

RESEARCH ARTICLE OPEN ACCESS

Biological Evaluation of *Swintonia floribunda* Leaf Extract Through Integrated GC-MS, *In Vivo*, *In Vitro*, and *In Silico* Approaches Targeting Diarrhea, Pain, Fever, and Inflammation

Farjana Akter Nipa^{1,2} | Mst. Riniara Khatun¹ | Israt Jahan³ | Md Shirajul Islam⁴ | Sabbir Khan⁵ | Shariful Islam⁵ | Jannatul Nisa Nipa⁶ | Mahathir Mohammad⁷ | Md. Anisul Islam³ | Trishala Dutta² | Md. Mustafiz Chowdhury^{6,8}  | Mycal Dutta² 

¹Department of Pharmacy, International Islamic University Chittagong, Chattogram 4318, Bangladesh | ²Department of Pharmaceutical Technology, University of Science & Technology Chittagong (USTC), Chittagong, Bangladesh | ³Department of Pharmacy, BGC Trust University, Chittagong 4381, Bangladesh | ⁴Regenerative Sustainability, University of Saskatchewan, Saskatoon, Canada | ⁵Department of Botany, University of Chittagong, Chittagong 4331, Bangladesh | ⁶Department of Pharmacy, Southern University Bangladesh, Chittagong, Bangladesh | ⁷Department of Chemistry, Chittagong University of Engineering & Technology, Chittagong 4349, Bangladesh | ⁸Department of Pharmacy, University of Chittagong, Chittagong 4331, Bangladesh

Correspondence: Mycal Dutta (mycal@ustc.ac.bd)

Received: 12 September 2025 | **Revised:** 25 November 2025 | **Accepted:** 3 March 2026

Academic Editor: Anupama Bose

Keywords: antidiarrheal | anti-inflammatory | antinociceptive | antipyretic | GI motility

ABSTRACT

Background: *Swintonia floribunda*, a member of the Anacardiaceae family, is a traditional medicinal plant in Bangladesh. The present study aimed to identify potential therapeutic agents from *Swintonia floribunda* leaves. Accordingly, we evaluated the methanolic extract of *Swintonia floribunda* leaves (MESFL) for anti-inflammatory, antinociceptive, antipyretic, and antidiarrheal activities using both in vitro and in vivo approaches.

Methods: The anti-inflammatory effect of MESFL was determined using protein denaturation and membrane stabilization assays. Antinociceptive activity was assessed in mice using acetic acid-induced writhing, formalin-induced paw-licking tests, and hot plate and tail immersion methods. The yeast-induced pyrexia model was used to evaluate antipyretic activity, while castor oil-induced gastrointestinal motility was assessed to evaluate antidiarrheal potential. GC-MS analysis confirmed the presence of bioactive compounds. Molecular docking and ADMET studies were conducted to elucidate binding mechanisms, pharmacokinetics, and safety profiles.

Results: MESFL exhibited significant antipyretic activity in vivo and moderate antidiarrheal activity by reducing intestinal motility. Both acetic acid-induced writhing inhibition, suppression of paw licking, and increased latency period in both the hot plate and tail immersion methods confirmed its strong antinociceptive effect. Anti-inflammatory activity was evident through inhibition of protein denaturation and protection against red blood cell lysis. GC-MS and docking studies revealed several active phytoconstituents; among them, gamma-tocopherol demonstrated the strongest binding affinities to COX-2 (PDB Code: 1CX2) and 5-lipoxygenase (PDB Code: 5Y3Z), while benzene derivatives showed interactions with heat shock protein 90 α (PDB Code: 4U14)

Farjana Akter Nipa and Mst. Riniara Khatun contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Copyright © 2026 Farjana Akter Nipa et al. *Journal of Food Biochemistry* published by John Wiley & Sons Ltd.

and voltage-gated sodium channel Nav1.7 (PDB Code: 4YK5). ADMET analysis confirmed favorable pharmacokinetic and safety characteristics, supporting the drug-likeness of the compounds.

Conclusion: MESFL exhibits notable anti-inflammatory properties, along with significant antinociceptive and antipyretic activities and moderate antidiarrheal potential. These findings highlight its promise as a natural source of novel therapeutic agents.

1 | Introduction

Natural sources of medications have been used for the treatment and recovery of various diseases since ancient times. Many plants with medicinal value have been studied for their various properties. Traditional plants are excellent sources of modern medicines and could lead to valuable therapeutic alternatives [1]. People in developing countries, particularly those with low incomes and living in rural areas, rely on traditional plants as the primary means of counteracting and curing illnesses [2].

Swintonia floribunda Griff is a medicinal plant that typically grows up to 30 m tall but can reach heights of 45 m. The trunk can be 50–90 cm in diameter, rarely with erect plank buttresses up to 2 m high. The plant's bark was processed to make juice, which is used as a CNS stimulant, and a paste of its leaves is also used [3]. The leaves and bark of this plant are used to ward off insects [4]. The plant is also characterized by a naturally high content of phenolic compounds, flavonoids, and other antioxidant-enriched phytochemicals, which are typically associated with dietary antioxidant potential and contribute to the overall nutritional quality of many wild species. In addition to these secondary metabolites, the leaf matrix contains primary nutrients such as carbohydrates and proteins, suggesting that, once edible parts are established, *Swintonia floribunda* could provide both energy-yielding macronutrients and protective micronutrient-like components relevant to functional food applications [5].

In considering the medicinal properties of such plants, it is essential to note that nociception is a localized or widespread unpleasant sensation that causes varying degrees of physical discomfort and emotional distress, often resulting from bodily discomforts (such as injury or illness). It arrives in diverse forms: intense, refractory, visceral, irritant, or neuropathic [6]. Pain-killers derived from traditional medicinal plants have been discovered and utilized to alleviate pain, as they contain various effective metabolites responsible for antinociceptive activity [7].

Alongside the relief of discomfort, another key aspect of traditional medicine is its role in reducing fever. Pyrexia is also known as a rise in body temperature above the normal range. An antipyretic is a substance that minimizes fever. Antipyretics influence the hypothalamus to counteract prostaglandin-induced temperature elevation, and most antipyretics act by suppressing the enzyme cyclooxygenase, as well as reducing the levels of PGE₂ within the hypothalamus [8].

In addition to pain and fever, plant-based medicines are often used to address digestive concerns such as diarrhea. Diarrhea is a loose, watery, and probably more frequent bowel movement and is a very common problem. Sometimes, it may be associated with other syndromes, such as nausea, vomiting, abdominal pain, or weight loss [9]. The antidiarrheal action may be attributed to secondary phytochemicals, and this study suggests the plant's use in treating diarrhea [10].

Extending beyond gastrointestinal relief, inflammation is a host defense mechanism and a fundamental process that enables organisms to react to pathogenic stimuli [11]. It suggests an important process as it provides a favorable physiology to attack harmful pathogens or stimuli. However, the process must be maintained as uncontrolled inflammation is dangerous for pathophysiological states like cancer [12]. There is compelling evidence indicating that the resistance of the cyclooxygenase enzyme is the key mechanism underlying the anti-inflammatory properties of nonsteroidal anti-inflammatory drugs (NSAIDs). A significant percentage of the rural people in tropical Africa and China found themselves dependent on traditional herbal treatments due to expense, limited availability, and adverse effects of contemporary synthetic pharmaceuticals [13]. In this regard, extensive research has been conducted on plant-based anti-inflammatory agents, and the findings appear favorable due to their fewer adverse effects compared to synthetic medications [14].

Therefore, based on the limited literature available and a local survey, the leaves of this plant were chosen for the evaluation of in vivo antipyretic, antidiarrheal, antinociceptive, and in vitro anti-inflammatory activities.

2 | Methodology

2.1 | Chemicals and Drugs

Chemicals, which were an essential part of our investigation, were purchased from the following sources: normal saline, diclofenac sodium, and indomethacin, which was acquired from a well-known pharmaceutical company in Bangladesh. All of the chemicals, which were of an analytical grade, were purchased from a supplier located in the immediate vicinity.

2.2 | Plant Identification, Collection, and Extraction

In January 2023, we carefully selected and harvested approximately 2 kg of fresh *Swintonia floribunda* leaves from the Hazarikhil Wildlife Sanctuary (22°40'00"N and 91°40'00"E) in the afternoon, located in Fatikchari, Chittagong, Bangladesh. This specific location was chosen for its unique environmental conditions, which contribute to the distinct properties of the plant [15]. A renowned botanist (Prof. Dr. Shaikh Bokhtear Uddin) carried out the authentication and identification of the sample. A specimen voucher (Accession No. FA-16012024-0012) was kept at the Flora of Bangladesh Chittagong University Herbarium Center for further analysis. The gathered leaves were meticulously cleaned before being dried under the shed for 30 days at room temperature. After combining in a commercial blender and filtering through test sieve No. 50 (Endecotts, UK), a fine powder was obtained. For 10 days, powder (290 g) was macerated in 750 mL of solvent (methanol) while being

periodically shaken. After that, the mixture of solvents was filtered using Whatman filter paper and a new cotton plug [16]. After that, we obtained a crude methanol extract of *Swintonia floribunda* using a hot water bath at 45°C. The yield value of the methanolic extract was 5.11% by weight. The animals were given a solution containing dried extracts and 1% Tween 80 in distilled water.

2.3 | Acute Toxicity Study

The study of the acute toxicity of the methanol extract of *Swintonia floribunda* was undertaken to identify the proper dose, in accordance with the OECD standards. The extract was administered orally at increasing dosages of 500, 1000, and 2000 mg/kg. The individuals were monitored for 48 h to detect any changes in behavior, toxicity, or death. There were no deaths or other acute adverse effects identified. The present research employed dosages of 200 and 400 mg/kg body weight to investigate analgesic and CNS depressive effects. The effective dosage will be lower than the median lethal dose (LD₅₀).

2.4 | Animals Used in Experiments

For this study, Swiss albino mice, 23–30 g and 6–7 weeks old, were collected from Rajshahi, Bangladesh. During their 14-day adaptation period, the mice were subjected to a temperature of 25 ± 2°C, a 12 h light/dark cycle, fresh water, and food pellets [17]. Every behavioral session was run from 8.30 a.m. to 2 p.m., and the animals were observed for an hour following the conclusion of each trial. All experimental protocols were reviewed and approved by the Planning and Development (P & D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh, with the ethical approval no IIUC/Nipa-Pharm2023 for animal use. For the human participant Planning and Development (P & D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh also gave the ethical approval with the reference no IIUC-Pharm-2023/045. Following treatments, the animals were anesthetized with a controlled, gradual-fill inhalation of carbon dioxide 20%–30% of the total chamber air volume and subsequently euthanized.

2.5 | Treatment Design

At random, we used four groups, each containing three animals: one serving as a control, one receiving standard medicine, and two serving as test subjects (MESFL at 200 and 400 mg/kg, orally). As standard drugs, diclofenac sodium (10 mg/kg b.w., orally) was used for pain relief and inflammation tests, indomethacin (4 mg/kg b.w., IP) was used for fever tests, and loperamide (5 mg/kg b.w., orally) was used for diarrhea tests. A 1% solution of Tween-80 was administered to the negative control group.

2.6 | Qualitative Analysis of Phytochemicals

For the purpose of preparing a stock solution, 1 g of MESFL was dissolved in 100 mL of methanol. A standard methodology was used to conduct a qualitative phytochemical study on the stock solution [18]. The purpose of this examination was to determine which secondary metabolites are present, namely, terpenoids, tannins, phenols, glycosides, flavonoids, carbohydrates, quinones, saponins, and steroids.

2.7 | Antidiarrheal Activity

2.7.1 | Gastrointestinal Tract (GIT) Motility

MESFL was investigated for its potential impact on GIT motility by Ahmad et al., with a modest modification [17]. We started by giving each mouse half a milliliter of castor oil. Following 60 min, mice were randomly assigned four groups according to the treatment design in Section 2.4 and weighed between 20 and 25 g. Following a 60-min treatment period, mice received a 0.5-mL oral aqueous charcoal suspension [19]. The flow of charcoal was measured in the small intestine, and the reduction of GI motility was calculated by applying a formula that is presented below. In this equation, LI stands for the length of the small intestine, while TC shows the total distance traveled by charcoal

$$\% \text{GIT motility} = \left[\frac{(\text{LI} - \text{TC})}{\text{LI}} \right] \times 100. \quad (1)$$

2.8 | Antipyretic Activity

To find out how well the methanol extract of *Swintonia floribunda* lowered body temperature, in order to produce pyrexia in mice, we administered Brewer's yeast (10 mL/kg, bw) through subcutaneous injection [20, 21]. Despite being granted unlimited access to water, the animal models in this study were prohibited from consuming sustenance overnight before the experiment. Using a digital thermometer, initial rectal temperatures were recorded. Mice were only chosen for the evaluation of their antipyretic properties if their rectal temperature had risen by 0.3–0.5°C within 18 h of receiving a subcutaneous injection of a 2% yeast solution. In accordance with the protocol for the treatment design, this experiment used four different mouse groups. Finally, a digital thermometer was used to record the rectal temperature at 0, 1, and 3 h following administration of the substance. The results were subsequently tabulated.

2.9 | Antinociceptive Activity

2.9.1 | Acetic Acid-Induced Writhing Test

The antinociceptive action of *Swintonia floribunda* was assessed following the established technique [17, 22]. In accordance with the protocol for the treatment design, this experiment used four different mouse groups. Thirty minutes later, acetic acid (1%) had been administered via intraperitoneal injection. For the subsequent 15 min following the acetic acid injection, abdominal writhes (contractions) were assessed.

2.9.2 | Formalin-Induced Paw-Licking Test

For this investigation, four distinct mouse groups were used in accordance with the experimental treatment design procedure. In the 30 min preceding the investigation, the animals were administered MESFL and standard medication, diclofenac sodium. The animals were given 20 µL of a solution containing formalin (2.5%) in NaCl solution. Then, this solution was administered to the underside of the paw (right hind), specifically to the ventral surface. As a marker of nociception, the licking time (sec) of the injected paw was noted. The animals were monitored during both the phase of inflammation (which lasted 15–30 min) and the neurogenic phase (which lasted the first 5 min) [23].

2.9.3 | Hot Plate Test

The antinociceptive activity of MESFL was evaluated using the hot plate test, which was conducted in accordance with the method of Eddy and Leimbach with some slight modifications [24–26]. The surface of Eddy's hot plate was maintained at a steady temperature of $55.0 \pm 0.5^\circ\text{C}$. Mice were divided into groups of three and given either a vehicle (1% Tween-80 in distilled water, given orally) or MESFL (200 and 400 mg/kg, given orally) 30 min before the experiment began. The mice had fasted the night before. Administered 15 min before the experiment, morphine sulfate acted as a positive control (5 mg/kg, i.p.). At 0, 30, 60, 90, and 120 min after being placed on the heated surface, the mice that had been treated were observed for their latency time to a nociceptive reflex, which could be characterized as forepaw licking, paw withdrawal, or jumping. To prevent damage to the paw tissue and ensure complete analgesia, a cutoff contact time of 20 s was employed. To double-check that the opioid receptor was involved, the technique was performed 15 min before administering morphine or MESFL with naltrexone hydrochloride (2 mg/kg, intraperitoneally).

2.9.4 | Tail Immersion Test

This experiment followed a previously described protocol in an effort to identify MESFL's potential central antinociceptive effects [27]. The temperature of the water bath was maintained at $55 \pm 0.5^\circ\text{C}$, allowing the mice to dip their tails in it. There were three groups of mice, each given a different treatment: a control group ($n = 6$), a standard medicine group (group II) that contained 5 mg/kg of morphine administered intraperitoneally, and test groups III and IV, which included 200 and 400 mg/kg of MESFL administered orally, respectively. Following that, a small portion, around 1–2 cm, of each mouse's tail was submerged in hot water. It was thought that the tail's abrupt withdrawal from the hot water was a reaction to pain. Reaction timings were noted for the length of duration of tail immersion as well as tail deflection at 0, 30, 60, 90, and 120 min following administration of all test samples. A maximum submersion time of 20 s was used to avoid tail tissue damage in mice. The procedure was identical to that of the hot plate test, with the addition of an intraperitoneal injection of naltrexone hydrochloride (2 mg/kg) before the trial began.

