60 ± 0.10***	57.2
Ethyl acetate	100	3.40 ± 0.62	4.63 ± 0.41	3.96 ± 0.47	5.83 ± 0.41	22.2
	200	3.50 ± 0.60	4.73 ± 0.90	4.40 ± 0.20	7.60 ± 0.60***	40.3
	300	3.66 ± 0.90	4.86 ± 0.28	6.80 ± 0.62***	9.80 ± 0.55***	53.7
Ethanol	100	3.46 ± 0.90	4.23 ± 0.45	4.33 ± 0.05	7.23 ± 0.95***	37.3
	200	3.63 ± 0.45	4.30 ± 0.80	5.50 ± 0.26**	8.80 ± 0.45***	48.5
	300	3.70 ± 0.85	4.50 ± 0.90	6.43 ± 0.25***	11.20 ± 0.40***	59.5

Values are expressed as mean ± standard deviation. Significance is shown as *significant, **more significant, and ***highly significant.

extract and ethyl acetate of *Albizia lebbeck* (L.) Benth. and *Senna sophera* (L.) Roxb. Several secondary metabolites such as steroids, tannins, flavonoids, terpenoids, and alkaloids might be responsible for the anti-inflammatory activity of *Z. mauritiana* and *O. biennis* (Table 1). Flavonoids inhibit significant enzymes involved in the biosynthesis of tissue activators, especially prostaglandins and arachidonic acid [37]. The triterpenes possess anti-inflammatory potentials and prevent the production of inflammatory mediators [38].

The effect of drugs on peristaltic movement can be tested by charcoal meal test [21]. The irritation and inflammation of intestinal mucosa can result from the hydrolysis of castor oil into ricinoleic acid which results in diarrhea. It results in the release of prostaglandins which provoke gastrointestinal motility and result in secretion of water and electrolytes [22]. The result showed that ethyl acetate ($p < 0.001$) and ethanolic extract ($p < 0.01$) of *Z. mauritiana* and *O. biennis* at a dose of 300 mg/kg showed a significant reduction in the distance travelled by charcoal meal when compared with atropine sulfate which also showed significant ($p < 0.001$) reduction in charcoal meal movement (Table 11). The extract showed a dose-dependent inhibition of charcoal meal motility. Thus, 300 mg/kg doses of both plants' extracts had more antimotility effect. The ethyl acetate extract of *Z. mauritiana* and *O. biennis* was more effective and showed higher activity than atropine sulfate. However, the maximum antimotility effect was shown by the ethyl acetate extract of *O. biennis* than the ethyl acetate extract of *Z. mauritiana*. The inhibition in the peristaltic movement of the gastrointestinal tract might be due to the inhibition of acetylcholine by the plant extracts resulting in the absorption of water and electrolytes [39]. The antispasmodic activity of *Z. mauritiana* and *O. biennis* might be due to the presence of secondary metabolites such as flavonoids, alkaloids, steroids, phenol, terpenoids, phytosterol, and tannins which were detected in plant extracts (Table 1). Tannins present in the extract form protein tannates by precipitating the proteins in the intestinal mucosa which helps in the protection of intestinal mucosa by making it more resistant to certain chemicals [40]. Flavonoids and steroids help in absorbing electrolytes by inhibiting secretion induced by castor oil. Alkaloids and terpenoids are known to inhibit the secretion induced by castor oil by inhibiting the release of autocoids and prostaglandin [41, 42].

