

Article

A Multifaceted Exploration of *Shirakiopsis indica* (Willd) Fruit: Insights into the Neuropharmacological, Antipyretic, Thrombolytic, and Anthelmintic Attributes of a Mangrove Species

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Abstract

Background: *Shirakiopsis indica* (Willd.) (Family: Euphorbiaceae), a mangrove species found in the Asian region, is a popular folkloric plant. Locally, the plant is traditionally used to treat various types of ailments, especially for pain relief. Therefore, the current study investigates the neuropharmacological, antipyretic, thrombolytic, and anthelmintic properties of the *S. indica* fruit methanolic extract (SIF-ME). **Methods:** The neuropharmacological activity was evaluated using several bioactive assays, and the antipyretic effect was investigated using the yeast-induced pyrexia method, both in Swiss albino mice models. Human blood clot lysis was employed to assess thrombolytic activity, while in vitro anthelmintic characteristics were tested on *Tubifex tubifex*. Insights into phytochemicals from SIF-ME have also been reported from a literature review, which were further subjected to molecular docking, pass prediction, and ADME/T analysis and validated the wet-lab outcomes. **Results:** In the elevated plus maze test, SIF-ME at 400 mg/kg demonstrated significant anxiolytic effects (200.16 ± 1.76 s in the open arms, $p < 0.001$). SIF-ME-treated mice exhibited increased head dipping behavior and spent a longer time in the light box, confirming strong anxiolytic activity in the hole board and light–dark box tests, respectively. It (400 mg/kg) also significantly reduced depressive behavior during forced swimming and tail suspension tests (98.2 ± 3.83 s and 126.33 ± 1.20 s, respectively). The extract induced strong locomotor activity, causing mice’s mobility to gradually decrease over time

in the open field and hole cross tests. The antipyretic effect of SIF-ME (400 mg/kg) was minimal using the yeast-induced pyrexia method, while it (100 µg/mL) killed *T. tubifex* in 69.33 ± 2.51 min, indicating a substantial anthelmintic action. SIF-ME significantly reduced blood clots by 67.74% ($p < 0.001$), compared to the control group's 5.56%. The above findings have also been predicted by in silico molecular docking studies. According to the molecular docking studies, the extract's constituents have binding affinities ranging from 0 to -10.2 kcal/mol for a variety of human target receptors, indicating possible pharmacological activity. **Conclusions:** These findings indicate that SIF-ME could serve as a promising natural source of compounds with neuropharmacological, anthelmintic, thrombolytic, and antipyretic properties.

Keywords: *Shirakiopsis indica*; anxiolytic; antidepressant; antipyretic; thrombolytic; anthelmintic

1. Introduction

In the world, anxiety and depression are the two most common psychiatric diseases and are complicated and heterogeneous [1]. According to a WHO report, anxiety disorders impact almost 4.4% of the world's population (around 300 million individuals) [2]. Genetic, epigenetic, and environmental factors can impact anxiety and depression, directing additional pathophysiological complexes in the right directions [3]. Sadness, a lack of interest or pleasure, guilt, low self-worth, restless nights or irregular eating patterns, fatigue, and poor concentration are all signs of depressive disorders, which can result in suicide. Human brain diseases can cause anxiety and significantly reduce one's ability to carry out daily tasks and live comfortably. Fear and anxiety are two of the symptoms of anxiety disorders [4,5]. Selective serotonin reuptake inhibitors, selective serotonin and norepinephrine reuptake inhibitors, pregabalin, tricyclic antidepressants, buspirone, benzodiazepines, and monoamine oxidase inhibitors are among the medications used to treat anxiety disorders [6]. Tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors, serotonin and norepinephrine reuptake inhibitors, norepinephrine and dopamine reuptake inhibitors, and serotonin antagonist and reuptake inhibitors are among the medications used in the pharmacological treatment of depressive disorders [7]. For example, TCAs relieve depression by boosting norepinephrine and serotonin levels in the brain and by preventing the enzymatic breakdown of serotonin, norepinephrine, and dopamine. MAOIs function as antidepressants by enhancing neurotransmission, which elevates mood [8]. Synthetic antidepressants and anxiolytics can cause serious adverse effects such as headaches, sexual dysfunction, addiction, seizures, and suicidal thoughts. To avoid dangerous side effects, many patients prefer to use herbal medicines to treat symptoms of depression, anxiety, stress, and other mental diseases that are on the rise globally [9].

Insomnia is a common problem affecting people of all ages worldwide. This common and serious disorder may negatively impact the daily functioning, health, and quality of life of individuals of any age [10]. People who are depressed and exhibit anxiety and worry are typically administered sedatives. Short-term use of these medicines may be beneficial, but long-term usage may lead to drug addiction. Consequently, studies are being conducted to find a safe and reliable natural drug source to eventually develop safe sedative medications [11].

An example of the body's intricate immunological physiological reaction to an infection or an inflammatory stimulus is fever (pyrexia), which sets off a chain reaction that produces various endogenous pyrogens through biochemical reactions [12]. Damaged tissue can lead to graft rejection, infection, cancerous tumors, other ailments, and fever or hyperpyrexia. Prostaglandin E2 (PGE2) is synthesized near the preoptic hypothalamic zone and can cause a rise in body temperature due to the high production of pro-inflammatory mediators like TNF- α , interleukin-1 β , and interleukin- α [13]. There are numerous commercially available medication moieties to lessen the effects of fever incidence, even though serious side effects such as disturbances of the stomach mucosa have, in rare circumstances, progressed to ulcerative states. Research on natural phytomedicines has recently become more interested in finding new herbal anti-pyretic chemicals without side effects to counteract these undesirable conditions that conventional medications have been shown to cause [14].