2.10 | In Vitro Anti-Inflammatory Activity

2.10.1 | Protein (Egg Albumin (EA)) Denaturation Assay

The anti-inflammatory property of *Swintonia floribunda* was evaluated by reducing the breakdown of EA using previously published methodologies with some alterations [28]. The reaction mixture, which consisted of 5 mL, contained 0.2 mL of EA, 2.8 mL of PBS (pH 6.4), and 2 mL of plant extract with varying concentrations of 31.25, 62.5, 125, 250, and 500 $\mu\text{g/mL}$. In the positive control, extract solutions were substituted with diclofenac sodium solutions. In the negative control, the extract solution was substituted with distilled water. After 15 min at $37 \pm 2^\circ\text{C}$, the final solutions were heated to 70°C in an incubator for 5 minutes. After the temperature was lowered to 30°C , the absorbance was measured at 660 nm. The method for estimating the % inhibition of protein denaturation is provided by Chandra et al. [29].

2.10.2 | Evaluation of the Membrane-Stabilizing Activity of MESFL

We conducted this to ascertain the effectiveness of *Swintonia floribunda*. As previously documented by Umukoro et al. and Ansari et al., the membrane-stabilizing effects of *Swintonia floribunda* were evaluated using a hypotonic solution to induce inhibition of HRBC hemolysis [30, 31]. Written informed consent was obtained from the participant with reference no IIUC-Pharm-2023/045. The human blood samples were combined with an equal volume of sterilized Alsever's solution, which was subsequently centrifuged. The packed cells underwent a total of three washes with iso saline (pH 7.2) to remove any remaining residue. A further 2 mL of hypotonic solution in 1 mL of saline (sodium phosphate buffer) with a pH of 7.4 was added to the RBC suspension (0.5 mL). Additionally, 0.5 mL of MESFL (31.25–500 $\mu\text{g/mL}$) or diclofenac sodium-10 (31.25–500 $\mu\text{g/mL}$) was added to the mixture. For the control, we used 0.5 mL of red blood cells (RBCs) that had been completely combined with a hypotonic-buffered saline solution. After 30 min of incubation at 37°C , the mixtures were centrifuged, and the absorbance of the supernatant was measured at 560 nm. The method for estimating the % inhibition of hemolysis is provided in a previously published article [32].

2.11 | GC-MS Analysis

The Shimadzu GC-MS-QP2020 was employed to perform the GC-MS analysis of MESFL using an RTX-5 MS capillary column with dimensions of $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ [33]. The carrier gas, helium (99.99%), was introduced at a rate of 1.72 mL/min. It all started with a 2-min preheating of the oven to 80°C . Afterward, the temperature was increased to 150°C for 3 min at a rate of 5°C per minute. Finally, the temperature was increased to 280°C for 5 min at a rate of 5°C per minute. Afterward, at a temperature of 220°C , a 1- μL sample was injected using splitless mode. An ion source with an energy level of 70 eV and a temperature of 280°C was maintained throughout the procedure using electron-impact ionization. To conduct broad-based profiling, the 254-nm TIC peaks were examined. Chemicals with mass spectra ranging from 45 to 350 m/z were identified using the NIST08 database, LIB database.

2.12 | ADMET Analysis

The quantitative study of a drug's ADME (absorption, distribution, metabolism, and excretion) characteristics, including dose and time, is known as pharmacokinetics. To optimize lead compounds into clinically viable therapies, computational drug design relies on early evaluation of pharmacokinetic parameters [34]. The Swiss-ADME web tool (<http://www.swissadme.ch/index.php>) was used to characterize the pharmacokinetic profiles of the selected drugs [35]. Drug development pipelines can benefit from this computational platform's ability to visualize and predict important pharmacokinetic and pharmacodynamic features.

2.13 | PASS Prediction

Based on the structural formulas of organic compounds, the PASS Online tool (<http://www.way2drug.com/passonline>) is a publicly available resource that predicts the biological activity spectra of organic compounds for over 4000 different types of biological activities [36]. This method utilizes structure–activity relationship

(SAR) analysis, which is based on a training set of approximately 300,000 compounds with known structures and biological activities, to achieve an accuracy of over 95%. By allowing researchers to prioritize substances for chemical synthesis and biological testing, the platform provides guidelines for evaluating prediction results. The practical relevance of PASS Online in drug discovery has been highlighted by Bezhtentsev et al. who noted that it helped identify novel compounds with pharmacological activity. In the field of medicinal chemistry and pharmacology, PASS Online will continue to be an indispensable tool for multidisciplinary research due to its strong prediction capabilities [37].

2.14 | Protein Retrieval and Preparation

Based on structural resolution ($\leq 2.5 \text{ \AA}$), biological significance, and literature evidence, this work focused on four critical therapeutic targets: μ -opioid receptor (PDB Code: 5Y3Z), cyclooxygenase-2 (PDB Code: 1CX2), heat shock protein 90 α (PDB Code: 4U14), and voltage-gated sodium channel Nav1.7 (PDB Code: 4YK5). PyMOL 2.4.1 was used to create high-resolution crystal structures from the RCSB PDB [38] by eliminating crystallographic water and heteroatoms. The structural analysis of cocrystallized ligands was performed using BIOVIA Discovery Studio version 19.1, which enabled the identification of binding sites and key interaction patterns. These scientifically confirmed sites ensured biologically meaningful spatial limitations [39], thereby guiding the construction of receptor grids in PyRx for subsequent docking studies.

2.15 | Molecular Docking

A molecular docking study was conducted using PyRx [40] to find compounds that could potentially bind to the target proteins. For virtual screening of extensive compound libraries against biological macromolecules, PyRx incorporates AutoDock 4 and AutoDock Vina, two open-source computational platforms. The docking technique was performed using AutoDock Vina, with the parameters set to their default conditions. Ligand preparation involved energy minimization in the MMFF94 force field using PyRx's conjugate gradient method, followed by conversion to PDBQT format. Centered at coordinates (18.81, 25.75, 14.27 $\text{\AA}$), a grid box with size $58.56 \times 53.92 \times 66.13 \text{ \AA}$ covered the whole binding region of both protein targets and ligands for the docking. This arrangement guaranteed that all potential binding positions were thoroughly sampled during the virtual screening procedure.

2.16 | Statistical Analysis

In order to analyze the data, the mean and standard error of the mean (SEM) were calculated. To make statistical judgments, Tukey's test was performed using a two-way analysis of variance (ANOVA). A statistically significant difference was reported between test values and the control group at the levels of "a" $p < 0.0001$, "b" $p < 0.001$, "c" $p < 0.01$, and "d" $p < 0.05$. GraphPad Prism (Version 10.2.3), and Microsoft Excel (2021) were employed to conduct statistical analysis.

3 | Results

3.1 | Phytochemical Screening

Following the completion of the qualitative phytochemical screening of MESFL, various compounds were identified, as

TABLE 1 | Results of qualitative phytochemical screening for MESFL.

Constituents	Test	Observation
1. Alkaloid	a. "Mayer" test	+
	b. "Wagner" test	+
2. Reducing sugar	a. "Fehling's" test	—
3. Protein and amino acids	a. "Xanthoproteic test"	+
4. Flavonoids	a. "Lead acetate test"	+
5. Phenolic	a. "Iodine test"	+
	b. "Ferric chloride test"	+
6. Carbohydrate	a. "Molish" test	+
7. Tannins	a. "10% NaOH" test	+
8. Phlobatannins	a. "HCL test"	—
9. Phytosterols	a. "Acetic anhydride" test	—
10. Coumarins	a. "NaOH" test	+
11. Steroids	a. "Salkowski" reaction	+
12. Cholesterol		—
13. Terpenoids		—
14. Saponins	a. "Frothing" test	+

Note: "+" represents present, while "—" represents absence.

shown in Table 1. The components involved included alkaloids, protein and amino acids, flavonoids, phenolic compounds, tannins, saponins, coumarins, carbohydrates, and steroids, while reducing sugars, phlobatannins, phytosterols, cholesterol, and terpenoids were absent (Table 1).

3.2 | Effects of MESFL on In Vivo Antidiarrheal Test

The antidiarrheal efficacy of MESFL on intestinal motility, as evidenced by the charcoal marker, demonstrated a considerable decrease in the peristalsis. The peristalsis index of the control, loperamide, MESFL-200, and MESFL-400 was observed to be 88.31 ± 1.75 , 50.13 ± 4.09^a , 65.17 ± 2.63^b , and 63.75 ± 6.17^b , respectively (Table 2). As shown in Figure 1, both MESFL doses produced a clear reduction in peristalsis compared to the control group. The 400-mg/kg dose of MESFL exhibited a $25.37 \pm 2.69\%$ inhibition of GI motility, near that of loperamide ($40.3 \pm 3.94\%$). In contrast, the 200-mg/kg dose of MESFL demonstrated a $17.91 \pm 3.25\%$ reduction of intestinal motility.

3.3 | Impact of MESFL on In Vivo Yeast-Induced Pyretic Assay

The rectal temperature was notably elevated following the subcutaneous administration of brewer's yeast solution for 18 h. A considerable ($p < 0.001$) dose-dependent reduction of pyrexia was recorded in mice administered indomethacin-4 and MESFL (200 and 400 mg/kg) when compared with the control group (Table 3). The inhibition rate of MESFL 400 mg/kg was nearly identical to that of indomethacin-4 (standard drug), as evidenced

TABLE 2 | Antidiarrheal activity of MESFL by castor oil-induced diarrhea test (charcoal marker).

Treatment (mg/kg)	Total length of intestine (cm)	Distance travel by charcoal (cm)	Peristalsis index (%)	Inhibition (%)
Control	50.67 ± 2.33	44.67 ± 1.20	88.31 ± 1.75	—
Loperamide (5)	53.33 ± 0.88	26.67 ± 1.76	50.13 ± 4.09 ^a	40.3 ± 3.94 ^a
MESFL 200	53 ± 4.04	33.33 ± 1.20	65.17 ± 2.63 ^b	17.91 ± 3.25 ^c
MESFL 400	56.33 ± 2.02	36.67 ± 1.45	63.75 ± 6.17 ^b	25.37 ± 2.69 ^a

Note: All the values are presented as mean ± SEM ($n = 6$); two-way analysis of variance (ANOVA) followed by Tukey's test. ^a $p < 0.05$, ^c $p < 0.01$, ^b $p < 0.001$, and ^a $p < 0.0001$ are considered significant compared with the control.

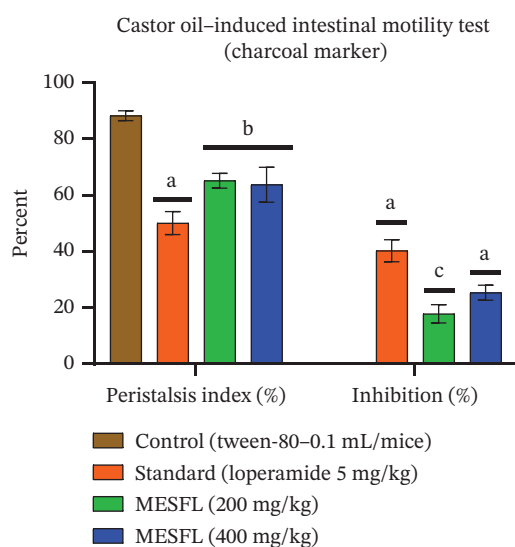
Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

by the most effective inhibition of pyrexia. As shown in Figure 2, both MESFL doses produced a gradual and time-dependent decline in rectal temperature after yeast-induced pyrexia. The 400-mg/kg group demonstrated the most prominent reduction at both 60 and 180 min, closely mirroring the temperature-lowering pattern of the standard drug.

3.4 | Effect of MESFL on In Vivo Antinociceptive Activity

3.4.1 | Acetic Acid-Induced Writhing Test

MESFL has a decreasing effect on the frequency of writhing in mice produced by acetic acid in a dose-dependent manner. Figure 3 illustrates the average frequency of writhing and the percentage of inhibition expressed by all test samples. MESFL slowed down the writhing reaction ($F(3, 16) = 20.4$; $p < 0.0001$), which is produced by acetic acid (Table 4). The average writhing inhibitions by both doses of MESFL were 25.64% and 56.41%, while diclofenac sodium-10 yielded 69.23%. There was a significant decrease in both MESFL concentrations ($p < 0.001$). However, a higher dose of MESFL is comparable to the standard drug diclofenac sodium. Figure 3 demonstrates a clear dose-dependent reduction in writhing, with MESFL-400 producing a substantial decline in nociceptive responses. The inhibition profile of the 400-mg/kg group closely aligns with that of diclofenac, highlighting the strong analgesic potential of the extract.

**FIGURE 1** | Castor oil-induced antidiarrheal test of MESFL.

3.4.2 | Formalin-Induced Paw-Licking Test

There is a dose-dependent relationship between the MESFL and the suppression of licking in the formalin-induced licking test. This suppression occurs in the early and late phases. The inhibition of paw licking that occurs on average during both phases is depicted in Table 5. In the early phase, the average percentage of hind paw inhibitions was 57.76% and 69.57%, while in the late phase, the percentages were 29.5% and 52.5% at doses of MESFL-200 and MESFL-400 mg/kg, respectively. On the other hand, it has been confirmed that the average percentage reduction by diclofenac-10 is 74.53% in the early phase and 67.5% in the late phase (Table 5). With a significant ($p < 0.001$) inhibition rate, MESFL-400, which is comparable to the standard medicine diclofenac sodium-10, measured the highest level of inhibition. As depicted in Figure 4, both MESFL doses attenuated paw-licking responses in the early and late phases. MESFL-200 mg/kg exhibited a meaningful reduction, whereas MESFL-400 mg/kg produced a substantially stronger inhibition, nearly matching the standard analgesic.

3.4.3 | Hot Plate Test

The hot plate test was used to assess analgesic activity, and the data were analyzed using two-way ANOVA ($\alpha = 0.05$). The study demonstrated a notable interaction between treatment groups and time ($F(12, 39) = 7.38$, $p < 0.0001$), indicating that treatment effects varied across the analysis intervals. After conducting post hoc comparisons, it was shown that the standard group displayed significantly longer reaction latencies at every time point. The response latencies for the standard group were as follows: 0 min, 8.33 ± 0.88 ; 30 min, 10.33 ± 0.67 ; 60 min, 14 ± 1.0 ; 90 min, 15 ± 0.58 ; 120 min, 19.67 ± 1.45 . Also, MESFL-200 mg/kg (10 ± 0.57 at 60 min, 11.33 ± 0.33 at 90 min, and 13.33 ± 0.33 at 120 min) and MESFL-400 mg/kg (8.33 ± 0.67 at 30 min, 10.67 ± 1.45 at 60 min, 12.33 ± 0.33 at 90 min, and 15 ± 0.58 at 120 min) showed increasing levels of analgesia that were dose-dependent, with significant levels ($p < 0.05$) compared to the control (Table 6). After 120 min, the higher dose of MESFL (400 mg/kg) had nearly the same effectiveness as the conventional dose ($15 \pm 0.58^{****}$ vs. $19.67 \pm 1.45^{****}$). These results confirm the strong, dose-responsive analgesic properties of MESFL, with statistical significance rigorously maintained over all critical intervals that are clearly illustrated in Figure 5.

3.4.4 | Tail Immersion Test

The findings of the tail immersion test indicated that MESFL had significant analgesic effects, which were dependent on both dose and time (two-way analysis of variance: interaction $F(12, 40)$

TABLE 3 | In vivo antipyretic activity of MESFL by brewer's yeast.

Treatment	Dose	Normal rectal temperature (°F)	Rectal temperature (°F) after yeast administration	Rectal temperature (°F) after drug administration	
				60 min	180 min
TWN-80	10 (mL/kg; p.o)	97.30 ± 0.15	102.067 ± 0.38	101.65 ± 0.09	101.133 ± 0.09
IDM	4 (mg/kg-B. w; i.p)	97.733 ± 0.09	101.133 ± 0.23	99.1 ± 0.12 ^a	96.033 ± 0.15 ^a
MESFL	200 (mg/kg; p.o)	97.33 ± 0.52	100.53 ± 0.21	99.8 ± 0.25 ^c	98.53 ± 0.29 ^a
MESFL	400 (mg/kg; p.o)	96.63 ± 0.59	100.13 ± 0.54 ^c	98.1 ± 0.12 ^a	97 ± 0.12 ^a

Note: All the values are presented as mean ± SEM ($n = 6$); two-way analysis of variance (ANOVA) followed by Tukey's test. ^a $p < 0.05$, ^c $p < 0.01$, ^b $p < 0.001$, and ^a $p < 0.0001$ are considered significant compared with the control.

Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

= 8.27, $p < 0.0001$). A progressive analgesia was observed in the standard treatment, reaching significance at 60 min (7 ± 1 s), 90 min (9 ± 0.58 s), and peaking at 180 min (9.33 ± 0.33 s), in contrast to the control group that maintained consistent response latencies throughout the observation period (0 min: 1.33 ± 0.33 s; 30 min: 1.33 ± 0.33 s; 60 min: 1.67 ± 0.33 s; 90 min: 1.33 ± 0.33 s; 180 min: 1 ± 0 s). Figure 6 provides a clear representation of these time-dependent differences among the groups. On the other hand, the MESFL-400 group revealed an earlier onset at 30 min (4 ± 0.58 s), with rising efficacy up to 180 min (8.67 ± 0.33 s) (Table 7). The MESFL-200 group exhibited significant effects beginning at 60 min (5.67 ± 0.33 s) and continuing through 180 min (6.33 ± 0.33 s). MESFL-400 notably confirmed its strong dose-dependent action by reaching 93% of the peak impact within 180 min (8.67 ± 0.33 s vs 9.33 ± 0.33 s).

3.5 | Effect of MESFL on In Vitro Anti-Inflammatory Activity

3.5.1 | EA Denaturation

Figure 7 provides a visual representation of the percentage of protein denaturation inhibition, highlighting the protective efficacy of the MESFL alongside the standard anti-inflammatory agent. Diclofenac sodium prescribed standard medicine, prescribed medication, demonstrated $43.88 \pm 1.282\%$, $47.66 \pm$

2.659% , $69.01 \pm 5.300\%$, $73.44 \pm 0.983\%$, and $76.56 \pm 1.479\%$ prevented the denaturation of EA at concentrations of 31–500 $\mu\text{g/mL}$, accordingly while *Swintonia floribunda* Leaves exhibited $42.97 \pm 0.597\%$, $47.66 \pm 0.677\%$, $56.51 \pm 2.034\%$, $58.85 \pm 1.584\%$, and $62.50 \pm 2.289\%$ prevention of the denatured state of EA (Table 8).

3.5.2 | Membrane Stabilization Method of MESFL

Figure 8 depicts the concentration-dependent membrane-stabilizing activity of MESFL, clearly demonstrating its protective effect against hypotonicity-induced hemolysis in comparison with the reference standard, diclofenac sodium. A hypotonic solution was used to promote hemolysis in human RBCs. According to the findings of the two-way ANOVA, there were significant differences between the treatment groups. Regarding hemolysis inhibition, the plant extract demonstrated $17.16 \pm 1.099\%$, $23.96 \pm 1.367\%$, $33.15 \pm 0.889\%$, $38.72 \pm 1.169\%$, and $44.30 \pm 1.397\%$ inhibition at concentrations of 31.25–500 $\mu\text{g/mL}$, respectively, while reference drug (diclofenac sodium) exhibited $18.82 \pm 1.408\%$, $24.35 \pm 0.555\%$, $35.26 \pm 2.258\%$, $42.35 \pm 0.565\%$, and $49.90 \pm 0.686\%$ inhibition of hemolysis (Table 9). MESFL and diclofenac sodium demonstrate significant inhibition of hemolysis at 125, 250, and 500 $\mu\text{g/mL}$ concentrations (Supporting Figure).

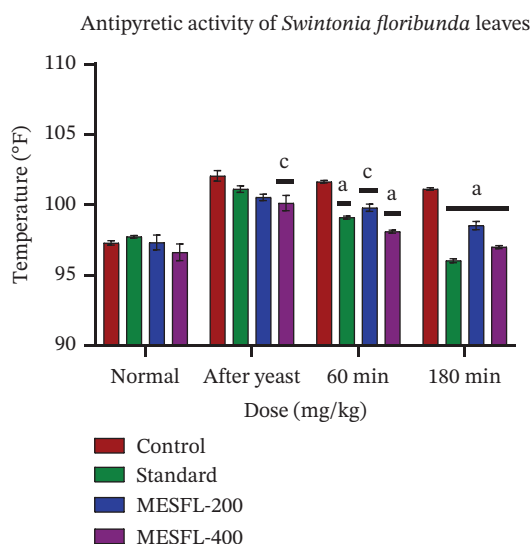
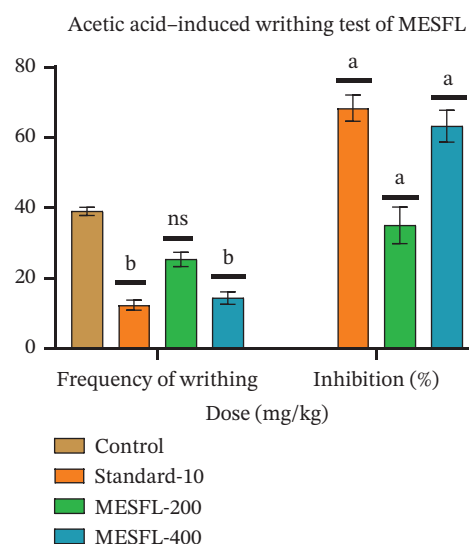
**FIGURE 2** | In vivo antipyretic activity of MESFL by brewer's yeast-induced method.**FIGURE 3** | Analgesic activity of MESFL by acetic acid-induced writhing test.

TABLE 4 | Analgesic activity of MESFL by acetic acid-induced writhing test.

Group	Dose (mg/kg- B. w)	Frequency of writhing (mean ± SEM)	Percent inhibition
Control		39.00 ± 1.15	—
Diclofenac-Na	10	12.33 ± 1.45 ^b	69.23 ^a
MESFL	200	25.33 ± 2.03	25.64 ^a
MESFL	400	14.33 ± 1.76 ^b	56.41 ^a

Note: All the values are presented as mean ± SEM (*n* = 6); two-way analysis of variance (ANOVA) followed by Tukey's test. ^a*p* < 0.05, ^c*p* < 0.01, ^b*p* < 0.001, and ^a*p* < 0.0001 are considered significant compared with the control.
Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

TABLE 5 | Formalin-induced paw-licking test of MESFL.

Groups	Dose (mg/kg)	Licking time (sec) (mean ± SEM)	
		1st phase (0–5 min)	Last phase (20–30 min)
Control (1% Tween)	—	53.67 ± 3.53	66.67 ± 2.96
Diclofenac-Na	10	13.67 ± 1.76 ^a	21.67 ± 2.03 ^a
MESFL	200	22.67 ± 1.415 ^a	47.00 ± 2.31 ^b
MESFL	400	16.33 ± 1.76 ^a	31.67 ± 1.45 ^a

Note: All the values are presented as mean ± SEM (*n* = 6); two-way analysis of variance (ANOVA) followed by Tukey's test. ^a*p* < 0.05, ^c*p* < 0.01, ^b*p* < 0.001, and ^a*p* < 0.0001 are considered significant compared with the control.
Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

3.6 | Results of GC-MS Analysis of MESFL

A wide variety of organic compounds were found in the MESFL through GC-MS analysis (Table 10 and Figure 9). These included alkoxysilanes (e.g., ethyl(dimethyl)ethoxy silane, 1.05%), aromatic alcohols (e.g., benzyl alcohol, 1.27%), unsaturated alcohols (e.g., 5-methyl-1,5-hexadien-3-ol, 1.12%), and organosilicon derivatives (e.g., trimethyl[2-methylene-1-(4-pentenyl) cyclopropyl]-, 0.10%). Saturated fatty acids such as n-hexadecanoic acid (palmitic acid, 12.65%) and octadecanoic acid (3.44%), as well as fatty acid esters such as methyl 10-trans,12-cis-octadecadienoate (1.22%) and methyl 7,11,14-eicosatrienoate (2.69%), were detected. Additionally, plasticizers (such as bis(2-ethylhexyl) phthalate, 0.73%), phenolic compounds (such as thymol, 0.10%), diterpenes (such as neophyte diene, 0.59%),

triterpenes (such as squalene, 1.52%), and vitamin E (γ-tocopherol, 3.64%) were found. The findings draw attention to the MESFL's complex chemical composition, which comprises a range of bioactive, structural, and industrially significant compounds.

3.7 | PASS Prediction

Their biological activity predictions showed the pharmacological potential of 15 compounds; thymol showed balanced activity across antipyretic (Pa: 0.524), anti-inflammatory (Pa: 0.549), analgesic (Pa: 0.584), and antidiarrheal (Pa: 0.244) properties (Table 11). Significant anti-inflammatory (*p* < 0.775) and moderate antipyretic (*p* < 0.324) effects were observed in hexanedioic acid (2-ethylhexyl) ester, whereas gamma-tocopherol displayed the most pronounced anti-inflammatory action. Among the compounds tested, 9-octadecenamide and eicosen-1-ol-9 showed the most promise as analgesics (Pa: 0.598 and 0.545, respectively), whereas octadecanoic acid and n-hexadecenoic acid had the same antipyretic, anti-inflammatory, analgesic, and antidiarrheal activity profiles (0.497, 0.526, 0.211, respectively). Particularly in the fields of anti-inflammatory and analgesic compounds, those with inadequate evidence (e.g., tetradecamethyl, phytol) still demonstrated selective activity. These findings reveal structurally varied options for additional experimental validation, which suggest that thymol and gamma-tocopherol may stand out as exciting multitarget agents.

3.8 | ADME/T Analysis

The study of 15 bioactive compounds using Lipinski's and Veber's rules, as well as bioavailability and toxicity parameters, showed that all the compounds met Lipinski's "violation ≤ 1" parameter, with molecular weights (MWs) ranging from 128.09 to 519.08 g/mol 9 (Table 12). However, tetradecamethyl (MW: 519.08 g/mol) and eicosen-1-ol-9 (Log *p*: 5.22) were found to exceed the ideal thresholds for molecular weight (MW < 500 g/mol) and Log *p* (≤ 5), respectively, while still exhibiting acceptable bioavailability (0.55).

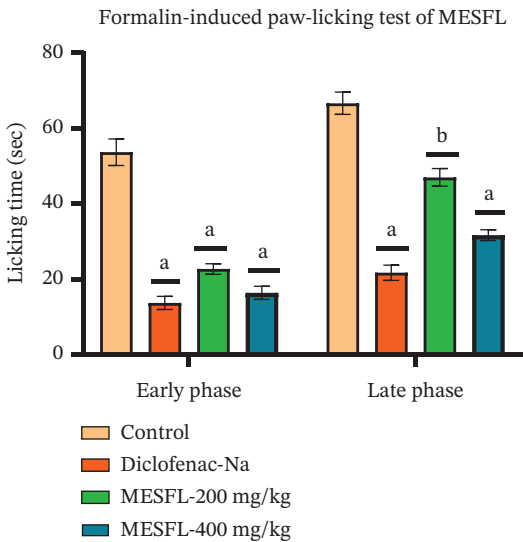


FIGURE 4 | Analgesic activity of MESFL by the formalin-induced paw-licking method.

TABLE 6 | Analgesic activity of MESFL by the hot plate test.

Group	0 min	30 min	60 min	90 min	120 min
Latent period of response time					
Control	1.33 ± 0.33	2.67 ± 0.67	2.67 ± 0.33	3 ± 0.58	2.67 ± 0.33
Standard	8.33 ± 0.88 ^a	10.33 ± 0.67 ^a	14 ± 1.0 ^a	15 ± 0.58 ^a	19.67 ± 1.45 ^a
MESFL-200	3 ± 0.58	5.33 ± 0.67	10 ± 0.57 ^a	11.33 ± 0.33 ^a	13.33 ± 0.33 ^a
MESFL-400	4.33 ± 0.88	8.33 ± 0.67 ^b	10.67 ± 1.45 ^a	12.33 ± 0.33 ^a	15 ± 0.58 ^a

Note: All the values are presented as mean ± SEM (n = 6); two-way analysis of variance (ANOVA) followed by Tukey's test. ^dp < 0.05, ^cp < 0.01, ^bp < 0.001, and ^ap < 0.0001 are considered significant compared with the control. Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

Aside from n-hexadecanoic acid and 9-octadecenamide, which showed higher rotational flexibility, the majority of compounds followed Veber's rules (nRotB ≤ 10, TPSA ≤ 140 Å²). Nevertheless, all of these compounds exhibited a favorable topological polar surface area (TPSA: 0–75.27 Å²), which supported membrane permeability. All compounds showed excellent human intestinal absorption (HIA > 0.9) and blood–brain barrier penetration (BBB: 0.73–0.99), indicating high bioavailability and potential central nervous system (CNS) activity. This, along with their lack of mutagenic or carcinogenic risks (Ames' toxicity: NAT) and acute oral toxicity classifications ranging from II (moderate) to IV (low), suggests that these compounds have drug-like properties and are suitable for further pharmacological investigation.

3.9 | Frontier Molecular Orbital (FMO) Evaluations

The DFT study revealed significant electronic differences between the two compounds (Table 13 and Figure 10). Compound-2 showed superior electron-donating capacity (higher HOMO: 0.0126 au vs 0.00388 au), and both compounds exhibited strong electron-accepting potential (negative LUMO energies). Compound-2's narrower energy

gap (−0.19583 au vs −0.20528 au) suggests greater reactivity and enhanced charge-transfer capabilities for biological interactions. While both compounds demonstrated thermodynamic stability (chemical potential: 0.09884 au and 0.085315 au), Compound-2's higher softness (11.72 vs 10.12 au^{−1}) indicates stronger noncovalent binding potential. Compound-1 displayed slightly greater electrophilicity (−0.0476 au vs −0.0372 au), suggesting better charge-transfer reactivity. These findings suggest that Compound-2 may exhibit improved bioavailability due to its favorable electronic profile, whereas Compound-1 may be more effective in charge-mediated interactions. The results provide crucial insights for SAR analysis and further optimization of these compounds for potential therapeutic applications.

3.10 | Molecular Docking Analysis of Selected Proteins and Ligands

Molecular docking studies of MESFL compounds revealed a diverse range of interaction patterns and strong binding affinities to key therapeutic targets (Table 14, Figures 11, 12, 13, and 14). With a binding energy of −7.6 kcal/mol against COX-2 (1CX2), gamma-tocopherol (92729) exhibited significant analgesic

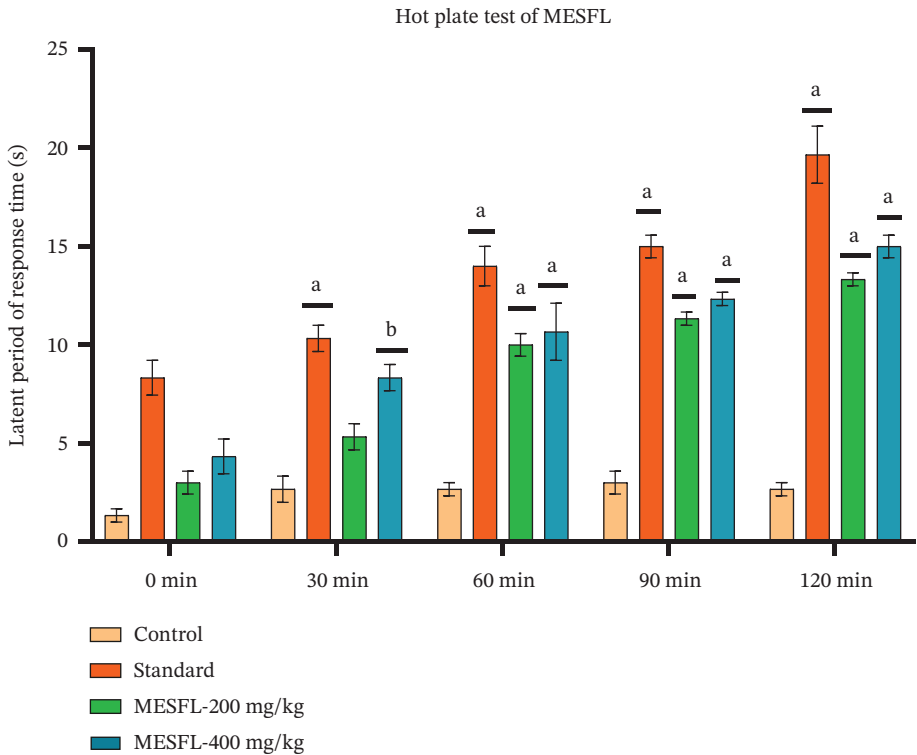


FIGURE 5 | Analgesic activity of MESFL by the hot plate method.