Table 9: Anti-inflammatory activity of *Ziziphus mauritiana.* var. *spontanea.*

Treatment	Dose (mg/kg)	Paw volume after drug administration (mean + SEM)				
		1 hour	2 hour	3 hour	4 hour	5 hour
Normal saline	10 ml/kg	0.90 ± 0.02	0.77 ± 0.08	0.86 ± 0.10	0.95 ± 0.02	1.50 ± 0.27
Diclofenac sodium	10 mg/kg	0.80 ± 0.04 (11.11%)	0.54 ± 0.04* (29.8%)	0.45 ± 0.03* (47.67%)	0.52 ± 0.04*** (45.2%)	0.38 ± 0.08*** (75.4%)
Ethyl acetate extract	100 mg/kg	0.86 ± 0.06 (4.44%)	0.68 ± 0.08 (15.58%)	0.75 ± 0.08 (12.7%)	0.65 ± 0.04** (31.57%)	0.53 ± 0.06*** (64.6%)
	200 mg/kg	0.88 ± 0.07 (2.22%)	0.57 ± 0.08 (26.0%)	0.66 ± 0.06 (23.2%)	0.55 ± 0.08*** (42.1%)	0.47 ± 0.05*** (68.6%)
	300 mg/kg	0.76 ± 0.09 (15.5%)	0.59 ± 0.06 (23.3%)	0.64 ± 0.07 (34.3%)	0.53 ± 0.09*** (44.2%)	0.45 ± 0.04*** (70.0%)
Ethanolic extract	100 mg/kg	0.87 ± 0.03 (3.33%)	0.71 ± 0.04 (7.79%)	0.75 ± 0.18 (12.7%)	0.70 ± 0.14* (26.3%)	0.64 ± 0.19*** (57.3%)
	200 mg/kg	0.86 ± 0.12 (4.44%)	0.66 ± 0.10 (23.2%)	0.70 ± 0.24 (26.3%)	0.64 ± 0.12** (57.3%)	0.59 ± 0.04*** (60.6%)
	300 mg/kg	0.84 ± 0.09 (6.66%)	0.63 ± 0.09 (18.1%)	0.67 ± 0.12 (22.0%)	0.73 ± 0.10 (23.1%)	0.51 ± 0.10*** (66.0%)

Values are expressed as mean ± standard deviation. Significance is shown as *significant, **more significant, and ***highly significant.

Table 10: Anti-inflammatory activity *Oenothera biennis* L.

Treatment	Dose (mg/kg)	Paw volume after drug administration (Mean + SEM)				
		After 1 hour	After 2 hour	After 3 hour	After 4 hour	After 5 hour
Normal saline		0.90 ± 0.02	0.77 ± 0.08	0.86 ± 0.10	0.95 ± 0.02	1.50 ± 0.27
Diclofenac sodium	10 mg/kg	0.80 ± 0.04 (11.11%)	0.54 ± 0.04* (29.8%)	0.45 ± 0.03* (47.67%)	0.52 ± 0.04*** (45.2%)	0.38 ± 0.08*** (75.4%)
Ethyl acetate extract	100 mg/kg	0.86 ± 0.01 (4.44%)	0.67 ± 0.07 (12.98%)	0.72 ± 0.15 (16.27%)	0.64 ± 0.10** (57.3%)	0.70 ± 0.11*** (53.3%)
	200 mg/kg	0.85 ± 0.02 (5.55%)	0.58 ± 0.08 (24.6%)	0.66 ± 0.05 (23.2%)	0.55 ± 0.11*** (42.1%)	0.48 ± 0.05*** (68.0%)
	300 mg/kg	0.83 ± 0.03 (7.77%)	0.61 ± 0.09 (20.7%)	0.68 ± 0.11 (20.9%)	0.49 ± 0.09*** (48.4%)	0.43 ± 0.02*** (71.3%)
Ethanolic extract	100 mg/kg	0.87 ± 0.07 (3.33%)	0.64 ± 0.11 (16.8%)	0.73 ± 0.15 (15.1%)	0.61 ± 0.07** (35.7%)	0.50 ± 0.06*** (66.6%)
	200 mg/kg	0.87 ± 0.06 (3.33%)	0.59 ± 0.11 (23.3%)	0.67 ± 0.100 (22.0%)	0.5733 ±0 .13051*** (26.0%)	0.4633 ±0 .06028*** (69.3%)
	300 mg/kg	0.84 ± 0.04 (6.66%)	0.57 ± 0.15 (26.0%)	0.63 ± 0.08 (18.1%)	0.56 ± 0.06*** (41.0%)	0.40 ± 0.02*** (73.3%)

Values are expressed as mean ± standard deviation. Significance is shown as *significant, **more significant, and ***highly significant.