Once again, thrombosis is a blood clot that can cause coronary blood abnormalities, such as acute myocardial infarction and fatal brain hemorrhages. Intravenous heparin is the first-line treatment for thrombus due to its viability, safety, and potency [15]. Because they are less expensive than other thrombolytic drugs, streptokinase and urokinase are frequently used; however, they are also dangerous because they can cause severe bleeding, re-occlusion, and re-infarction [16].

Infestation by parasitic nematodes, trematodes, cestodes, and other larval insects can result in bronchitis, anemia, eosinophilia, malnourishment, and many other symptoms in humans and other animals. It is known as helminthiasis [17]. The idea of immunological regulation by parasites is worldwide and encompasses the processes of immune response suppression, pathogen distraction, and conversion. During this period, the immune response frequently progresses to generate pathologic alterations, the leading cause of disease in many helminth infections [18]. Synthetic medications, such as pyrantel pamoate, an agonist of the nicotinic acetylcholine receptor, β -tubulin, and benzimidazoles (mebendazole and albendazole), are used to control helminthic disease in various parts of the world. These medications are very effective at curing helminthiasis because they block the closure of glutamate-gated chloride channels, causing hyperpolarization, although they have several side effects [19]. Drug resistance is a significant issue in several parasite disorders brought on by the prolonged use of synthetic anthelmintic/larvicidal medications. The crude compounds obtained from plants are comparatively free of adverse effects, but are less effective at curing parasite disorders. In undeveloped nations, the traditional treatment for helminthiasis involves using numerous medicinal plants. Thus, there is a growing interest in the use of plant-derived medications to treat parasite infections [20].

Researchers in the field of biology are currently generating enormous opportunities through computational biology, which validates their findings. Computer-simulated screening can accurately describe the pharmacological activities of phytochemicals. Computer-aided drug development (CADD) techniques and molecular docking have been demonstrated through an *in silico* process to develop and investigate drug design within a limited period. A successful molecular docking process should be able to determine the ligands' status at the binding site and the protein structure's physicochemical relationship [8,21].

Since medicinal plants include distinctive compounds that have a variety of activities to treat different ailments, their significance in drug discovery is constantly growing. Herbal or conventional medications are used by 80% of people worldwide, either directly or indirectly [22]. Plant extracts' physiological and medicinal properties may be attributed to certain compounds that have been found through phytochemical investigation [23]. Natural drugs are similar to synthetic drugs in their ability to treat ailments, but they have fewer adverse effects [24]. The current state of finding substitute medications for mental

disorders derived from Shirakiopsis has significantly improved. In East Asia, *Shirakiopsis indica* (Willd.) is a member of the Shirakiopsis genus and the Euphorbiaceae family. It can be found in various water features, including rivers, beaches, and mangrove forests. Studies on the medicinal qualities of Shirakiopsis species have included antioxidant, anti-inflammatory, analgesic, cytotoxic, and anti-H. pylori effects [25]. The fruit of *Shirakiopsis indica*, called SaMor-Ta-Lay in Thai, is the most popular plant used to treat gastrointestinal disorders [26]. The previously published optimum IC₅₀ values showed that the ethanolic extract of *Shirakiopsis indica* had the strongest cytotoxic effects on the Kato III gastric cancer cell line and the most notable inhibition of nitric oxide synthesis. The fruit of *S. indica* possesses significant analgesic, anti-inflammatory, and antioxidant activities [25]. Significant pharmacological actions in in vitro and in vivo studies, along with proven ethnobotanical importance, make it a more suitable choice for further studies to pave the way for the exploration of new therapeutics.

The goal of this study is to evaluate the bioactive phytochemicals and pharmacological effects of the *S. indica* fruits, as there has not been any previous research on its anxiolytic, anti-depressant, locomotor, antipyretic, thrombolytic, and anthelmintic properties. This study fills a unique research void in medicinal plant-based medicines by presenting empirical data supporting the *S. indica* fruit's therapeutic potential. It bridges the gap between conventional medical knowledge and contemporary scientific confirmation by proving its efficacy in treating anxiety, depression, sleep disorders, fever, blood clotting ailments, and helminthiasis, opening the door for the creation of innovative treatments in these fields.

2. Results

2.1. Acute Toxicity Study

The first thing we saw was the purification of the compound. The plant was harvested in the natural environment. Following the oral administration of 1000, 2000, and 4000 mg/kg, no unfavorable behavioral changes, morbidity, or death occurred over the 8 h and 3-day observation phases following treatment with the SIF-ME extract.

2.2. Neuropharmacological Activity

2.2.1. Anxiolytic Activity

Elevated Plus Maze Test

The elevated plus maze method, or EPM, is a well-recognized test for assessing the anxiolytic effects of substances; it evaluates psychomotor skills and affective components in mice and analyzes exploratory behavior in an anxiety-inducing environment. The basis of the EPM test is the concept that exposure to an open arm of EPM causes a far greater approach–avoidance conflict than exposure to an enclosed arm. Mice treated with SIF-ME at a dose of 400 mg/kg spent 200.16 ± 1.76 s in the open arms of the elevated plus maze (EPM), significantly more than the control group, which spent only 126.14 ± 2.41 s ($p < 0.001$). The standard treatment, diazepam, resulted in mice spending an even longer duration in the open arms (234.87 ± 2.72 s, $p < 0.001$). However, compared to the control group, mice treated with SIF-ME (400 mg/kg) spent 59.69% of their time in the open arms, while diazepam-treated mice spent 87.20% of their time there.

At both doses of 200 and 400 mg/kg, SIF-ME caused mice to spend more time in the closed arms and less time in the open arms compared to diazepam-treated mice. Conversely, SIF-ME-treated mice spent more time in the open arms and less time in the closed arms compared to the control group. Based on these findings, it can be concluded that SIF-ME demonstrates significant anxiolytic activity (Supplementary Table S1, Figure 1A).