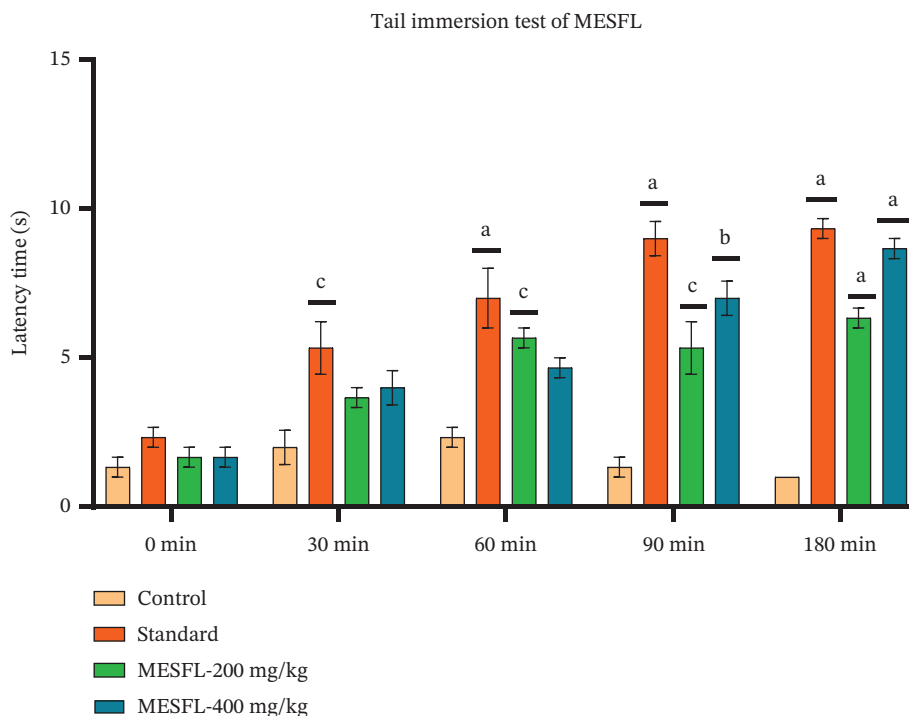


FIGURE 6 | Analgesic activity of MESFL by the tail immersion method.

potential, forming traditional hydrogen bonds with ASN39 and several hydrophobic contacts with CYS36, PRO153, and ALA156. It demonstrated remarkable binding (-7.9 kcal/mol) to 5-lipoxygenase (5Y3Z) through pi-sigma interactions with LEU264 and broad alkyl/pi-alkyl contacts with ALA194, VAL240, and MET242 for anti-inflammatory activity. In addition to its antidiarrheal activity (-7.9 kcal/mol against 4U14), the bisphenol derivative (61780) exhibited notable antipyretic activity (-5.8 kcal/mol against 4YK5) via carbon-hydrogen bonds with THR21, and it engaged in pi-donor hydrogen bonds with TYR506 and pi-sulfur interactions with CYS532. Comparative investigation using reference medications revealed competitive binding: gamma-tocopherol's COX-2 affinity (-7.6 kcal/mol) approached that of diclofenac (-7.4 kcal/mol), while the bisphenol's antidiarrheal binding (-7.9 kcal/mol) surpassed that of loperamide (-9.4 kcal/mol). Through hydrogen-bonding, pi-stacking (e.g., TRP502/TRP525), and alkyl interactions, other

compounds—including thymol (-6.5 kcal/mol for 1CX2) and phenolic derivatives—showed modest but consistent multi-target activity (Table 15). These findings, backed by comprehensive interaction maps at the residue level (e.g., HIS277 for 5Y3Z, TYR506/TRP503 for 4U14), demonstrate the polypharmacological promise of MESFL. They show that certain compounds can engage targets just as effectively as conventional medicines but with broader activity spectra. The therapeutic value of the extract for pain, inflammation, diarrhea, and fever regulation is underscored by its strong binding energies (< -5 kcal/mol across all targets) and varied interaction types (hydrogen bonds, hydrophobic contacts, π -effects).

4 | Discussion

A wide range of pathological disorders, such as fever, diarrhea, pain, and inflammation, have traditionally been treated with

TABLE 7 | Analgesic activity of MESFL by the tail immersion test.

	0 min	30 min	60 min	90 min	180 min
Latent period of response time (sec)					
Control	1.33 ± 0.33	1.33 ± 0.33	1.67 ± 0.33	1.33 ± 0.33	1 ± 0
Standard	2.33 ± 0.33	5.33 ± 0.88 ^c	7 ± 1 ^a	9 ± 0.58 ^a	9.33 ± 0.33 ^a
MESFL-200	1.67 ± 0.33	3.67 ± 0.33	5.67 ± 0.33 ^c	5.33 ± 0.8 ^c	6.33 ± 0.33 ^a
MESFL-400	1.67 ± 0.33	4 ± 0.58	4.67 ± 0.33	7 ± 0.58 ^b	8.67 ± 0.33 ^a

Note: All the values are presented as mean ± SEM ($n = 6$); two-way analysis of variance (ANOVA) followed by Tukey's test. ^d $p < 0.05$, ^c $p < 0.01$, ^b $p < 0.001$, and ^a $p < 0.0001$ are considered significant compared with the control

Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

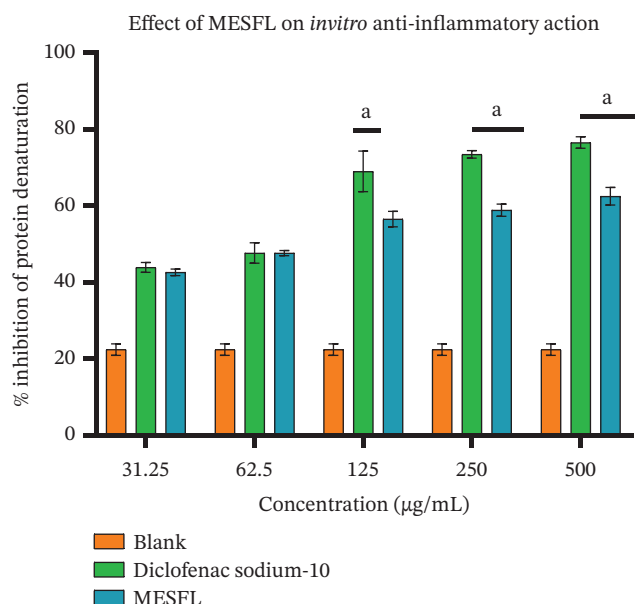


FIGURE 7 | Anti-inflammatory activity by the protein denaturation method.

medications derived from plants that contain therapeutically useful compounds. Furthermore, plants with medicinal properties are key resources for the creation of novel medications since it is speculated that these products include bioactive chemicals responsible for curing different ailments more effectively and economically without having adverse consequences [41].

Phytochemical groups such as phenolics, terpenoids, fatty acid derivatives, and steroid-like compounds are known to play key roles in mediating antidiarrheal, antipyretic, analgesic, and anti-inflammatory activities in medicinal plants. These bioactive molecules influence several molecular pathways, including the suppression of prostaglandins, modulation of cytokine release, attenuation of oxidative stress, and stabilization of cellular membrane mechanisms that align with the pharmacological actions evaluated in the present study [42]. In particular, steroidal and terpenoid constituents have been recognized for their capacity to inhibit protein denaturation and regulate enzymes involved in inflammatory cascades [43]. Furthermore, plant substances that are high in antioxidants show notable

TABLE 8 | In vitro anti-inflammatory activity by the protein denaturation method.

Concentration	Inhibition (mean ± SEM)	
	Diclofenac Na	MESFL
31.25	43.88 ± 1.282	42.97 ± 0.597
62.5	47.66 ± 2.659	47.66 ± 0.677
125	69.01 ± 5.300 ^a	56.51 ± 2.034
250	73.44 ± 0.983 ^a	58.85 ± 1.584 ^a
500	76.56 ± 1.479 ^a	62.50 ± 2.289 ^a

Note: All the values are presented as mean ± SEM ($n = 3$); two-way analysis of variance (ANOVA) followed by Tukey's test. ^d $p < 0.05$, ^c $p < 0.01$, ^b $p < 0.001$, and ^a $p < 0.0001$ are considered significant compared with the control. Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

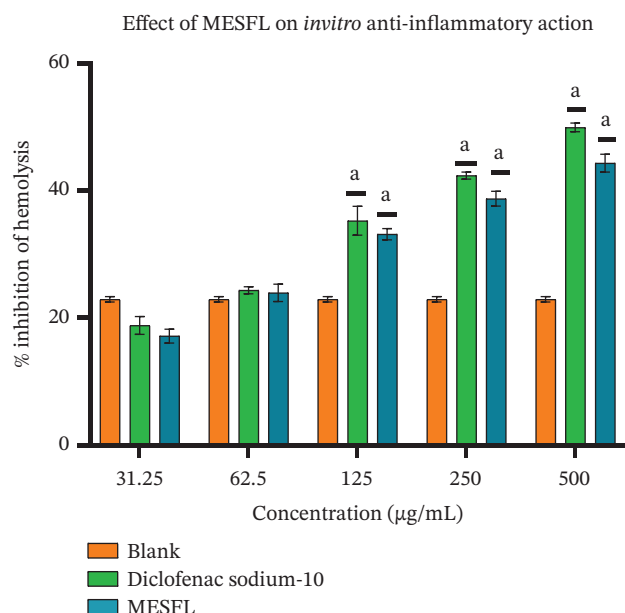


FIGURE 8 | Anti-inflammatory activity by the membrane-stabilizing method.

immunomodulatory and cytokine-reducing actions, underscoring their extensive therapeutic significance [44].

In this research, the plant MESFL is one of the better-reported plants, offering numerous benefits. It was assessed for its anti-diarrheal, antipyretic, antinociceptive, and anti-inflammatory actions. Research into the phytochemical composition of *Swintonia floribunda* has revealed a diverse array of phytochemicals, including steroids, alkaloids, carbohydrates, proteins, tannins, saponins, flavonoids, polyphenols, and coumarins. Researchers suggested that plant materials containing flavonoids, alkaloids, saponins, tannins, and phenolic acids are responsible for various pharmacological effects through opioid mechanisms, inhibition of arachidonic acid peroxidation, tumor necrosis factor α , and prostaglandin synthesis [45, 46].

In the current investigation, castor oil was used as an inducing agent to assess the in vivo antidiarrheal activity of the crude extract, which produces an irritating laxative effect due to the inadequate absorption of ricinoleic acid, released by intestinal lipases. Ricinoleic acid encourages prostaglandin synthesis from

TABLE 9 | In vitro anti-inflammatory activity by membrane stabilization assay.

Concentration	Inhibition (mean ± SEM)	
	Diclofenac Na	MESFL
31.25	18.82 ± 1.408	17.16 ± 1.099
62.5	24.35 ± 0.555	23.96 ± 1.367
125	35.26 ± 2.258 ^a	33.15 ± 0.889 ^a
250	42.35 ± 0.565 ^a	38.72 ± 1.169 ^a
500	49.90 ± 0.686 ^a	44.30 ± 1.397 ^a

Note: All the values are presented as mean ± SEM ($n = 3$); two-way analysis of variance (ANOVA) followed by Tukey's test. ^d $p < 0.05$, ^c $p < 0.01$, ^b $p < 0.001$, and ^a $p < 0.0001$ are considered significant compared with the control. Abbreviation: MESFL = methanol extract of *Swintonia floribunda* leaves.

TABLE 10 | Compound from MESFL through GC-MS.

Name of the compounds	Molecular weight	Retention time (RT)	% area	Nature
Ethyl(dimethyl)ethoxysilane	132	6.37	1.05	Alkoxysilane
Benzyl alcohol	108	7.63	1.27	Aromatic alcohol
5-methyl-1,5-hexadien-3-ol	112	9.42	1.12	Unsaturated alcohol
Trimethyl[2-methylene-1-(4-pentenyl)cyclopropyl]-	194	11.16	0.10	Organosilicon
Acetic acid, phenylmethyl ester	150	11.48	1.90	Organic ester
Trans-2-methyl-4-n-pentylthiane, S,S-dioxide	218	12.28	0.27	Sulfone
Octadecane-1,2-diol	287	14.84	0.49	Long-chain aliphatic diol
Thymol	150	16.27	0.10	Monoterpene phenol
Dodecamethyl	445	16.63	0.38	Cyclic siloxane
Phenol, 2,6-dimethoxy-	154	17.58	0.22	Methoxy-substituted phenol
Trans-2-methyl-4-n-pentylthiane, S,S-dioxide	218	18.67	0.07	Sulfur-containing heterocyclic compound
[(1,1-dimethyl-2-propenyl)oxy]dimethyl-	143	18.87	0.18	Organosilicon compound
Benzene, 1,1'-(1-methylethylidene)bis[4-methoxy-	256	19.42	4.50	Bisphenol derivative
Benzene, [(3-chloro-2-propenyl)oxy]-	168	20.86	0.38	Organic compound
2-methyl-3-o-tolyl-6-hydroxy-4 (3 h)quinazolinone	266	21.26	0.28	Quinazolinone derivatives
Tetradecamethyl-	519	21.99	0.69	Cyclic siloxane compound
2-methyl alcohol	72	22.42	7.83	Organic compound
D-gluco-hexodialdodifuranoside,	351	24.12	0.47	Monosaccharide
Tetrahydro-1-naphthylamine, n-heptafluorobutyl-	343	24.98	1.38	Organic intermediate
Phthalimidine	133	25.63	0.43	Bicyclic organic compound
2,4,6 (1 h, 3 h, 5 h)-pyrimidinetrione	128	26.70	1.02	Barbituric acid (organic compound)
Eicosen-1-ol, cis-9-	296	27.99	0.32	Long-chain fatty alcohol
Neophytadiene	278	31.21	0.59	Diterpene hydrocarbon
Phytol tetradecanoate	506	32.27	0.39	Saturated fatty acid
Tetradecanoic acid, 10,13-dimethyl-,methyl ester	270	33.37	5.50	Fatty acid ester
N-hexadecanoic acid	256	34.34	12.65	Saturated fatty acid (palmitic acid)
Ethane, 1,2-bis(1-phenylcyclopropyl)-	262	36.49	0.64	Organic compound
Methyl 10-trans,12-cisoctadecadienoate	294	37.28	1.22	Ester derivative of conjugated linoleic acid (CLA)
Methyl 7,11,14-eicosatrienoate	320	37.43	2.69	Ester of eicosatrienoic acid (polyunsaturated fatty acid ester)
Phytol	296	37.69	0.50	Acyclic diterpene alcohol
Heptadecanoic acid, 16-methyl-,methyl ester	298	38.01	0.92	Branched-chain saturated fatty acid methyl ester
Propyl 11-octadecenoate	324	38.31	4.03	Fatty acid ester
Octadecanoic acid	284	38.84	3.44	Saturated long-chain fatty acid

(Continues)

TABLE 10 | (Continued)

Name of the compounds	Molecular weight	Retention time (RT)	% area	Nature
(Z)-dec-4-enyl (e)-2-methylbut-2- enoate	238	41.97	0.40	Ester compound
Tetracontane-1,40-diol	594	42.25	0.77	Long-chain aliphatic diol
9-octadecenamide, (z)-	281	42.96	0.86	Fatty acid amide (Oleamide)
Hexanedioic acid, bis(2-ethylhexyl) ester	370	43.72	7.26	DEHA ((di (2-ethylhexyl) adipate)))
Cyclohexanone, 2-ethyl-4-methoxy-	156	44.30	0.59	Organic compound
(Z)-3-(pentadec-8-en-1-yl)phenol	302	45.36	1.25	Phenolic compound
Heptacosanoic acid, 25-methyl-,methyl ester	438	46.35	1.39	Long-chain fatty acid ester
Bis(2-ethylhexyl) phthalate	390	46.78	0.73	Plasticizer
Gamma-tocopherol	416	48.33	3.64	Vitamin E
3-((4z,7z)-heptadeca-4,7-dien-1-yl)phenol	328	50.01	1.08	Phenolic compound
Z)-3-(heptadec-10-en-1-yl)phenol	330	50.78	3.20	Phenol
Hentriacontane	436	50.99	3.12	Long-chain alkane hydrocarbon
Hexadecamethyl	533	51.66	1.07	Siloxane compounds
Methyl 18-fluoro-octadecanoate	316	52.11	0.53	Ester
Triacotane	422	54.73	0.34	Saturated hydrocarbon
1,6-dimethyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine	165	55.36	1.18	Heterocyclic compound
Squalene	410	55.97	1.52	Heterocyclic compound
Tricyclo[6.3.0.0 (2,7)]undecane-1,3,7-triol	226	56.62	1.18	Sesquiterpene
Fumaric acid, nonyl 3-methylphenyl ester	332	59.64	0.17	Ester derivative of fumaric acid

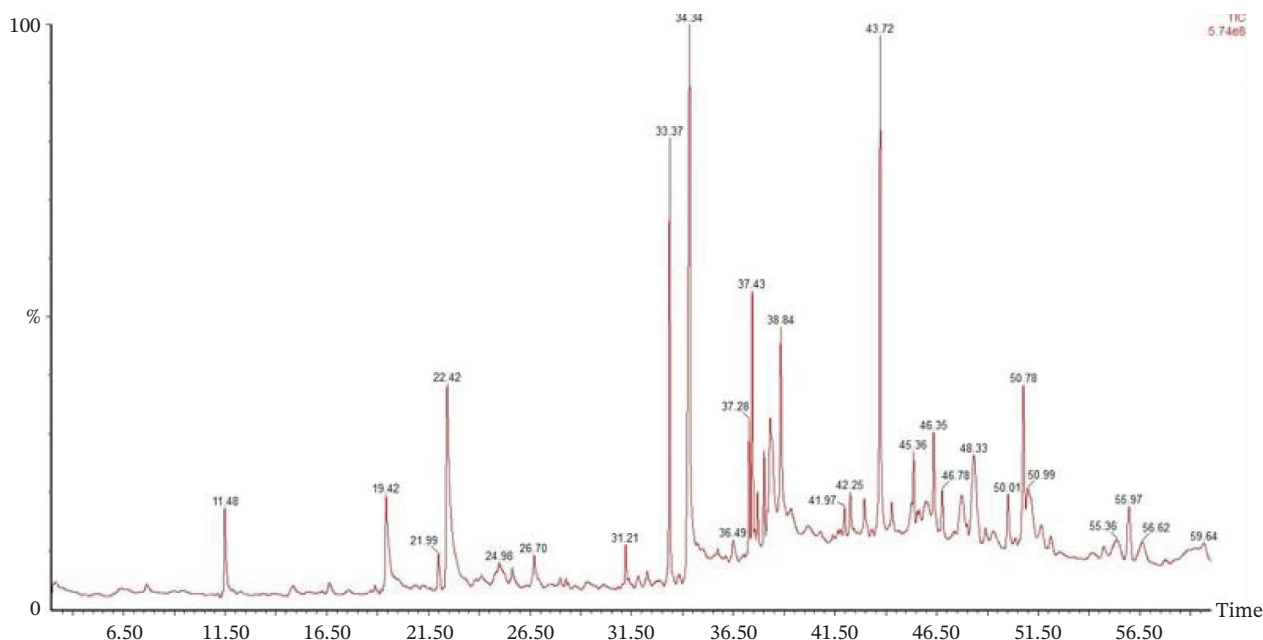


FIGURE 9 | GC-MS chromatogram of MESFL.