Table 11: Antispasmodic activity of *Ziziphus mauritiana* var. *spontanea* and *Oenothera*.

Treatment	Dose	*Ziziphus mauritiana* var. *spontanea*			*Oenothera biennis* L.		
		The mean length of the intestine	Mean distance travelled by charcoal	Percent inhibition (%)	The mean length of the intestine	Mean distance travelled by charcoal	Percent inhibition (%)
Normal saline + castor oil	10 ml/kg	55.2	46.06 ± 2.51	16.6	55.2	46.06 ± 2.51	16.6
Atropine sulfate	10 mg/kg	54.3	20.60 ± 0.70***	62.0	54.3	20.60 ± 0.70***	62.0
Ethyl acetate extract	100 mg/kg	50.6	34.63 ± 3.61	31.6	49.5	36.63 ± 10.66	26.0
	200 mg/kg	51.3	29.13 ± 3.82*	43.2	54.3	30.80 ± 4.96*	43.2
	300 mg/kg	52.3	18.65 ± 0.21***	64.4	54.6	17.90 ± 2.26***	67.2
Ethanolic extract	100 mg/kg	52.5	37.3 ± 3.70	28.9	53.2	34.80 ± 15.39	34.5
	200 mg/kg	49.4	29.3 ± 2.72*	40.6	54.4	32.30 ± 9.83	40.6
	300 mg/kg	54.4	23.56 ± 1.85**	56.8	52.2	25.70 ± 2.64**	50.7

Values are expressed as mean ± standard deviation. Significance is shown as *significant, **more significant, and ***highly significant.

5. Conclusion

In the current research, both plants showed important phytochemicals and therapeutic potential. From the results, it can be concluded that *Z. mauritiana* and *O. biennis* contained important chemical constituents including alkaloids, tannins, saponins, flavonoids, steroids, and triterpenoids as determined by phytochemical screening. GCMS revealed the presence of pharmacologically active compounds such as hexadecanoic acid, 4-vinyl-2-methoxy-phenol, n-eicosane, and 2,3,6-trimethyldecane which may be responsible for the significant anti-inflammatory, analgesic, and antispasmodic activity. Hence, these plants can be used for alleviating pain and treating various inflammatory and diarrhoeal disorders. Both plants can be used in the future for drug development and various herbal formulations having fewer side effects.

Data Availability

The data such as the source file associated with this finding are available from the corresponding author upon request.

Disclosure

The authors declare that they have no conflict of interest.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

A.A. administrated the project and proposed methodology, M.A. wrote the original draft, and M.N. edited, supervised, and visualized the study. F.Z.F. developed software, collected data, and provided funding.

Acknowledgments

The authors are highly grateful for receiving support from the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Grant No. IFPRC-187-130-2020.

References

[1] B. Patwardhan, *Traditional Medicine: A Novel Approach for Available, Accessible and Affordable Health Care*, vol. 13, World Health Organization, Geneva, Switzerland, 2005.

[2] A. J. Xie, H. S. Yin, H. M. Liu, C. Y. Zhu, and Y. J. Yang, "Chinese quince seed gum and poly (N, N-diethylacryl amide-co-methacrylic acid) based pH-sensitive hydrogel for use in drug delivery," *Carbohydrate Polymers*, vol. 185, pp. 96–104, 2018.

[3] L. Wang, H. M. Liu, C. Y. Zhu, A. J. Xie, B. J. Ma, and P. Z. Zhang, "Chinese quince seed gum: flow behaviour, thixotropy and viscoelasticity," *Carbohydrate Polymers*, vol. 209, pp. 230–238, 2019.