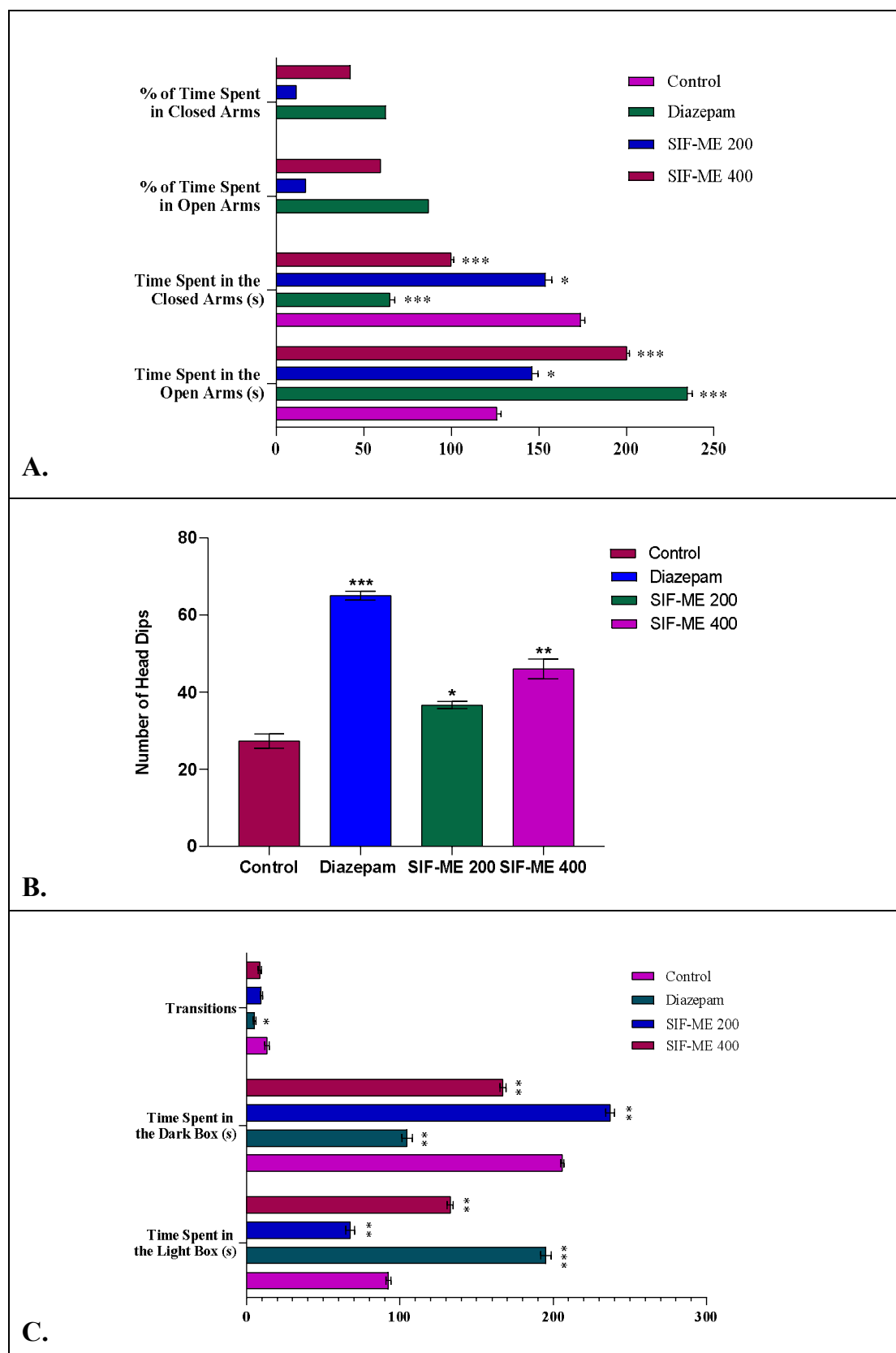


Figure 1. The elevated plus maze test (A), hole board test (B), and light–dark box test (C) were employed to examine the anxiolytic properties of SIF-ME. The results are shown as the means $\pm$ SEMs. Symbols in this section indicate statistical significance relative to the control group: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

Hole Board Test

The frequency of head dipping behavior was found to increase as the extract dose increased in the hole board test. Significant head dipping was observed in the groups treated with SIF-ME at the 200 and 400 mg/kg dosages (36.66 ± 0.88 and 46 ± 2.51) when compared to the control group (27.33 ± 1.86). In comparison to the control group, the diazepam group also demonstrated a substantial number of head dips (65 ± 1.15 , $p < 0.001$). In comparison to the control group (9 ± 0.58 s), the group treated with SIF-ME at 400 mg/kg doses demonstrated a rapid response in the first head dipping (3.67 ± 0.33 s). It can be assumed that SIF-ME at 400 mg/kg dosages exhibited strong anxiolytic activity because the frequency and latency of the first head drop were much higher than those of the control treatment (Figure 1B).

Light–Dark Box Test

In the light–dark test, mice treated with SIF-ME at a dose of 400 mg/kg spent more time (132.86 ± 2.05 s; $p < 0.01$) in the light box compared to the control-treated mice (92.5 ± 1.69 s). The standard diazepam treatment showed significant anxiolytic activity ($p < 0.001$), as the mice spent considerable time (195.24 ± 3.41 s) in the light box. The other dose of 200 mg/kg did not show anxiolytic activity, as the mice spent less time in the light box (62.73 ± 2.91 s) and more time in the dark box (237.267 ± 2.91 s) in comparison to the control mice. However, transitions were lowered by the standard diazepam treatment and SIF-ME at both doses (Table 1, Figure 1C).