TABLE 11 | PASS prediction results of compounds of MESFL.

Compound name	Biological activity			
	Antipyretic	Anti-inflammatory	Analgesic	Antidiarrheal
	Pa	Pa	Pa	Pa
Octadecane-1,2-diol	0.311	0.442	0.441	—
Thymol	0.524	0.549	0.584	0.244
Tetradecamethyl	—	0.342	0.374	—
2,4,6 (1 h, 3 h, 5 h)-pyrimidinetrione	0.194	0.319	0.438	0.254
Eicosen-1-ol-9	0.285	0.616	0.545	0.201
N-hexadecanoic acid	0.497	0.515	0.526	0.211
Phytol	—	0.458	0.300	—
9-octadecenamide,	0.231	0.384	0.598	0.180
Hexanedioic acid,(2-ethylhexyl)ester	0.324	0.622	0.301	—
Gamma-tocopherol	—	0.775	—	—
3-(heptadec-10-en-1-yl)phenol	0.410	0.475	0.553	0.209
1,6-dimethyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine	—	—	0.442	—
Neophytadiene	—	0.286	0.391	—
Octadecanoic acid	0.497	0.515	0.526	0.211
Benzene, 1,1'-(1- methylethylidene)bis[4-methoxy-	0.325	0.505	0.521	0.521

arachidonic acid. As a result, it increases gastrointestinal motility and net water and electrolyte secretion, leading to diarrhea and a hypersecretory response [47, 48]. When compared to the negative control, the extract of *Swintonia floribunda* leaves at all tested doses (200 mg/kg and 400 mg/kg) considerably inhibited the movement of the charcoal marker ($p < 0.01$ and $p < 0.001$, respectively). It also demonstrated that the extract can affect intestinal peristaltic movement by decreasing GI motility and its secretions. Concerning this, various investigations reported that medicinal plants showed antidiarrheal activities by enhancing fluid and electrolytic absorption, which are mostly due to flavonoids and tannins via inhibiting autacoids and prostaglandin

release and reducing intracellular Ca^{2+} , respectively [48–50]. Therefore, the presence of flavonoid and tannin in *Swintonia floribunda* may be linked to the antimotility action seen at both doses of crude extract [51].

Fever, an immunological reaction, may cause severe brain damage, which is initiated by several pyrogenic cytokines, including macrophage protein-1, tumor necrosis factor- α , interferon- α , and interleukin 1β , followed by COX-2-mediated prostaglandin synthesis. Subcutaneous injection of Brewer's yeast induces pyrexia with a notable increase in proinflammatory cytokines, transcription factors, and prostaglandin-producing

TABLE 12 | ADME/T analysis of selected compound of MESFL.

Compound name	Lipinski rules					Veber's rules				Toxicity parameters			
	MW (g/mol) < 500	HBA < 10	HBD < 5	Log p ≤ 5	Bioavailability score	Lipinski's violation ≤ 1	n RB ≤ 10	TPSA ≤ 140 (Å²)	Ames toxicity	Carcinogens	Acute oral toxicity	Human intestinal absorption	Blood-brain barrier (BBB)
Octadecane-1,2-diol	132.28	1	0	2.66	0.55	0	3	9.23	NAT	NC	IV	0.9883	0.7336
Thymol	150.22	1	1	2.32	0.55	0	1	20.23	NAT	NC	III	0.9955	0.9381
Tetradecamethyl	519.08	7	0	5.32	0.55	1	0	64.61	NAT	NC	II	0.9655	0.9583
2, 4, 6 (1 h, 3 h, 5 h)-pyrimidinetrione	128.09	2	3	−0.08	0.55	0	0	75.27	NAT	NC	III	0.8111	0.9744
Eicosen-1-ol-9	296.53	1	1	5.22	0.55	1	17	20.23	NAT	NC	III	0.9974	0.9625
N-hexadecanoic acid	256.42	1	2	3.85	0.85	1	14	37.30	NAT	NC	IV	0.9888	0.9488
Phytol	296.53	1	1	4.85	0.55	1	13	20.23	NAT	NC	III	0.9846	0.9375
9-octadecenamide,	281.48	1	1	4.1	0.55	1	15	43.09	NAT	NC	III	1	0.9972
Hexanedioic acid, (2-ethylhexyl) ester	258.35	4	1	2.79	0.85	0	12	63.60	NAT	NC	III	0.9	0.9267
Gamma-tocopherol	416.68	2	1	5.16	0.55	1	12	29.46	NAT	NC	III	0.9795	0.9767
3-(heptadec-10-en-1-yl) phenol	330.55	1	1	4.97	0.55	1	15	20.23	NAT	NC	III	1	0.9305
1,6-dimethyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-a]pyrimidine	165.19	2	0	1.75	0.55	0	12	38.13	NAT	NC	III	0.9973	0.9823
Neophytadiene	278.52	0	0	5.05	0.55	1	13	0.00	NAT	NC	III	0.975	0.9425
Octadecanoic acid	284.48	2	1	4.3	0.85	1	16	37.30	NAT	NC	IV	0.9888	0.9488
Benzene, 1,1'-(1-methylethylidene) bis [4-methoxy-	256.34	2	0	3.36	0.55	0	4	18.46	NAT	NC	III	1	0.9016

Note: Italic numerals represent toxicity categories based on LD₅₀ (lethal dose) values. With the increasing class number, the LD₅₀ values also increase, which determine less toxicity of the compounds.

TABLE 13 | Molecular orbital and reactivity descriptor analysis.

Compound	Compound-1 (617780)	Compound-2 (92729)
A = HOMO	0.00388	0.0126
I = LUMO	−0.20148	−0.18323
E Gap = (I − A)	−0.20528	−0.19583
Chemical potential (μ) = −(I + A)/2	0.09884	0.085315
Hardness (η) = (I − A)/2	−0.10264	−0.097915
Softness (σ) = 1/ μ	10.117361	11.721268
Electronegativity (X) = (I + A)/2	−0.09884	−0.085315
Electrophilicity (ω) = $\mu^2/2\eta$	−0.047590343	−0.037168203

enzyme synthesis in the periphery and brain [52, 53]. Like indomethacin, the mechanistic effect of the antipyretic action can be the inhibition of prostaglandin formation, which is achieved by downregulating the expression of the COX enzyme. In our study, MESFL demonstrated a dose-dependent reduction in yeast-induced fever, with the 400-mg/kg dose significantly reducing the rectal temperature ($p < 0.001$) in febrile mice. Based on these findings, it can be inferred that the antipyretic effect of MESFL extract is likely due to its inhibitory effect on prostaglandin synthesis. Therefore, the presence of alkaloids, flavonoids and tannins in *Swintonia floribunda* may be linked to the antipyretic activity observed at both doses of the crude extract, as these phytochemicals are known to modulate inflammatory mediators and inhibit prostaglandin biosynthesis [54].

We also investigated MESFL’s potential as an in vivo analgesic by using a mice model to perform the acetic acid-induced writhing test. Our mice’s abdomens became constricted after the acetic acid treatment, causing them to writhe. This test sheds light on pain perception by synthesizing proinflammatory endogenous mediators (such as cytokines, COX, serotonin, and prostaglandin) in peripheral tissues and fluids. These mediators exacerbate inflammation by promoting prostaglandin stimulation between the peritoneal compartments through an increase in capillary permeability [55]. Thus, the fundamental mechanisms of the analgesic and anti-inflammatory effects are similar [14]. In the current study, MESFL decreased acetic acid-induced abdominal discomfort, accompanied by a reduction in writhing activity. In the current study, MESFL significantly decreased acetic acid-induced abdominal discomfort, accompanied by a reduction in writhing activity at doses of 200 mg/kg and 400 mg/kg ($p < 0.001$). These findings suggest that prostaglandin production may be regulated by MESFL, which would have a significant analgesic impact. The presence of active phytochemicals like flavonoids and tannins in the extract is likely responsible for modulating inflammatory pathways, thus decreasing pain perception through mechanisms similar to those of traditional analgesics [56].

A formalin-induced paw-licking experiment was also used to investigate whether there were any differences in the analgesic activity of MESFL mediated by peripheral and central pathways. By creating a protracted pain experience, the formalin-induced paw-licking experiment can be used to study a biphasic nociceptive response (both early and late phases), which is mediated by distinct mechanisms [57]. Due to the progressive chemical stimulation of sensory C-fibers, intraperitoneal injection of mice triggered the

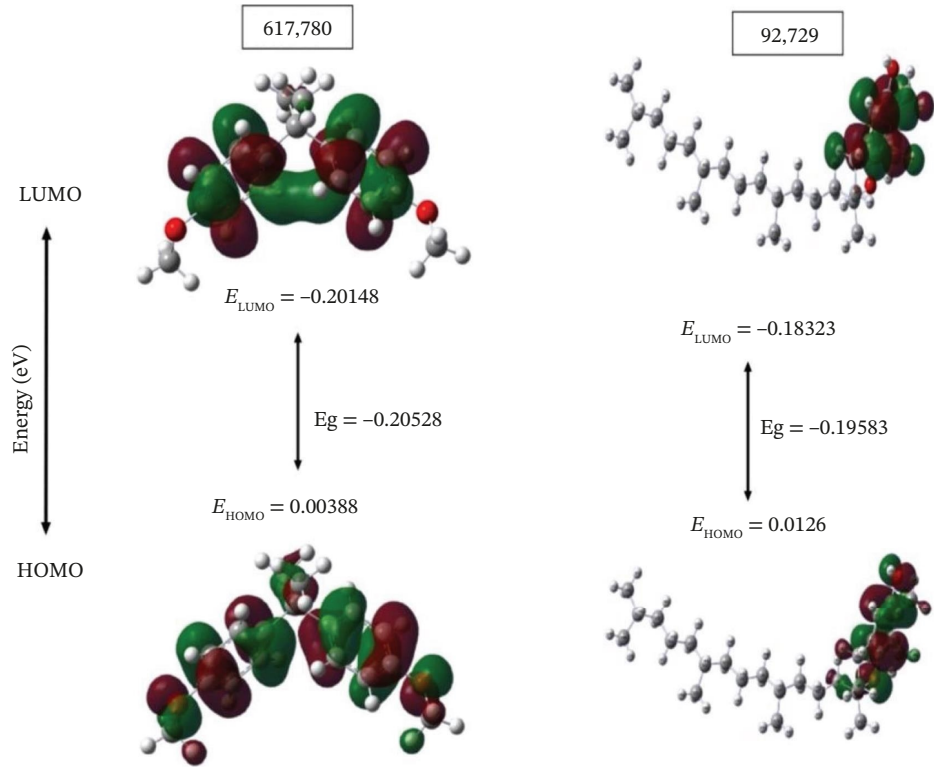


FIGURE 10 | Frontier molecular orbital (FMO) evaluations.

TABLE 14 | Binding affinity and nonbonding interaction of the selected compound of MESFL against 1CX2, 5Y3Z, 4U14, and 4YK5.

Receptor	Compound	Binding affinity (kcal/mol)	Bond type	Amino acids
Analgesic	1CX2	92729	Conventional Hydrogen Bond	ASN39
			Alkyl	CYS36, PRO153, ALA156, CYS47, CYS47, PRO153, CYS36, MET48, PRO154
			Pi-Alkyl	TYR136, CYS47, PRO153
			Carbon-Hydrogen Bond	ASN375, HIS226, GLY533, GLY536
Anti-Inflammatory	5Y3Z	617780	Pi-Pi T-shaped	PHE142
			Pi-Alkyl	PHE142, PRO538
			Conventional Hydrogen Bond	ASN375
			Pi-Sigma	GLN374
Antidiarrheal	4U14	92729	Alkyl	LEU145
			Pi-Alkyl	PHE142, PRO538
			Pi-Sigma	LEU264
			Alkyl	ALA194, VAL240, MET242, ALA273, ALA270, ALA273, ALA273, ARG150, LEU264, LEU264, MET242, MET246, ILE274, LEU264, ILE274, ILE274
Anti-Inflammatory	5Y3Z	617780	Pi-Alkyl	HIS277, HIS277, HIS277, ALA194
			Carbon-Hydrogen Bond	LEU263
			Alkyl	MET242, MET246, ILE274, VAL240, MET242
			Pi-Cation	ARG238
Anti-Inflammatory	5Y3Z	617780	Pi-Sigma	ALA194
			Pi-Alkyl	LEU264
			Conventional Hydrogen Bond	SER192, SER192, SER262
			Pi-Sigma	LEU264
Anti-Inflammatory	5Y3Z	3033	Pi-Pi T-shaped	HIS277
			Alkyl	ARG150, LEU264
			Pi-Alkyl	HIS277, ALA194, VAL240, MET242, VAL145, ARG150
Antidiarrheal	4U14	92729	Pi-Donor Hydrogen Bond	TYR506
			Pi-Sulfur	CYS532
			Pi-Pi Stacked	TRP503, TRP503
			Pi-Pi T-shaped	TYR506
Antidiarrheal	4U14	92729	Alkyl	ALA235, ALA238
			Pi-Alkyl	TYR148, TRP199, ALA235, VAL510
			Alkyl	LEU198, ALA202, ALA237, ALA237
			Pi-Alkyl	TRP199, TRP199, PHE205, TRP206, TRP206, PHE209, PHE209, PHE209, PHE224, LEU198, ALA238
Antidiarrheal	4U14	3955	Conventional Hydrogen Bond	TYR148, ILE222
			Pi-Pi T-shaped	TRP525, TRP525, TRP525, TRP525
			Alkyl	ALA238, VAL155
			Pi-Alkyl	TRP503, ALA238

(Continues)

TABLE 14 | (Continued)

Receptor	Compound	Binding affinity (kcal/mol)	Bond type	Amino acids
Antipyretic	617780	−5.8	Carbon–Hydrogen Bond	THR21
			Pi–Pi T-shaped	PHE16
			Amide–Pi Stacked	LEU83, PHE84
			Alkyl	LEU83
			Pi–Alkyl	LEU83
	92729	−5.7	Pi–Sigma	LEU83, TYR80
			Alkyl	LEU83, VAL24, LEU83, LEU83, LEU83
			Pi–Alkyl	PHE16, TYR80, PHE87
			Pi–Sigma	LEU83, LEU83
			Pi–Pi Stacked	PHE84
	3715	−6.5	Pi–Pi T-shaped	PHE87
			Alkyl	VAL88
			Pi–Alkyl	PHE16, PHE84, PHE87, PHE87

onset of the early preparatory phase (0–5 min). While centrally acting anti-inflammatory drugs may reduce the ending phase (15–30 min), which is anticipated to cause an inflammatory response, some opioid-based drug types are susceptible to neurogenic solid inflammation [58]. The formalin experiment demonstrated that MESFL injection greatly decreased the formalin-induced pain reaction in both the early and late stages in a dose-dependent manner ($p < 0.001$). This implies that the extract has both central and peripheral analgesic effects.