[4] L. Wang, H. M. Liu, A. J. Xie, X. D. Wang, C. Y. Zhu, and G. Y. Qin, "Chinese quince (Chaenomeles sinensis) seed gum: structural characterization," *Food Hydrocolloids*, vol. 75, pp. 237–245, 2018.

[5] G. S. Gnintoungbe, T. C. M. Medehouenou, F. Adounkpe, C. Akpovi, and F. Loko, "Phytochemical screening, antioxidant activity and safety of Petroselinum crispum (mill.) AW hill apiaceae leaves grown in Benin," *Open Journal of Applied Sciences*, vol. 13, no. 01, pp. 36–50, 2023.

[6] E. Ashenafi, T. Abula, S. M. Abay, M. Arayaselassie, S. Taye, and R. A. Muluye, "Analgesic and anti-inflammatory effects of 80% methanol extract and solvent fractions of the leaves of vernonia auriculifera hiern. (Asteraceae)," *Journal of Experimental Pharmacology*, vol. 15, pp. 29–40, 2023.

[7] N. B. Situmorang and S. Widya Ningsih, "Analgesic activity test of waru (Hibiscus tiliaceus L.) leaves ethanol extract in male white miscules (*Mus musculus*)," *Jurnal Farmasimed (JFM)*, vol. 5, no. 1, pp. 22–25, 2022.

[8] F. Alam, M. Hanif, A. U. Rahman, S. Ali, and S. Jan, "In vitro, in vivo and in silico evaluation of analgesic, anti-inflammatory, and anti-pyretic activity of salicylate rich fraction from Gaultheria trichophylla Royle (Ericaceae)," *Journal of Ethnopharmacology*, vol. 301, Article ID 115828, 2023.

[9] S. Piazza, F. Colombo, C. Bani et al., "Evaluation of the potential anti-inflammatory activity of black rice in the framework of celiac disease," *Foods*, vol. 12, no. 1, p. 63, 2022.

[10] A. Xie, Y. Dong, Z. Liu et al., "A review of plant-based drinks addressing nutrients, flavor, and processing technologies," *Foods*, vol. 12, no. 21, p. 3952, 2023.

[11] A. Xie, S. Zhao, Z. Liu et al., "Polysaccharides, proteins, and their complex as microencapsulation carriers for delivery of probiotics: a review on carrier types and encapsulation techniques," *International Journal of Biological Macromolecules*, vol. 124784, 2023.

[12] X. Shen, A. Xie, Z. Li et al., "Research progress for probiotics regulating intestinal Flora to improve functional Dyspepsia: a review," *Foods*, vol. 13, no. 1, p. 151, 2024.

[13] M. Li, Q. Li, H. Dong et al., "Pilose antler polypeptides enhance chemotherapy effects in triple-negative breast cancer by activating the adaptive immune system," *International Journal of Biological Macromolecules*, vol. 222, pp. 2628–2638, 2022.

[14] M. M. Islam, M. J. Hossain, M. S. Zahan, F. Nur, M. A. Al Mansur, and M. A. Rashid, "Stixis suaveolens (roxb.) fruit extract deciphered antidepressant and antidiarrheal effects via in vivo approach," *Bangladesh Pharmaceutical Journal*, vol. 26, no. 1, pp. 28–35, 2023.

[15] S. Paul Roy, "Formulation and evaluation of a novel herbal-based face wash by using hydra-africana (subfamily-Hydnoraceae) fruit extract," 2023.

[16] M. R. Paudel, M. R. Poudeyal, and H. P. Devkota, "Ziziphus spp. (Ziziphus jujuba mill., Ziziphus mauritiana lam.)," in *Himalayan Fruits and Berries*, pp. 491–497, Academic Press, Cambridge, MA, USA, 2023.