Table 1. Evaluation of anxiolytic activity through the light–dark box test. All values are shown as the means $\pm$ SEMs, and the statistical analysis was performed using one-way analysis of variance (ANOVA). Subsequently, $n = 5$ as employed for Dunnett’s multiple comparison test, with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in comparison to the control group.

Treatment (mg/kg)	Time Spent in the Light Box (s)	Time Spent in the Dark Box (s)	Transitions
Control	92.5 ± 1.69	205.83 ± 1.20	13.33 ± 1.33
Diazepam	195.24 ± 3.41 ***	104.75 ± 3.41 **	5.33 ± 0.88 *
SIF-ME 200	62.73 ± 2.91 **	237.267 ± 2.91 **	9.66 ± 0.66
SIF-ME 400	132.86 ± 2.05 **	167.13 ± 2.05 **	8.66 ± 1.20

2.2.2. Antidepressant Activity

Forced Swimming Test

In this test, SIF-ME showed significant antidepressant activity in a dose-dependent manner. SIF-ME at both 200 and 400 mg/kg doses reduced the immobility time of the mice (137.87 ± 2.77 s and 98.2 ± 3.83 s, respectively). The standard fluoxetine treatment reduced the immobility time significantly (89.26 ± 1.57 s, $p < 0.001$) in comparison to the control (187.47 ± 2.46 s). The immobility (%) was increased by the 400 mg/kg doses of SIF-ME (47.62%) when compared to the control (Supplementary Table S2, Figure 2A).

Tail Suspension Test

SIF-ME at both 200 and 400 mg/kg reduced the immobility time significantly (182.66 ± 2.40 s and 126.33 ± 1.20 s, respectively) when compared to the control (214.66 ± 3.28 s). Fluoxetine also reduced the immobility time significantly (71.33 ± 2.33 s, $p < 0.001$). When compared to the control, SIF-ME at a 400 mg/kg dose showed a reduction in immobility of 41.15%. All this result suggests that SIF-ME had significant antidepress-

sant activity compared to the control but less significant compared to the positive control fluoxetine (Figure 2B).

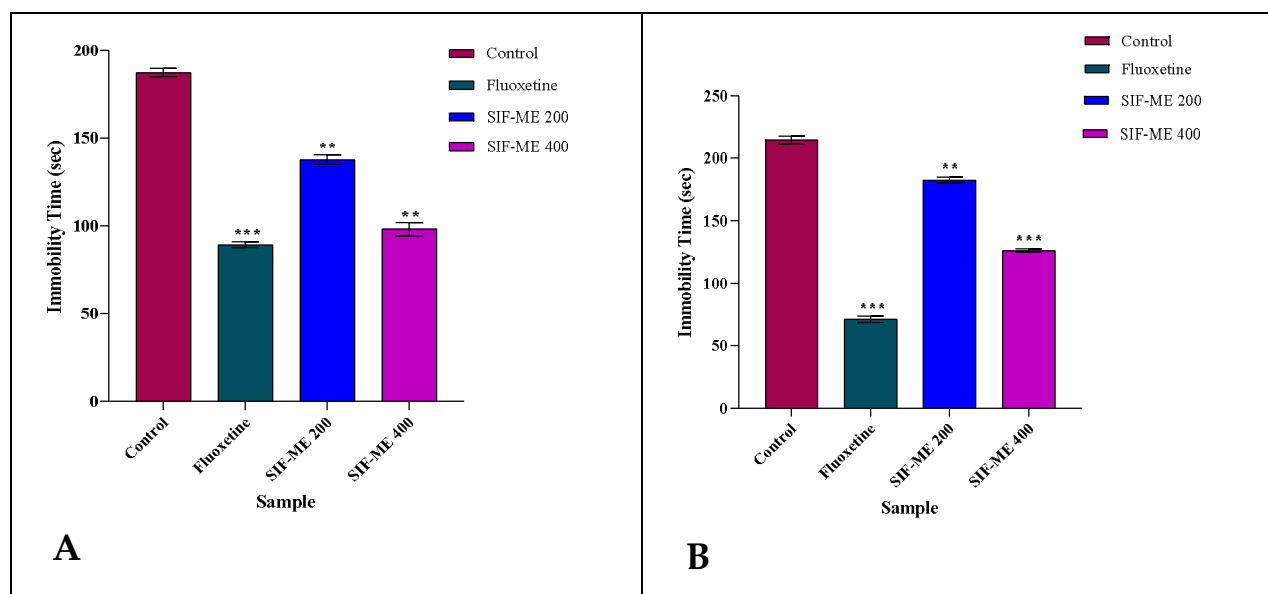


Figure 2. The forced swimming (A) and tail suspension (B) tests were employed to examine the antidepressant properties of SIF-ME. The results are shown as the means $\pm$ SEMs. Symbols in this section indicate statistical significance relative to the control group: ** $p < 0.01$; and *** $p < 0.001$.

2.2.3. Locomotor Activity

Open Field Test

In the open field test, SIF-ME-treated mice (both 200 and 400 mg/kg) significantly reduced the number of crossed squares (38.33 ± 1.33 and 25.33 ± 1.45 , respectively) in comparison to the control mice (66.33 ± 1.76) at 120 min. The standard treatment diazepam, also reduced the movement of mice significantly (26.66 ± 2.60 , $p < 0.001$). As the movement of mice was reduced with time, and it was significant with the control, this extract had a significant effect on locomotor activity (Supplementary Table S3, Figure 3A).

Hole Cross Test

Locomotor activity was also assessed by the hole-cross test. In this test, SIF-ME-induced mice showed significant locomotor activity in a dose-dependent manner. At 120 min, SIF-ME (400 mg/kg dose) significantly reduced the hole crossing of mice (7.33 ± 0.33 , $p < 0.01$) when compared to the control, whereas diazepam resulted in 4.33 ± 0.88 number of hole crossing (Table 2, Figure 3B).