This work also employed the hot plate and tail immersion tests, two well-established models for evaluating centrally mediated analgesia, to examine the antinociceptive effects of MESFL on the CNS. MESFL significantly increased response latency in the hot plate test ($p < 0.001$), suggesting its potential to modulate supraspinal pain pathways, including μ_1 , κ_3 , δ_1 , and σ_2 opioid receptors. Likewise, MESFL extended withdrawal latency in the tail immersion test ($p < 0.001$), which is a selective model for spinal reflex-mediated analgesia, further demonstrating its central analgesic effect through μ_2 , κ_1 , and δ_2 opioid receptors [59]. These tests are unaffected by analgesics with a peripheral action, suggesting that MESFL likely engages several opioid receptor subtypes in its central pathways to produce its antinociceptive actions [22]. The presence of active flavonoids, tannins, and other bioactive phytochemicals in the extract is likely responsible for its modulation of pain pathways. These compounds are known to interact with various molecular targets, including opioid receptors, contributing to MESFL’s analgesic effects [60]. These results demonstrate MESFL’s potential as a novel central analgesic drug, which justifies further pharmacological investigation to elucidate its exact molecular targets and clinical relevance.

To assess the anti-inflammatory impact of the MESFL extract, we chose the method of protein denaturation and membrane stabilization. In protein denaturation assay, heat treatment during the antidenaturation procedure denatures the EA. Certain antigens associated with type-III hypersensitivity responses, which cause glomerulonephritis and serum sickness, are expressed by denatured proteins [61]. The modification of disulfide, hydrophobic, hydrogen, and electrostatic bonds—all of which preserve the proteins’ three-dimensional structure—is one potential mechanism for protein denaturation. It has been noted that traditional NSAIDs, such as phenylbutazone and indomethacin, prevent proteins from becoming denatured [61]. The MESFL extract showed considerable anti-inflammatory efficacy in the current investigation ($p < 0.001$), suggesting that it can regulate autoantigen formation.

In the membrane stabilization assay, hemolytic action is shown in the hypotonic solution. Hemolysis results from the rupture of the RBC membrane caused by fluid buildup inside the cells [62]. When the membrane of RBCs becomes damaged, they are more susceptible to subsequent injury. This damage is brought on by free radical-induced lipid peroxidation [63]. The passage of fluids and serum proteins into the tissue can be stopped by membrane stabilization. The effective suppression of hemolysis by the methanolic leaf extract of *Swintonia floribunda* may preserve the RBC membrane by halting the production of lytic enzymes and other strong inflammatory mediators ($p < 0.001$). The presence of active flavonoids, tannins, and phenolic compounds in the extract may be responsible for the observed membrane stabilization, as

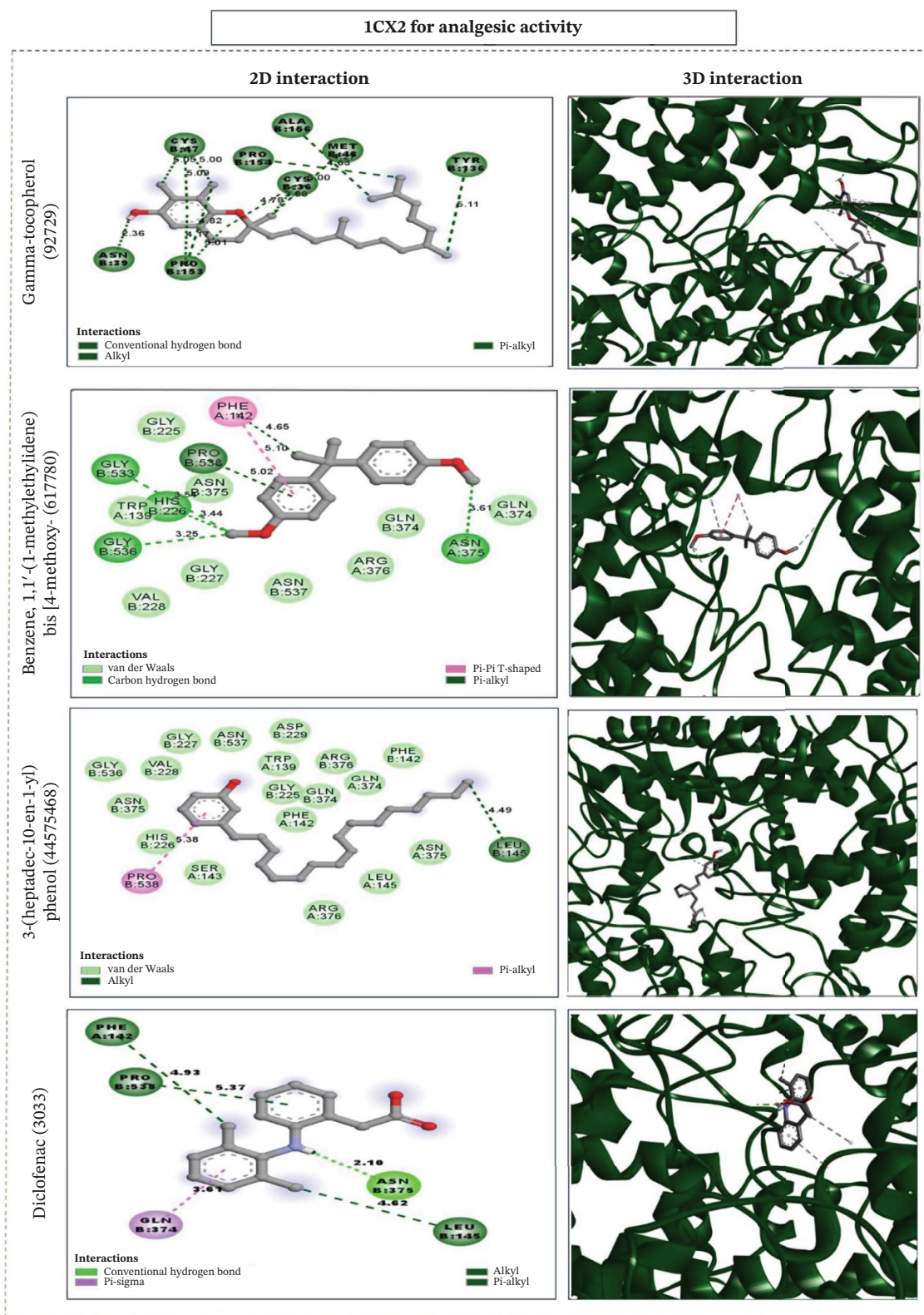


FIGURE 11 | Binding site and nonbonding interaction with 1CX2.

these phytochemicals are known to modulate inflammatory pathways and inhibit free radical-induced damage [64].

Combining MESFL's GC-MS profile with molecular docking data provides a scientifically supported basis for its multitarget therapeutic potential. With gamma-tocopherol (3.64% relative

abundance), benzyl alcohol (1.27%), and thymol (0.10%), the GC-MS analysis identified 32 bioactive chemicals, substances well documented for their pharmacological effects [65].

Notably, the docking studies revealed a remarkable binding affinity of gamma-tocopherol to both COX-2 (−7.6 kcal/mol) and

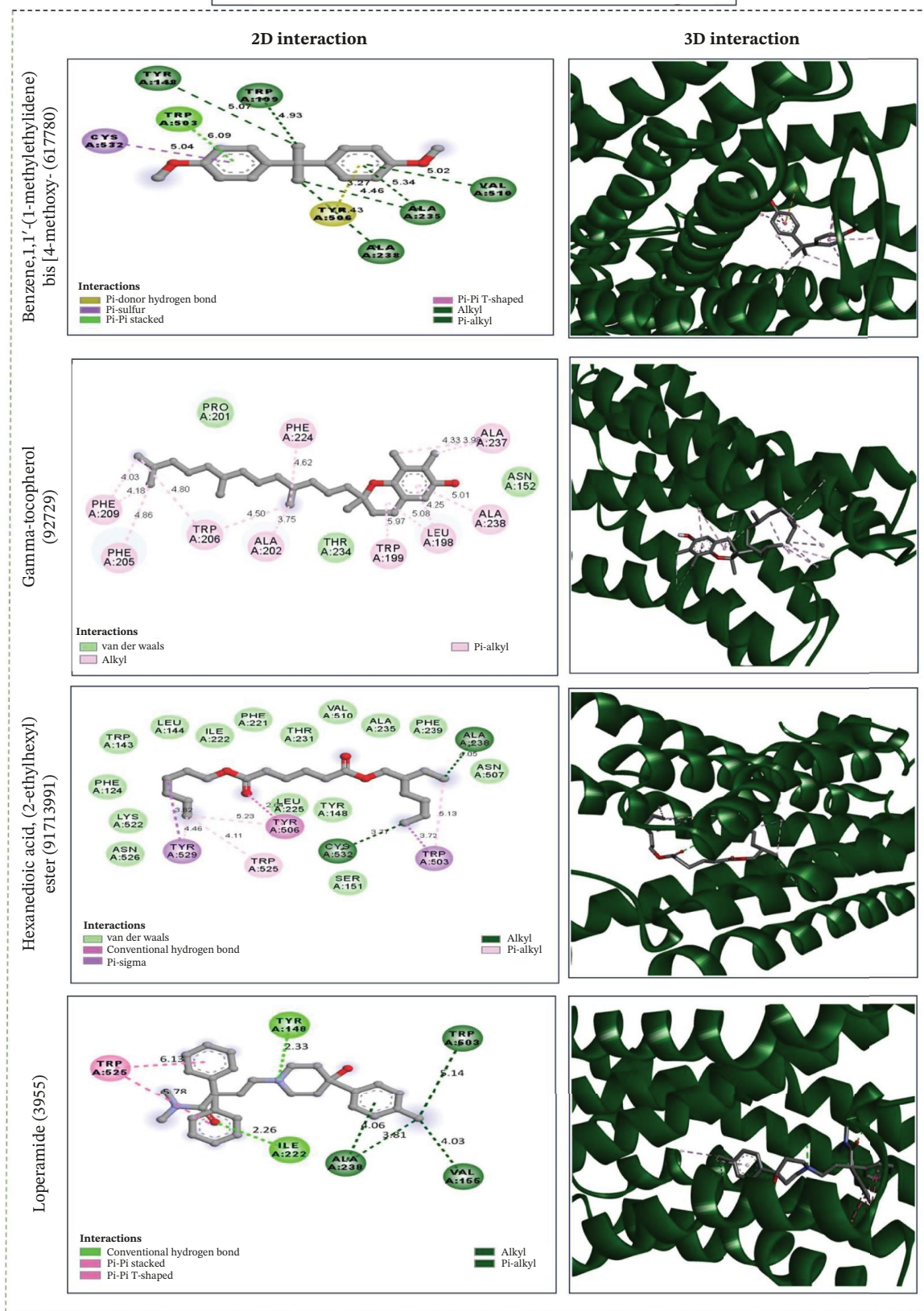


FIGURE 12 | Binding site and nonbonding interaction with 4U14.

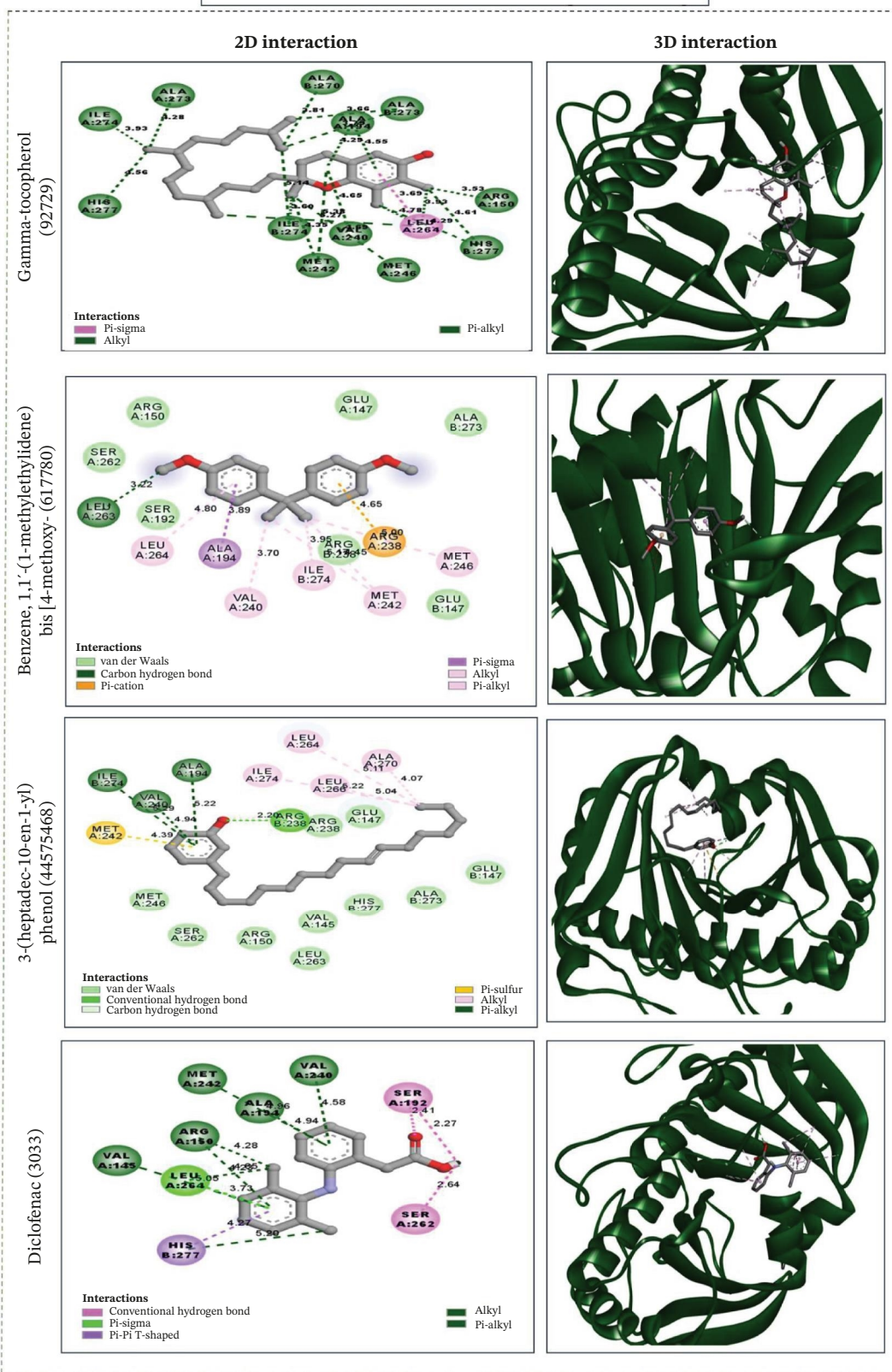


FIGURE 13 | Binding site and nonbonding interaction with 5Y3Z.