[17] R. Mohankumar, S. E. L. Prakash, N. Irfan, S. Mohanraj, and C. Kumarappan, "Evaluation of analgesic, anti-inflammatory, and antipyretic activities of Ziziphus Mauritania Lam leaves in animal models," *Pharmacological Research-Modern Chinese Medicine*, vol. 4, Article ID 100153, 2022.

[18] M. K. Ramar, K. Chidambaram, B. Chandrasekaran, and R. Kandasamy, "Standardization, in-silico, and in-vivo safety assessment of methanol extract of Ziziphus mauritiana Lam leaves," *Regulatory Toxicology and Pharmacology*, vol. 131, Article ID 105144, 2022.

[19] R. Fecker, I. Z. Magyari-Pavel, I. Cocan et al., "Oxidative stability and protective effect of the mixture between helianthus annuus L. And Oenothera biennis L. Oils on 3D tissue models of skin irritation and phototoxicity," *Plants*, vol. 11, no. 21, p. 2977, 2022.

[20] S. Montserrat-de la Paz, M. A. Fernández-Arche, M. Ángel-Martín, and M. D. García-Giménez, "Phytochemical characterization of potential nutraceutical ingredients from Evening Primrose oil (Oenothera biennis L.)," *Phytochemistry Letters*, vol. 8, pp. 158–162, 2014.

[21] M. I. Qadir, K. Abbas, R. Hamayun, and M. Ali, "Analgesic, anti-inflammatory, and antipyretic activities of aqueous ethanolic extract of Tamarix aphylla L. (Saltcedar) in mice," *Pakistan journal of pharmaceutical sciences*, vol. 27, no. 6, pp. 1985–1988, 2014.

[22] M. Adil, G. Dastagir, J. Bakht, and A. Ambrin, "Phytochemical screening and antimicrobial activity of medicinally important *Achillea millefolium* l and chaerophyllum villosum wall EXDC," *Pakistan Journal of Botany*, vol. 52, no. 3, pp. 971–974, 2020.

[23] J. Liu, J. Zhang, M. Zeng et al., "Anti-pulmonary fibrosis activities of triterpenoids from Oenothera biennis," *Molecules*, vol. 27, no. 15, p. 4870, 2022.

[24] N. H. Mat, S. N. S. Bakar, V. Murugaiyah, M. C. Chawarski, and Z. Hassan, "Analgesic effects of main indole alkaloid of kratom, mitragynine in acute pain animal model," *Behavioural Brain Research*, vol. 439, Article ID 114251, 2023.

[25] M. Bilal, A. Naz, A. S. Khan, R. Ghaffar, R. Ghaffar, and A. Abrar, "Assessment of Iris albicans lange as potential antimicrobial and analgesic agent," *PLoS One*, vol. 18, no. 1, Article ID e0280127, 2023.

[26] M. A. Aziz, S. Naher, M. I. Akter, S. M. Rahman, and S. R. Sajon, "Analgesic, anti-inflammatory, and antipyretic activities of methanolic extract of Cordyline fruticosa (L.) A. Chev. leaves," *Journal of Research in Pharmacy*, vol. 23, no. 2, pp. 198–207, 2019.

[27] T. Shamala, B. S. Surendra, M. V. Chethana, G. bolakatti, and S. Shanmukhappa, "Extraction and isolation of Isoflavonoids from stem bark of Bauhinia purpurea (L): its biological antipsychotic and analgesic activities," *Smart Materials in Medicine*, vol. 3, pp. 179–187, 2022.

[28] S. Madièye, S. B. Firmin, S. Abdou et al., "Anti-inflammatory and analgesic activities of methanolic extract of Elaeis guineensis Jacq. leaves (Arecaceae) and its fractions," *African Journal of Pharmacy and Pharmacology*, vol. 17, no. 2, pp. 43–51, 2023.

[29] A. Sumitha, R. Dhanasekaran, A. Archana, S. Sa, S. Thamizharasan, and B. Cs, "Phyllanthus seeds Methanolic extract: in vivo evaluation of Analgesic activity," *Research Journal of Pharmacy and Technology*, vol. 15, no. 2, pp. 713–716, 2022.