Table 2. Evaluation of locomotor activity through the hole cross test.

Treatment Dose (mg/mL)	Number of Holes Crossed				
	0 min	30 min	60 min	90 min	120 min
Control	17.33 ± 0.88	18.33 ± 0.88	16.33 ± 1.20	14 ± 1.15	12.33 ± 0.33
Diazepam	10.66 ± 1.45	7.33 ± 0.88 **	11.66 ± 0.88 **	9.33 ± 0.66	4.33 ± 0.88 **
SIF-ME 200	15.33 ± 0.88 ***	10.33 ± 1.20 *	13.33 ± 1.20 ***	10.66 ± 0.66 *	9.33 ± 0.88 *
SIF-ME 400	11.33 ± 1.20 *	7.66 ± 1.20 **	10.66 ± 0.88 **	9.66 ± 0.88 *	7.33 ± 0.33 **

All values are shown as the means $\pm$ SEMs, and the statistical analysis was performed using one-way analysis of variance (ANOVA). Subsequently, $n = 5$ was employed for Dunnett's multiple comparison test, with * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ in comparison to the control group.

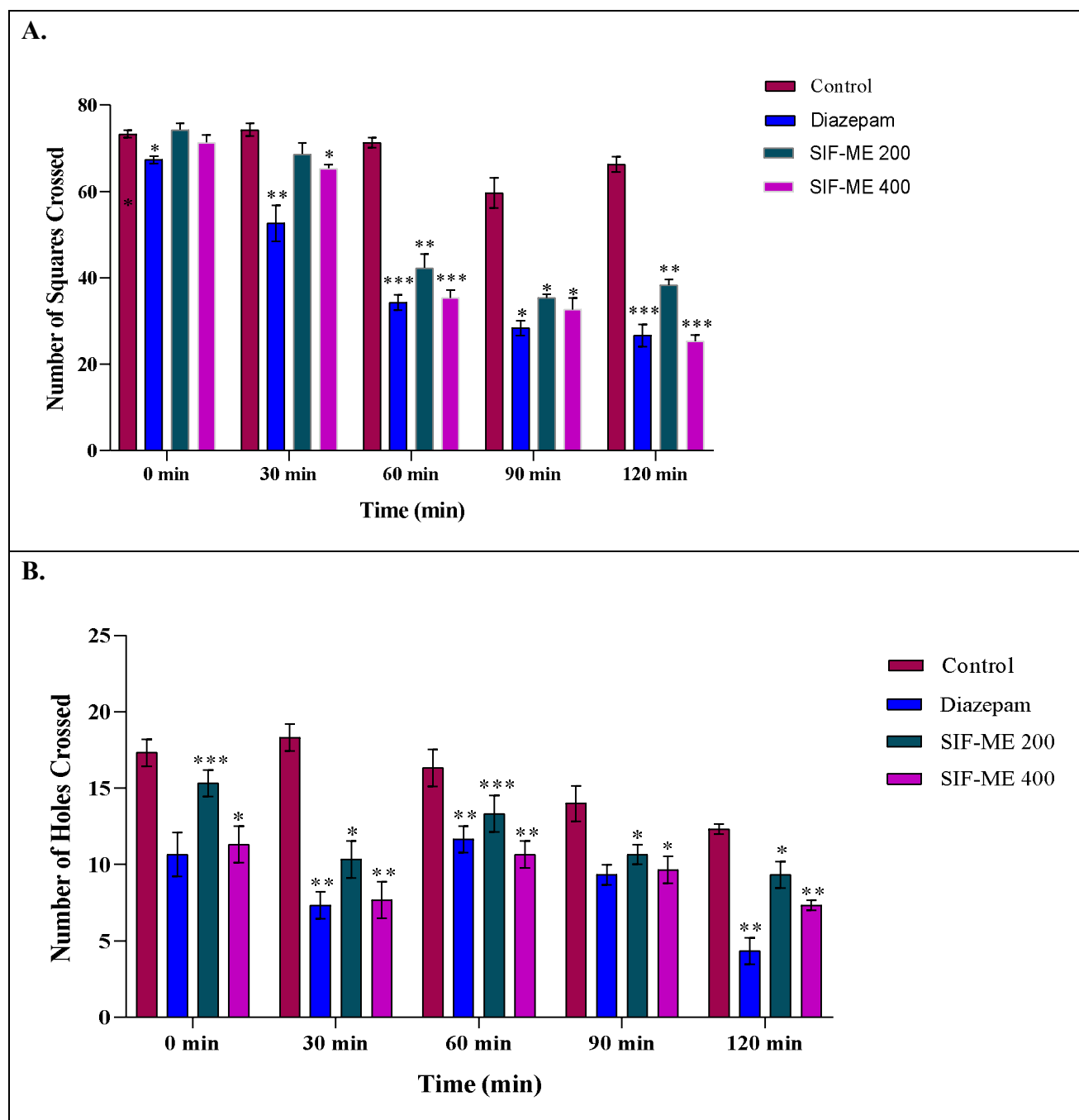


Figure 3. The open field test (A) and hole cross test (B) were employed to examine the locomotor properties of SIF-ME. The results are shown as the means $\pm$ SEMs. Symbols in this section indicate statistical significance relative to the control group: * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$.

2.3. Antipyretic Activity

Yeast-Induced Pyrexia Method

In this test, SIF-ME demonstrated a modest antipyretic effect compared to the control. During this study, pyrexia was caused by administering a yeast suspension subcutaneously. SIF-ME (400 mg/kg) lowered the rectal temperature to 99.3 ± 0.53 °F after 180 min of treatment, compared to 99.53 ± 0.96 °F in the control group. At 120 min, paracetamol significantly reduced rectal temperature to 99.2 ± 1.12 °F ($p < 0.05$) (Table 3, Figure 4A).