5-LOX (−7.9 kcal/mol), resulting in persistent contacts with the catalytic residues ASN39 and LEU264, respectively. These results are under the findings reported by [66] regarding tocopherols as

inhibitors of both COX and LOX. Through precise interactions with TYR506—a fundamental residue in opioid receptor binding—the bisphenol derivative (Compound 61780) displayed

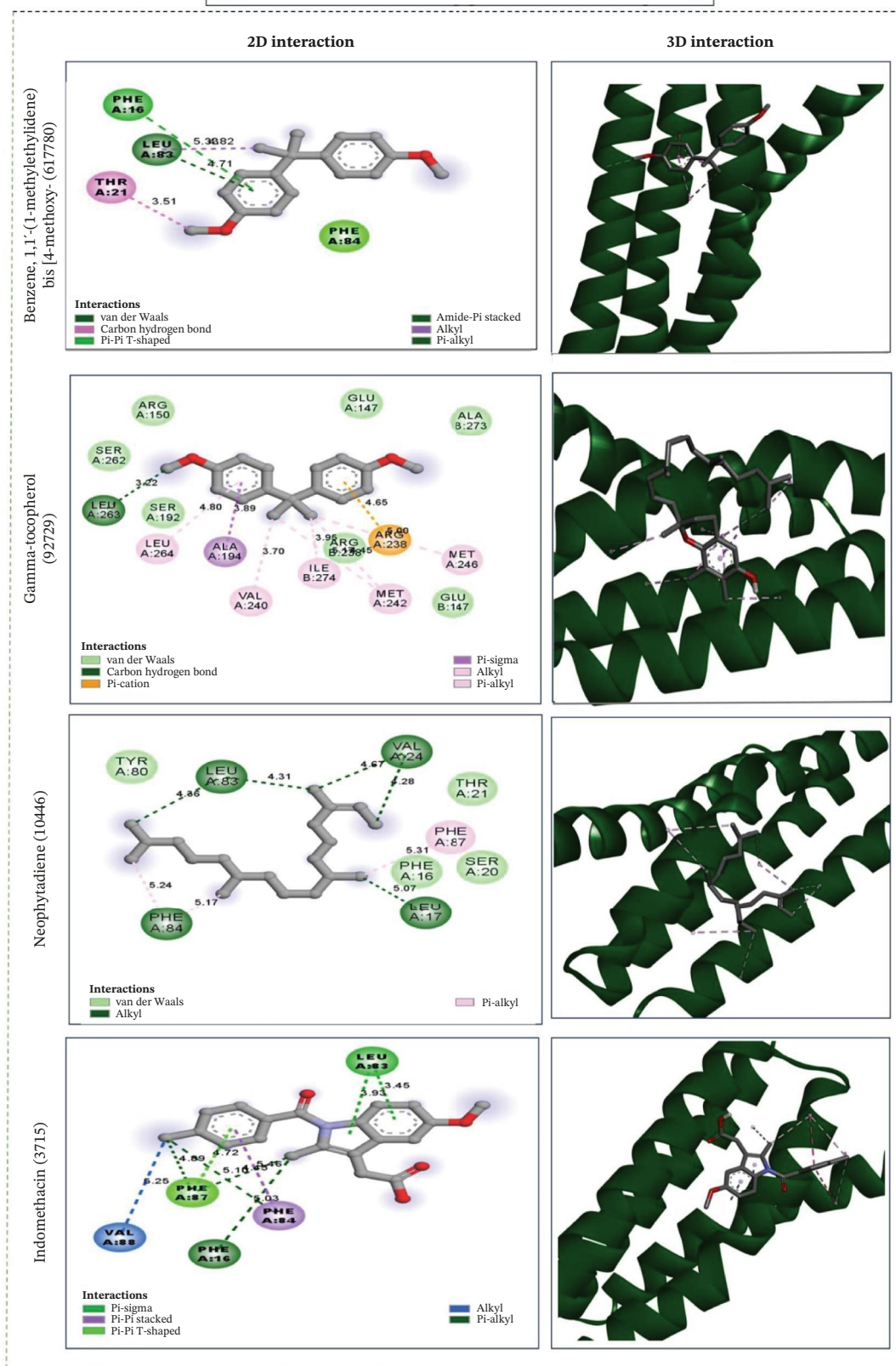


FIGURE 14 | Binding site and nonbonding interaction with 4YK5.

TABLE 15 | No. of different bonds for different activities.

Protein	Compound	Compound Gibbs free energy (ΔG)	No. of hydrogen bonds	H-bond interaction sites
Analgesic				
1CX2	92729	−7.6	1	ASN39
	617780	−7.3	4	ASN375, HIS226, GLY533, GLY536
	3033	−7.4	1	ASN375
Anti-Inflammatory				
5Y3Z	92729	−7.9	0	No h-bond interactions
	617780	−7	1	LEU263
	3033	−7.8	3	SER192, SER192, SER262
Antidiarrheal				
4U14	617780	−7.9	1	TYR506
	92729	−6.9	0	No h-bond interactions
	3955	−9.4	2	TYR148, ILE222
Antipyretic				
4YK5	617780	−5.8	1	THR21
	92729	−5.7	0	No h-bond interactions
	3715	−6.5	0	No h-bond interactions

excellent antidiarrheal potential (−7.9 kcal/mol against 4U14), equivalent to loperamide (−9.4 kcal/mol).

Multiple drugs with moderate but consistent binding affinities (−5 to −7 kcal/mol) across all targets suggest potential synergistic action, thereby confirming [67] polypharmacology theory. Experimental validation is necessary for these computational predictions, as pointed out by [68]. This is especially true when considering the GC-MS profile, which shows complex matrix effects due to the presence of lipophilic compounds and fatty acid esters (e.g., methyl 7,11,14-eicosatrienoate, 2.69%).

It is essential to highlight that, despite the encouraging results of the study on the pharmacological potential of MESFL, several limitations exist. The presence of dose-dependent effects does not negate the need for a larger dose–response investigation to establish the optimal therapeutic dosage. Further research using a range of animal models, as well as well-designed clinical trials, is warranted by this work. Despite these limitations, the study highlights the therapeutic potential of MESFL and encourages further investigation into its use as an alternative medicine for various diseases.

5 | Conclusion

The current research investigated the phytochemical profile and biological activities of *Swintonia floribunda* leaves, identifying that they exhibit strong anti-inflammatory, antidiarrheal, and antinociceptive effects in biological models. The in silico molecular docking experiments supported to uncover other necessary bioactive chemicals; gamma-tocopherol had the strongest binding affinity for target proteins 1CX2 and 5Y3Z, while benzene and 1,1'-(1-methyl ethylidene) interacted strongly with 4U14 and 4YK5, respectively. These results lend credence to *Swintonia floribunda*'s long-standing use in managing pain and inflammation and raise the possibility that it could be developed into an antipyretic medication. The current focus is on identifying and characterizing its bioactive components to understand how they function and pave the way for

potential pharmaceutical applications in the future. This research provides supportive evidence for the use of *Swintonia floribunda* in evidence-based herbal medicine, opening the door to new avenues for the plant's potential as a source of medicinal compounds.

6 | Limitations of the Study

The use of GC-MS, which only detects volatile and semivolatile chemicals, limits this study's ability to reflect the whole phytochemical profile of *Swintonia floribunda* leaves. Nonvolatile components were not decisively characterized owing to a lack of modern methods such as LC-MS/MS and NMR. The preliminary phytochemical screening was qualitative and suggestive only, with no individual chemicals isolated, restricting conclusive structural confirmation and direct linkage with biological activity. The molecular docking study was based entirely on GC-MS-detected chemicals and should be seen as supportive rather than conclusive. Further research into chemical separation, fractionation, and sophisticated analytical procedures is consequently necessary.

Nomenclature

MESFL	Methanolic extract of <i>Swintonia floribunda</i> leaves
EA	Egg albumin
PBS	Phosphate buffer solution
<i>n</i>	the number of mice
b.w.	body weight
SEM	Standard error mean
i.p.	intraperitoneal
p.o.	Orally
HRBC	Human red blood cell

Author Contributions

Farjana Akter Nipa: conceptualization and formal analysis.

Mst. Riniara Khatun: investigation, methodology, software, and supervision.

Israt Jahan: investigation, methodology, visualization, writing—original draft, and writing—review and editing.

Md Shirajul Islam: methodology, resources, software, and visualization.

Sabbir Khan: data curation, software, visualization, writing, review, and editing.

Shariful Islam: methodology, visualization, writing—original draft, and writing—review and editing.

Jannatul Nisa Nipa: data curation, formal analysis, project administration, and resources.

Mahathir Mohammad: data curation, software, and validation.

Md. Anisul Islam: software, visualization, writing, review, and editing.

Trishala Dutta: data curation, software, visualization, writing, review, and editing.

Md. Mustafiz Chowdhury: data curation, formal analysis, investigation, methodology, resources, software, writing—original draft, and writing—review and editing.

Mycal Dutta: conceptualization, data curation, formal analysis, project administration, resources, supervision, validation, and visualization.

Acknowledgments

The authors have nothing to report.

Funding

This study was self-funded.

Ethics Statement

All experimental protocols were reviewed and approved by the Planning and Development (P & D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh, with the ethical approval no IIUC/Nipa-Pharm2023 for animal use. For the human participant Planning and Development (P & D) Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh also gave the ethical approval with the reference no IIUC-Pharm-2023/045. Following treatments, the animals were anesthetized with controlled, gradual-fill inhalation of carbon dioxide 20%–30% of the total chamber air volume and subsequently euthanized, which was carried out in accordance with the guidelines established by the National Institutes of Health.

Permission to reproduce material from other sources is not available.

Consent

Consent has been obtained from the participant.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The datasets used and/or analyzed during the present study are available upon reasonable request from the relevant author, and they were also discovered online by searching Google Scholar, PubMed, websites, book chapters, etc.

References

1. S. Maqsood, P. Singh, M. H. Samoon, and A. K. Balange, “Effect of Dietary Chitosan on Non-Specific Immune Response and Growth of

Cyprinus carpio Challenged With *Aeromonas hydrophila*,” *International Aquatic Research* 2, no. 2 (2010): 77.

2. M. I. Haque, A. A. Chowdhury, M. Shahjahan, and M. G. D. Harun, “Traditional Healing Practices in Rural Bangladesh: A Qualitative Investigation,” *BMC Complementary and Alternative Medicine* 18, no. 1 (2018): 62, <https://doi.org/10.1186/s12906-018-2129-5>.

3. A. Rahman, A. Sikder, A. Kaisar, C. M. Hasan, and M. A. Rashid, “Total Phenolics and Antioxidant Activity of *Swintonia floribunda* (Griff.),” *Bangladesh Pharmaceutical Journal* 13, no. 2 (2010): 20–24.

4. D. H. Phu, N. X. Hai, N. T. Nhan, and N. T. T. Mai, “Chemical Constituents of the Stem Barks of *Swintonia floribunda* Griff.(Anacardiaceae),” *Journal of Natural Sciences* 2, no. 1 (2018): 71–75.

5. P. García-Herrera, P. Morales, M. Cámara, V. Fernández-Ruiz, J. Tardío, and M. C. Sánchez-Mata, “Nutritional and Phytochemical Composition of Mediterranean Wild Vegetables After Culinary Treatment,” *Foods* 9, no. 12 (2020): 1761, <https://doi.org/10.3390/foods9121761>.

6. C. M. Uritu, C. T. Mihai, G. D. Stanciu, et al., “Medicinal Plants of the Family Lamiaceae in Pain Therapy: A Review,” *Pain Research and Management* 2018, no. 1 (2018): 7801543–7801544, <https://doi.org/10.1155/2018/7801543>.

7. T. Dutta, A. Paul, M. Majumder, R. A. Sultan, and T. B. Emran, “Pharmacological Evidence for the Use of *Cissus assamica* as a Medicinal Plant in the Management of Pain and Pyrexia,” *Biochemistry and Biophysics Reports* 21 (2020): 100715, <https://doi.org/10.1016/j.bbrep.2019.100715>.

8. M. J. Timbadiya, K. Nishteswar, R. Acharya, and M. B. Nariya, “Experimental Evaluation of Antipyretic and Analgesic Activities of Amalakyadi Gana: An Ayurvedic Formulation,” *AYU (An International Quarterly Journal of Research in Ayurveda)* 36, no. 2 (2015): 220–224, <https://doi.org/10.4103/0974-8520.175554>.

9. L. Terefe, A. Nardos, A. Debella, et al., “Antidiarrheal Activities of the Methanol Leaf Extracts of *Olinia rochetiana* (Oliniaceae) Against Castor Oil-Induced Diarrhea in Mice,” *Journal of Experimental Pharmacology* 15 (2023): 485–495, <https://doi.org/10.2147/jep.s441555>.

10. A. Degu, E. Engidawork, and W. Shibeshi, “Evaluation of the Anti-Diarrheal Activity of the Leaf Extract of *Croton macrostachyus* Hocsht. ex Del.(Euphorbiaceae) in Mice Model,” *BMC Complementary and Alternative Medicine* 16, no. 1 (2016): 379, <https://doi.org/10.1186/s12906-016-1357-9>.

11. L. Ferrero-Miliani, O. Nielsen, P. Andersen, and S. Girardin, “Chronic Inflammation: Importance of NOD2 and NALP3 in Interleukin-1 β Generation,” *Clinical and Experimental Immunology* 147, no. 2 (2007): 227–235, <https://doi.org/10.1111/j.1365-2249.2006.03261.x>.

12. D. Schottenfeld and J. Beebe-Dimmer, “Chronic Inflammation: A Common and Important Factor in the Pathogenesis of Neoplasia,” *CA: A Cancer Journal for Clinicians* 56, no. 2 (2006): 69–83, <https://doi.org/10.3322/canjclin.56.2.69>.

13. J. R. Vane, “Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-Like Drugs,” *Nature New Biology* 231, no. 25 (1971): 232–235, <https://doi.org/10.1038/newbio231232a0>.

14. A. Karbab, K. Mokhnache, S. Ouhida, et al., “Anti-Inflammatory, Analgesic Activity, and Toxicity of *Pituranthos Scoparius* Stem Extract: An Ethnopharmacological Study in Rat and Mouse Models,” *Journal of Ethnopharmacology* 258 (August 2020): 112936, <https://doi.org/10.1016/j.jep.2020.112936>.

15. M. Rahman, M. Alim, M. Hossain, and M. Hossain, “Prospect of Natural Regeneration of Tree Species in Hazarikhil Wildlife Sanctuary of Chattogram, Bangladesh,” *Journal of Biodiversity Conservation and Bioresource Management* 5, no. 2 (2019): 1–12, <https://doi.org/10.3329/jbcbm.v5i2.44909>.

16. R. Rahman, T. Siddique, F. A. Nipa, et al., “Bark Extract of *Chaetocarpus castanocarpus* (Roxb.) Exhibits Potent Sedative, Anxiolytic, and