[30] D. Venkatachalam and B. S. Thavamani, "Evaluation of analgesic activity of ethanolic and aqueous extracts of leaf of Plumeria rubra in albino rat," *Pharmaceutical and Biological Evaluations*, vol. 5, no. 2, pp. 52–58, 2018.

[31] E. Z. Yassine, B. Dalila, E. M. Latifa et al., "Phytochemical screening, anti-inflammatory activity, and acute toxicity of hydro-ethanolic, flavonoid, tannin and mucilage extracts of Lavandula stoechas L. from Morocco," *International Journal of Pharmaceutical and Phytopharmacological Research*, vol. 8, no. 1, pp. 31–37, 2016.

[32] A. Bouyahya, F. E. Guaouguaou, N. El Omari et al., "Anti-inflammatory and analgesic properties of Moroccan medicinal plants: phytochemistry, in vitro and in vivo investigations, mechanism insights, clinical evidence, and perspectives," *Journal of Pharmaceutical Analysis*, vol. 12, no. 1, pp. 35–57, 2022.

[33] M. Shahed-Al-Mahmud, T. Jahan, and M. Towhidul Islam, "Antidiarrheal activities of hydroalcoholic extract of Sida cordifolia roots in Wister albino rats," *Oriental Pharmacy and Experimental Medicine*, vol. 18, no. 1, pp. 51–58, 2018.

[34] M. Wahid, F. Saqib, M. Qamar, and Z. M. Ziora, "Antispasmodic activity of the ethanol extract of *Citrullus lanatus* seeds: justifying ethnomedicinal use in Pakistan to treat asthma and diarrhea," *Journal of Ethnopharmacology*, vol. 295, Article ID 115314, 2022.

[35] F. Tasleem, "Biomedical analysis on phytopharmaceuticals," Doctoral dissertation, University of Karachi Karachi, Karachi, Pakistan, 2016.

[36] R. Ventura-Martinez, G. E. Angeles-Lopez, M. E. Gonzalez-Trujano, O. F. Carrasco, and M. Deciga-Campos, "Study of antispasmodic and antidiarrheal activities of Tagetes lucida (Mexican Tarragon) in experimental models and its

mechanism of action," *Evidence-based Complementary and Alternative Medicine*, vol. 2020, Article ID 7140642, 10 pages, 2020.

[37] A. Rauf, M. Akram, P. Semwal et al., "Antispasmodic potential of medicinal plants: a comprehensive review," *Oxidative Medicine and Cellular Longevity*, vol. 2021, Article ID 4889719, 12 pages, 2021.

[38] E. Tadesse, E. Engidawork, T. Nedi, and G. Mengistu, "Evaluation of the anti-diarrheal activity of the aqueous stem extract of Lantana camara Linn (Verbenaceae) in mice," *BMC Complementary and Alternative Medicine*, vol. 17, no. 1, pp. 190–198, 2017.

[39] R. Koster, M. Anderson, and E. De Beer, "Acetic acid-induced analgesic screening," *Federation Proceedings*, vol. 18, p. 412, 1959.

[40] N. B. Eddy and D. Leimbach, "Synthetic analgesics. II. Dithienylbutenyl-and dithienylbutylamines," *Journal of Pharmacology and Experimental Therapeutics*, vol. 107, no. 3, pp. 385–393, 1953.

[41] C. A. Winter and C. C. Porter, "Effect of alterations in side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone Esters∗∗Merck institute for therapeutic research, west point, Pa," *Journal of the American Pharmaceutical Association*, vol. 46, no. 9, pp. 515–519, 1957.

[42] N. Mascolo, A. A. Izzo, G. Autore, F. Barbato, and F. Capasso, "Nitric oxide and castor oil-induced diarrhea," *Journal of Pharmacology and Experimental Therapeutics*, vol. 268, no. 1, pp. 291–295, 1994.