Table 3. Evaluation of antipyretic activity through the yeast-induced pyrexia method.

Treatment	Normal Rectal Temperature (°F)	Temperature After Pyrexia (°F)	Rectal Temperature (°F) After Drug Administration		
			60 min	120 min	180 min
Control	99.06 ± 0.58	103.1 ± 1.92	99.74 ± 1.08	101.63 ± 1.7	99.53 ± 0.96
Paracetamol	98.69 ± 0.42	100.98 ± 2.58	100.63 ± 2.5	99.1 ± 0.6 *	99.2 ± 1.12
SIF-ME 200	98.56 ± 0.15	100.56 ± 1.86	103.86 ± 1.35 *	101.26 ± 2.14	103.1 ± 0.52 *
SIF-ME 400	99.15 ± 0.6	100.96 ± 1.68	101.06 ± 1.56	100.7 ± 2.1	99.3 ± 0.53

All values are shown as the means ± SEMs, and the statistical analysis was performed using one-way analysis of variance (ANOVA). Subsequently, n = 5 was employed for Dunnett's multiple comparison test, with * $p < 0.05$ in comparison to the control group.

2.4. Thrombolytic Activity

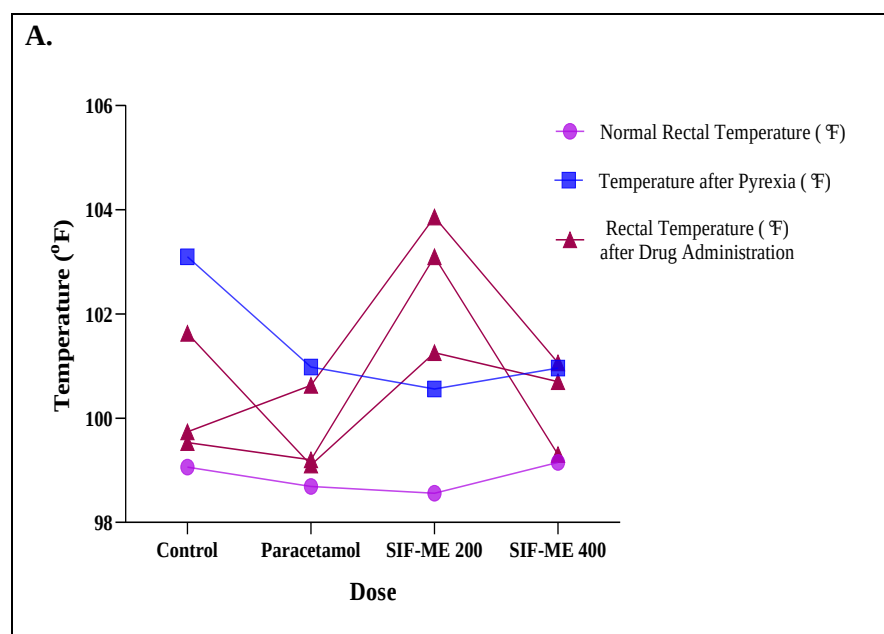
Human Blood Clot Lysis Method

The thrombolytic activity of the extract SIF-ME was evaluated through the human blood clot lysis method. In this test, SIF-ME demonstrated significant blood clot lysis of 67.74% ($p < 0.001$), whereas the control group showed 5.56%. The standard streptokinase showed significant clot lysis of $74.19 \pm 0.66\%$ ($p < 0.001$). This result suggests that SIF-ME had strong thrombolytic activity (Figure 4B).

2.5. Anthelmintic Activity

In Vitro Anthelmintic Test on *Tubifex tubifex*

Comparing SIF-ME to the reference medication albendazole, this study showed that SIF-ME had dose-dependent anthelmintic activity. At doses of 10, 25, 50, and 100 µg/mL, the extract immobilized *Tubifex tubifex* for 79.33 ± 2.51 , 71 ± 3.60 , 60.66 ± 3.21 , and 38.33 ± 3.51 min, respectively. The parasite was rendered immobile by albendazole (10 µg/mL) in 19.33 ± 1.52 min. *Tubifex tubifex* was killed by the extract in 150.33 ± 2.51 , 123.66 ± 2.88 , 99.33 ± 3.21 , and 69.33 ± 2.51 min at doses of 10, 25, 50, and 100 µg/mL, respectively. At 10 µg/mL, the standard albendazole killed the parasite in 46.33 ± 2.081 min (Figure 4C).