- Antidepressant Effects Through an In Vivo Approach in Swiss Albino Mice,” *European Review for Medical and Pharmacological Sciences* 28, no. 3 (February 2024): 1202–1212, https://doi.org/10.26355/eurev_202402_35359.
17. B. Ahmad, S. Naz, A. Rauf, et al., “In Vivo Study on Analgesic, Gastrointestinal Tract (GIT) Motility, and Anti-Termite Potential of Methanolic Extract of *Sarcococca saligna* (D. Don) Muell. Fruits,” *South African Journal of Botany* 114 (January 2018): 40–43, <https://doi.org/10.1016/j.sajb.2017.10.013>.
 18. J. R. Shaikh and M. Patil, “Qualitative Tests for Preliminary Phytochemical Screening: An Overview,” *International journal of chemical studies* 8, no. 2 (March 2020): 603–608, <https://doi.org/10.22271/chemi.2020.v8.i2i.8834>.
 19. M. M. Billah, R. Islam, H. Khatun, et al., “Antibacterial, Antidiarrhoeal, and Cytotoxic Activities of Methanol Extract and Its Fractions of *Caesalpinia Bonducella* (L.) Roxb Leaves,” *BMC Complementary and Alternative Medicine* 13, no. 1 (December 2013): 101, <https://doi.org/10.1186/1472-6882-13-101>.
 20. N. U. Emon, S. Alam, S. Rudra, et al., “Antidepressant, Anxiolytic, Antipyretic, and Thrombolytic Profiling of Methanol Extract of the Aerial Part of *Piper nigrum*: in Vivo, in Vitro, and in Silico Approaches,” *Food Science and Nutrition* 9, no. 2 (February 2021): 833–846, <https://doi.org/10.1002/fsn3.2047>.
 21. M. Gupta, U. Mazumder, R. S. Kumar, et al., “Anti-Inflammatory, Analgesic and Antipyretic Effects of Methanol Extract From *Bauhinia racemosa* Stem Bark in Animal Models,” *Journal of Ethnopharmacology* 98, no. 3 (April 2005): 267–273, <https://doi.org/10.1016/j.jep.2005.01.018>.
 22. A. K. Gupta, D. Parasar, A. Sagar, et al., “Analgesic and Anti-Inflammatory Properties of Gelsolin in Acetic Acid Induced Writhing, Tail Immersion and Carrageenan Induced Paw Edema in Mice,” *PLoS One* 10, no. 9 (2015): e0135558, <https://doi.org/10.1371/journal.pone.0135558>.
 23. M. Uddin, S. Ahmed, M. Kabir, M. Rahman, R. Sultan, and T. Emran, “In Vivo Analgesic, Anti-Inflammatory Potential in Swiss Albino Mice and in Vitro Thrombolytic Activity of Hydroalcoholic Fruits Extract From *Daemonorops robusta* Warb,” *Journal of Applied Pharmaceutical Science* (2017): 104–113, <https://doi.org/10.7324/JAPS.2017.70114>.
 24. M. Adnan, M. Nazim Uddin Chy, A. Mostafa Kamal, et al., “Evaluation of Anti-Nociceptive and Anti-Inflammatory Activities of the Methanol Extract of *Holigarna caustica* (Dennst.) Oken Leaves,” *Journal of Ethnopharmacology* 236 (May 2019): 401–411, <https://doi.org/10.1016/j.jep.2019.01.025>.
 25. L. P. Coelho, P. A. Reis, F. L. de Castro, et al., “Antinociceptive Properties of Ethanolic Extract and Fractions of *Pterodon pubescens* Benth. Seeds,” *Journal of Ethnopharmacology* 98, no. 1–2 (April 2005): 109–116, <https://doi.org/10.1016/j.jep.2005.01.014>.
 26. N. B. Eddy and D. Leimbach, “Synthetic Analgesics. II. Dithienylbutenyl- and Dithienylbutylamines,” *Journal of Pharmacology and Experimental Therapeutics* 107, no. 3 (March 1953): 385–393, [https://doi.org/10.1016/s0022-3565\(25\)05180-8](https://doi.org/10.1016/s0022-3565(25)05180-8).
 27. P. A. Janssen, C. J. Niemegeers, and J. G. Dony, “The Inhibitory Effect of Fentanyl and Other Morphine-Like Analgesics on the Warm Water Induced Tail Withdrawal Reflex in Rats,” *Arzneimittelforschung* 13 (June 1963): 502–507.
 28. A. Saleem, M. Saleem, and M. F. Akhtar, “Antioxidant, Anti-Inflammatory and Antiarthritic Potential of *Moringa oleifera* Lam: An Ethnomedicinal Plant of Moringaceae Family,” *South African Journal of Botany* 128 (January 2020): 246–256, <https://doi.org/10.1016/j.sajb.2019.11.023>.
 29. S. Chandra, P. Chatterjee, P. Dey, and S. Bhattacharya, “Evaluation of in Vitro Anti-Inflammatory Activity of Coffee Against the Denaturation of Protein,” *Asian Pacific Journal of Tropical Biomedicine* 2, no. 1 (January 2012): S178–S180, [https://doi.org/10.1016/S2221-1691\(12\)60154-3](https://doi.org/10.1016/S2221-1691(12)60154-3).
 30. P. Ansari, M. J. Uddin, M. M. Rahman, et al., “Anti-Inflammatory, Anti-Diarrheal, Thrombolytic and Cytotoxic Activities of an Ornamental Medicinal Plant: *Persicaria orientalis*,” *Journal of Basic and Clinical Physiology and Pharmacology* 28, no. 1 (June 2016): 51–58, <https://doi.org/10.1515/jbcpp-2016-0023>.
 31. S. Umukoro, A. O. Alabi, A. T. Eduviere, A. M. Ajayi, and O. G. Oluwole, “Anti-Inflammatory and Membrane Stabilizing Properties of Methyl Jasmonate in Rats,” *Chinese Journal of Natural Medicines* 15, no. 3 (March 2017): 202–209, [https://doi.org/10.1016/S1875-5364\(17\)30036-5](https://doi.org/10.1016/S1875-5364(17)30036-5).
 32. D. G. Joshi, R. K. Jat, and Dr. S. B. Patil, “In Vitro Protein Denaturation and Membrane Stabilizing Anti-Arthritic Activity of Aqueous Extracts of Bark of *Ficus benghalensis* L. Against Methotrexate,” *The Pharma Innovation* 10, no. 4 (April 2021): 689–692, <https://doi.org/10.22271/tpi.2021.v10.i4j.6038>.
 33. I. Jahan, M. R. Tona, S. Sharmin, et al., “GC-MS Phytochemical Profiling, Pharmacological Properties, and in Silico Studies of *Chukrasia velutina* Leaves: A Novel Source for Bioactive Agents,” *Molecules* 25, no. 15 (August 2020): 3536, <https://doi.org/10.3390/molecules25153536>.
 34. F. Cheng, W. Li, Y. Zhou, et al., “admetSAR: A Comprehensive Source and Free Tool for Assessment of Chemical ADMET Properties,” *Journal of Chemical Information and Modeling* 52, no. 11 (November 2012): 3099–3105, <https://doi.org/10.1021/ci300367a>.
 35. A. Daina, O. Michielin, and V. Zoete, “SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules,” *Scientific Reports* 7, no. 1 (March 2017): 42717, <https://doi.org/10.1038/srep42717>.
 36. E. S. Ozdemir, F. Halakou, R. Nussinov, A. Gursoy, and O. Keskin, “Methods for Discovering and Targeting Druggable Protein-Protein Interfaces and Their Application to Repurposing,” in *Methods in Molecular Biology, Computational Methods for Drug Repurposing*, ed. Q. Vanhaelen, 1903 (New York, NY: Springer New York, 2019), 1–21, https://doi.org/10.1007/978-1-4939-8955-3_1.
 37. V. M. Bezhtentsev, O. A. Tarasova, A. V. Dmitriev, et al., “Computer-Aided Prediction of Xenobiotic Metabolism in Humans,” *Russian Chemical Reviews* 85, no. 8 (August 2016): 854–879, <https://doi.org/10.1070/RCR4614>.
 38. Y. Yueniwati, M. F. R. Syaban, N. E. Erwan, G. F. A. Putra, and A. D. Krisnayana, “Molecular Docking Analysis of *Ficus religiosa* Active Compound With Anti-Inflammatory Activity by Targeting Tumour Necrosis Factor Alpha and Vascular Endothelial Growth Factor Receptor in Diabetic Wound Healing,” *Open Access Macedonian Journal of Medical Sciences* 9, no. A (January 2021): 1031–1036, <https://doi.org/10.3889/oamjms.2021.7068>.
 39. R. Zainul, R. Verawati, R. S. Rita, et al., “Computational Evaluation of the Potential of Salicylate Compound From *Syzygium aromaticum* on Carbonic Anhydrase I as a Gastric Acid Stimulant,” *Pharmacognosy Journal* 15, no. 4 (2023): 489–493, <https://doi.org/10.5530/pj.2023.15.107>.
 40. F. B. Tessema, Y. H. Gonfa, T. B. Asfaw, M. G. Tadesse, and R. K. Bachheti, “In Silico Molecular Docking Approach to Identify Potential Antihypertensive Compounds From *Ajuga integrifolia* Buch.-Ham. Ex D. Don (Armagusa),” *Advances and Applications in Bioinformatics and Chemistry* 17 (December 2024): 47–59, <https://doi.org/10.2147/AABC.S392878>.
 41. M. A. Ghauri, L. Iqbal, A. Raza, U. Hayat, N. Atif, and A. Javeed, “In Vivo Anti-inflammatory, Antipyretic, Analgesic Activity and in Vitro Anti-Proliferative Activity of Aqueous Methanolic Extract of *Euphorbia granulata* Forssk,” *Future Journal of Pharmaceutical Sciences* 7, no. 1 (February 2021): 34, <https://doi.org/10.1186/s43094-021-00184-9>.
 42. S. Misra, A. M. A. Ikbal, D. Bhattacharjee, et al., “Validation of Antioxidant, Antiproliferative, and in Vitro Anti-Rheumatoid Arthritis Activities of Epigallo-Catechin-Rich Bioactive Fraction From *Camellia sinensis* Var. *Assamica*, Assam Variety White Tea, and Its Comparative Evaluation With Green Tea Fraction,” *Journal of Food Biochemistry* 46, no. 12 (2022): e14487, <https://doi.org/10.1111/jfbc.14487>.

43. N. Das, A. Bhattacharya, S. Kumar Mandal, et al., "Ichnocarpus frutescens (L.) R. Br. Root Derived Phyto-Steroids Defends Inflammation and Algesia by Pulling Down the Pro-Inflammatory and Nociceptive Pain Mediators: An In-Vitro and In-Vivo Appraisal," *Steroids* 139 (2018): 18–27, <https://doi.org/10.1016/j.steroids.2018.09.005>.
44. P. P. Partha Palit, S. Mandal, and B. B. Biswanath Bhunia, "Total Steroid and Terpenoid Enriched Fraction From *Euphorbia neriifolia* Linn. Offers Protection Against Nociceptive-Pain, Inflammation, and in Vitro Arthritis Model: An Insight of Mechanistic Study," *International Immunopharmacology* 41 (2016): 106–115, <https://doi.org/10.1016/j.intimp.2016.10.024>.
45. O. U. Estella, A. C. William, O. Patrick, et al., "Evaluation of the Analgesic and Antipyretic Activity of Methanol Extract of *Combretum bauchiense* Hutch & Dalziel (Combretaceae) Leaves," *Phytomedicine Plus* 2, no. 1 (February 2022): 100166, <https://doi.org/10.1016/j.phyplu.2021.100166>.
46. M. S. Reza, M. Jashimuddin, J. Ahmed, et al., "Pharmacological Investigation of Analgesic and Antipyretic Activities of Methanol Extract of the Whole Part of *Aeginetia indica*," *Journal of Ethnopharmacology* 271 (May 2021): 113915, <https://doi.org/10.1016/j.jep.2021.113915>.
47. Y. A. Ferede, W. S. Zewdu, M. M. Zeleke, and M. A. Alemu, "Evaluation of Antidiarrheal Activity of 80% Methanolic Extract of the Leaves of *Cordia africana* (Lamiaceae) in Mice," *Evidence-Based Complementary and Alternative Medicine* 2021, no. 1 (2021): 10, article 3627878, <https://doi.org/10.1155/2021/3627878>.
48. B. Mosisa Gudeta, G. Melesie Taye, T. Abula, and D. Alemayehu Gadisa, "Evaluation of Anti-Diarrheal Activity of 80% Methanol Extracts of *Vernonia amygdalina* Delile (Asteraceae) Leaves in Mice," *Journal of Experimental Pharmacology* 12 (November 2020): 455–462, <https://doi.org/10.2147/JEP.S282669>.
49. F. Bamiaye, A. A. Odutuga, J. Minari, J. O. Dairo, B. S. Fagbohunka, and O. M. Oluba, "Phytochemical Constituents and Antidiarrhoeal Effects of the Aqueous Extract of *Terminalia superba* Leaves on Wistar Rats | Request PDF," (2024), https://www.researchgate.net/publication/285694232_Phytochemical_constituents_and_antidiarrhoeal_effects_of_the_aqueous_extract_of_Terminalia_superba_leaves_on_Wistar_rats.
50. S. Meite, J. D. N'guessan, C. Bahi, H. F. Yapi, A. J. Djaman, and F. G. Guina, "Antidiarrheal Activity of the Ethyl Acetate Extract of *Morinda morindoides* in Rats," *Tropical Journal of Pharmaceutical Research* 8, no. 3 (2009): <https://doi.org/10.4314/tjpr.v8i3.44533>.
51. A. Kumar, A. C. Rana, B. Parashar, A. Kumar, and D. K. Semwal, "Anti-Diarrheal Activity of the Aqueous Extract of Stem Bark of *Myrica nagi* in Albino Rats," *Journal of Exploratory Research in Pharmacology* 8, no. 1 (2023): 20–24.
52. H. Ilmi, I. R. Pamungkas, L. Tumewu, A. F. Hafid, and A. Widyawaruyanti, "Analgesic and Antipyretic Activities of Ethyl Acetate Fraction Tablet of *Andrographis paniculata* in Animal Models," *Evidence-Based Complementary and Alternative Medicine* 2021 (2021): 8, article 8848797, <https://doi.org/10.1155/2021/8848797>.
53. S. Rezq, M. F. Mahmoud, A. M. El-Shazly, M. A. El Raey, and M. Sobeh, "Anti-Inflammatory, Antipyretic, and Analgesic Properties of *Potamogeton perfoliatus* Extract: In Vitro and In Vivo Study," *Molecules* 26, no. 16 (August 2021): 4826, <https://doi.org/10.3390/molecules26164826>.
54. S. Irshad, I. Anjum, M. N. Mushtaq, et al., "Anti-Inflammatory, Analgesic and Anti-Pyretic Activity of *Fagonia bruguieri* DC in Rats," *Pakistan journal of pharmaceutical sciences* 35, no. 4 (Special) (2022): 1209–1213.
55. M. A. R. B. Shovo, M. R. Tona, J. Mouah, et al., "Computational and Pharmacological Studies on the Antioxidant, Thrombolytic, Anti-Inflammatory, and Analgesic Activity of *Molineria Capitulata*," *Current Issues in Molecular Biology* 43, no. 2 (June 2021): 434–456, <https://doi.org/10.3390/cimb43020035>.
56. M. T. Ahsan, N. N. Maria, U. Tahmida, A. A. Jasmin, and D. U. S. Chowdhury, "Anxiolytic, Analgesic and Anti-Inflammatory Effects of *Citrus Maxima* (Burm.) Merr. Seed Extract in Swiss Albino Mice Model," *Clinical Phytoscience* 9, no. 1 (2023): 2, <https://doi.org/10.1186/s40816-023-00354-7>.
57. J. A. Guerrero-Solano, M. Bautista, J. V. Espinosa-Juárez, et al., "Differential Antinociceptive Efficacy of Peel Extracts and Lyophilized Juices of Three Varieties of Mexican Pomegranate (*Punica granatum* L.) in the Formalin Test," *Plants* 12, no. 1 (December 2022): 131, <https://doi.org/10.3390/plants12010131>.
58. I. Hirota, Y. Koyama, and S. Shimada, "Histochemical Analysis of the Biphasic Properties of Formalin Pain-Induced Behavior," *Biochemistry and Biophysics Reports* 34 (July 2023): 101467, <https://doi.org/10.1016/j.bbrep.2023.101467>.
59. D. Srebro, K. Rajković, B. Dožić, et al., "Investigation of the Antinociceptive Activity of the Hydroethanolic Extract of *Junglas nigra* Leaf by the Tail-Immersion and Formalin Pain Tests in Rats," *Dose-Response* 20, no. 3 (July 2022): 155932582211198, <https://doi.org/10.1177/15593258221119877>.
60. K. Lu, C.-Y. Yang, and Z.-H. Yan, "Analgesic and Anti-Inflammatory Activity of *Cotinus coggygria* Scop. Extracts in Vivo," *Asian Pacific Journal of Tropical Biomedicine* 14, no. 6 (2024): 225–235, https://doi.org/10.4103/apjtb.apjtb_132_24.
61. A. Lekouaghet, A. Boutefnouchet, C. Bensaïci, L. Gali, K. Ghenaïet, and L. Tichati, "In Vitro Evaluation of Antioxidant and Anti-Inflammatory Activities of the Hydroalcoholic Extract and Its Fractions From *Leuzea conifera* L. Roots," *South African Journal of Botany* 132 (August 2020): 103–107, <https://doi.org/10.1016/j.sajb.2020.03.042>.
62. R. S. N. A. K. K. Chaitanya, S. S. D. Banji, V. Ravindran, and S. Murali, in *HRBC Membrane Stabilizing Property of Root, Stem and Leaf of *Glochidion velutinum* | Request PDF* (2024), https://www.researchgate.net/publication/284148206_HRBC_Membrane_Stabilizing_Property_of_Root_Stem_and_Leaf_of_Glochidion_velutinum.
63. S. Yesmin, A. Paul, T. Naz, et al., "Membrane Stabilization as a Mechanism of the Anti-Inflammatory Activity of Ethanolic Root Extract of *Choi* (Piper chaba)," *Clinical Phytoscience* 6, no. 1 (August 2020): 59, <https://doi.org/10.1186/s40816-020-00207-7>.
64. Y. H. Gonfa, F. B. Tessema, A. Bachheti, et al., "Anti-Inflammatory Activity of Phytochemicals From Medicinal Plants and Their Nanoparticles: A Review," *Current Research in Biotechnology* 6 (2023): 100152, <https://doi.org/10.1016/j.crbiot.2023.100152>.
65. B. Aryal, B. K. Raut, S. Bhattarai, et al., "Potential Therapeutic Applications of Plant-Derived Alkaloids Against Inflammatory and Neurodegenerative Diseases," *Evidence-Based Complementary and Alternative Medicine* 2022 (March 2022): 18, article 7299778, <https://doi.org/10.1155/2022/7299778>.
66. N.-Y. Park, S. Im, and Q. Jiang, "Different Forms of Vitamin E and Metabolite 13'-carboxychromanols Inhibit Cyclooxygenase-1 and Its Catalyzed Thromboxane in Platelets, and Tocotrienols and 13'-Carboxychromanols Are Competitive Inhibitors of 5-Lipoxygenase," *The Journal of Nutritional Biochemistry* 100 (February 2022): 108884, <https://doi.org/10.1016/j.jnutbio.2021.108884>.
67. M. Herranz-López, M. Losada-Echeberriá, and E. Barrajón-Catalán, "The Multitarget Activity of Natural Extracts on Cancer: Synergy and

Xenohormesis,” *Medicines* 6, no. 1 (March 2019): 6, <https://doi.org/10.3390/medicines6010006>.

68. G. Sliwoski, S. Kothiwale, J. Meiler, and E. W. Lowe, “Computational Methods in Drug Discovery,” *Pharmacological Reviews* 66, no. 1 (2014): 334–395, <https://doi.org/10.1124/pr.112.007336>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. (*Supporting Information*)

Supporting Figure: Clot lysis observed before and after the experiment. The figure illustrates the visual difference between the intact blood clot prior to treatment and the extent of clot degradation following the experimental procedure, demonstrating the clot-lysing activity of the tested sample.