**Figure 4.** Cont.

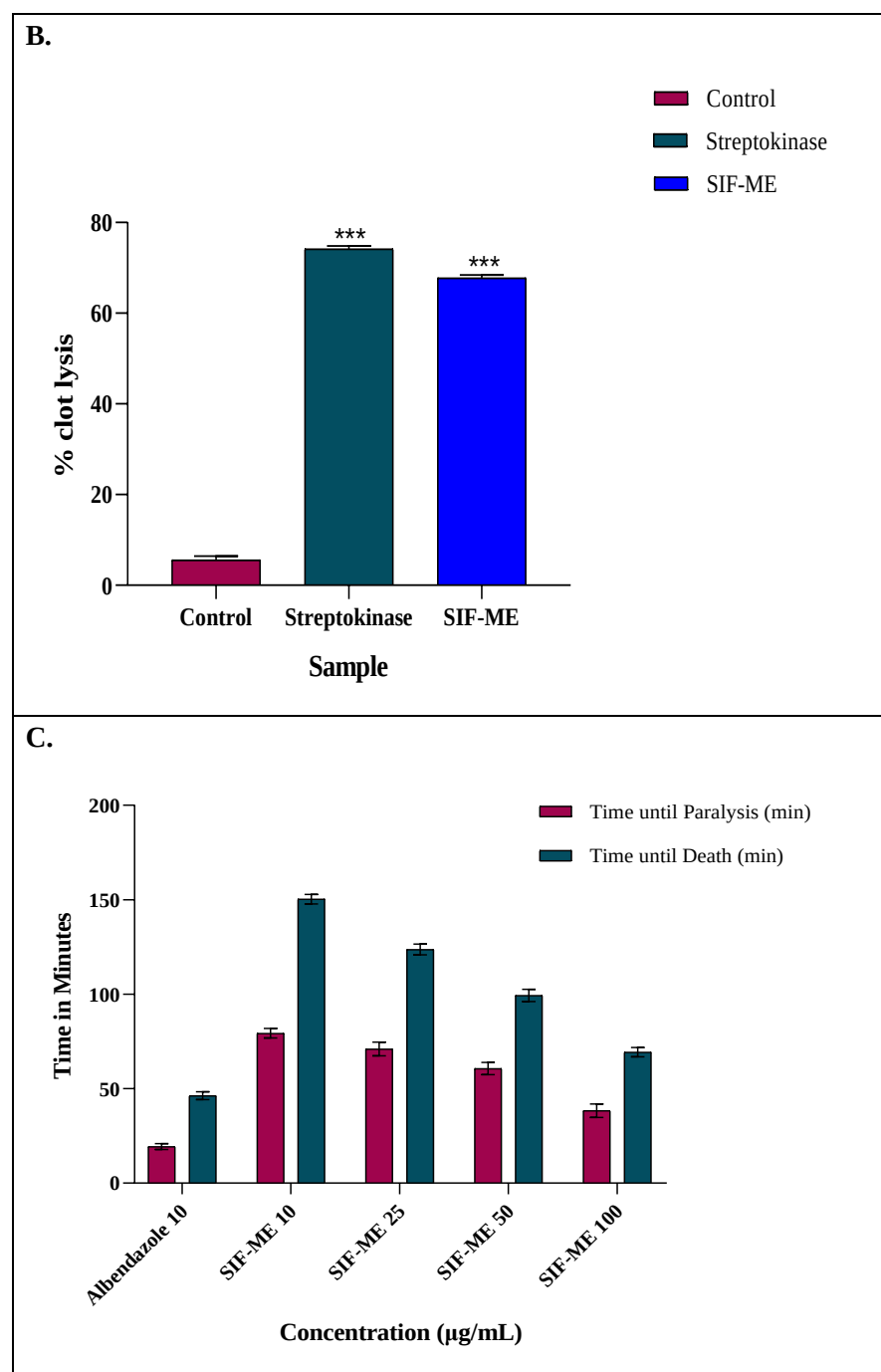


Figure 4. The yeast-induced pyrexia test (A), human blood clot lysis test (B), and in vitro Test on the *Tubifex tubifex* parasite (C) were employed to examine the antipyretic, thrombolytic, and anthelmintic properties of SIF-ME. The results are shown as the means $\pm$ SEMs. Symbols in this section indicate statistical significance relative to the control group: *** $p < 0.001$.

2.6. PASS Prediction Study

A PASS prediction analysis serves as a valuable tool for identifying and prioritizing compounds with desired biological activities, guiding experimental design, and improving the efficiency and ethical standards of preclinical research. The PASS web-based application evaluated 18 specially chosen SIF-ME compounds for their neuropharmacological, antipyretic, thrombolytic, and anthelmintic properties. Based on the results, molecules with Pa values higher than the Pi showed a lot of molecular potential (Table 4).

Table 4. PASS prediction of SIF-ME's suggested bioactive compounds.

Compounds	Biological Activity											
	Anxiolytic		Antidepressant		Locomotor		Antipyretic		Thrombolytic		Anthelmintic	
	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
Butanoic acid, 3-methyl	-	-	-	-	0.214	0.062	0.324	0.038	0.265	0.018	0.291	0.041
Phenol, 2-methoxy	0.127	0.013	0.196	0.062	0.139	0.100	0.472	0.017	0.264	0.019	0.308	0.035
2-Methoxy-4-vinyl phenol	0.229	0.005	-	-	-	-	0.427	0.022	0.200	0.073	0.291	0.041
Phenol, 2,6-dimethoxy	0.097	0.021	0.155	0.085	0.167	0.065	0.395	0.026	0.291	0.011	0.278	0.046
Vanillin	0.073	0.042	-	-	0.142	0.095	0.447	0.020	0.174	0.110	0.369	0.021
Phenol, 2-methoxy-4-propyl	0.098	0.020	-	-	-	-	0.492	0.014	0.258	0.021	0.236	0.065
Phenol, 4-ethenyl-2,6-dimethoxy	0.157	0.009	-	-	-	-	0.293	0.048	0.220	0.049	0.277	0.046
Benzaldehyde, 3-hydroxy-4-methoxy	0.073	0.042	-	-	0.142	0.095	0.447	0.020	0.174	0.110	0.369	0.021
5-Methyl-3-phenyl-1,3-oxazolidine	-	-	-	-	-	-	-	-	-	-	0.161	0.135
2,6-Dimethoxyhydroquinone	0.111	0.016	-	-	-	-	0.344	0.034	0.292	0.011	0.293	0.040
Benzaldehyde, 4-hydroxy-3,5-dimethoxy	-	-	-	-	0.180	0.053	0.306	0.043	0.196	0.079	0.349	0.025
Phenol, 2,6-dimethoxy-4-(2-propenyl)	0.143	0.011	-	-	-	-	0.393	0.027	0.193	0.083	0.268	0.050
2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)	0.066	0.053	-	-	-	-	0.334	0.036	0.209	0.062	0.325	0.086
Retinoic acid	-	-	-	-	-	-	-	-	-	-	0.306	0.035
Octahydro-9-phenanthrene methanol	0.069	0.048	0.229	0.046	-	-	0.275	0.056	-	-	0.166	0.129
Epoxylathyrol	-	-	-	-	-	-	-	-	-	-	0.259	0.054
Beta-Sitosterol	-	-	-	-	-	-	-	-	-	-	-	-
24-Noroleana-3,12-diene	-	-	-	-	-	-	-	-	0.319	0.007	-	-

2.7. In Silico Study

2.7.1. ADME/T Study

The purpose of ADME/T (absorption, distribution, metabolism, excretion, and toxicity) studies in molecular docking is to evaluate the drug-likeness and safety of potential compounds before experimental validation. An ADME/T analysis predicts how a compound behaves in the body, ensuring that it has favorable pharmacokinetic and toxicity profiles. Based on that study, each molecule meets Lipinski's requirements and can be consumed orally. The online admetSAR server (<http://lmmd.ecust.edu.cn/admetSAR2/>, accessed on 11 September 2024) and the pKCSM online tool (<http://biosig.unimelb.edu.au/pkcsM/>, accessed on 11 September 2024) were also used to predict the toxicological features of the 18 chemicals. The findings of the analysis show that the tested substances are not carcinogenic or poisonous (Table 5).

Table 5. In silico AdmetSAR and drug-likeness study of selected compounds in the SIF-ME.

Compound Name	Absorption		Distribution		Metabolism	Excretion	Toxicity		Drug Likeness	Bioavailability
	Water Solubility (log mol/L)	Intestinal Absorption (Human) (% Absorbed)	VDss (Human) (log L/kg)	BBB Permeability (log BB)	CYP3A4 Substrate	Total Clearance (log mL/min/kg)	AMES Toxicity	Hepatotoxicity		
Butanoic acid, 3-methyl	−0.811	88.82	−0.937	−0.227	No	0.391	No	No	Yes	0.85
Phenol, 2-methoxy	−1.264	93.374	0.174	−0.226	No	0.219	No	No	Yes	0.55
2-Methoxy-4-vinyl phenol	−1.958	91.965	0.118	0.289	No	0.233	Yes	No	Yes	0.55
Phenol, 2,6-dimethoxy	−1.4	93.789	−0.129	−0.204	No	0.213	No	No	Yes	0.55
Vanillin	−1.308	84.976	−0.152	−0.243	No	0.601	No	No	Yes	0.55
Phenol, 2-methoxy-4-propyl	−1.628	92.829	0.36	0.387	No	0.244	No	Yes	Yes	0.55
Phenol, 4-ethenyl-2,6-dimethoxy	−1.925	92.945	0.206	0.396	No	0.241	No	No	Yes	0.55
Benzaldehyde, 3-hydroxy-4-methoxy	−1.295	89.886	−0.165	−0.243	No	0.599	No	No	Yes	0.55
5-Methyl-3-phenyl-1,3-oxazolidine	−2.304	97.289	0.259	0.498	No	0.282	No	No	Yes	0.55
2,6-Dimethoxyhydroquinone	−1.715	85.96	0.169	−0.342	No	0.63	No	No	Yes	0.55
Benzaldehyde, 4-hydroxy-3,5-dimethoxy	−1.481	90.106	−0.042	−0.281	No	0.621	No	No	Yes	0.55
Phenol, 2,6-dimethoxy-4-(2-propenyl)	−2.069	92.959	0.272	0.362	No	0.293	No	No	Yes	0.55
2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)	−1.291	83.06	−0.345	−0.254	No	0.26	No	No	Yes	0.55
Retinoic acid	−4.924	94.419	−0.51	0.236	Yes	1.443	No	Yes	NF	NF
Octahydro-9-phenanthrene methanol	−3.862	95.247	0.995	0.61	Yes	1.176	No	No	Yes	0.55
Epoxylathyrol	−4.273	97.394	0.16	−0.571	No	0.666	No	NO	Yes	0.55
Beta-Sitosterol	−6.773	94.464	0.193	0.781	Yes	0.628	No	No	Yes	0.55
24-Noroleana-3,12-diene	−6.85	96.674	0.44	0.848	Yes	0.073	No	No	Yes	0.55

2.7.2. Molecular Docking Study

Molecular docking was used to investigate the interactions between phytochemical elements in the SIF-ME with a specific protein target. Supplementary Table S4 displays the combined docking score for each activity. The in silico binding affinities and non-bonding interactions of selected phytochemicals of SIF-ME with anxiolytic, antidepressant, locomotor, antipyretic, thrombolytic, and anthelmintic activities are displayed in Supplementary Table S5.

The anxiolytic efficacy of selected SIF-ME substances was assessed by examining their interactions with human monoamine oxidase (PDB: 2Z5X). Retinoic acid (−10.2 kcal/mol) showed the highest binding affinity, followed by dro-9-phenanthrene methanol (−9.3 kcal/mol) and beta-sitosterol (−8 kcal/mol). Retinoic acid formed 18 hydrophobic bonds with key residues, indicating strong binding and a high docking score (Supplementary Figure S1). For antidepressant efficacy, beta-sitosterol (−9.7 kcal/mol) exhibited stronger binding to the human serotonin transporter (PDB: 5I6X) than fluoxetine (−9.1 kcal/mol). It formed eight hydrophobic interactions with key residues, indicating a strong binding affinity, followed by 24-noroleana-3,12-diene (−9.3 kcal/mol) and retinoic acid (−9.2 kcal/mol) (Supplementary Figure S2). 24-Noroleana-3,12-diene (−6.3 kcal/mol) showed the highest binding affinity for the human GABA_A receptor alpha1-beta2-gamma2 subtype, surpassing diazepam (−4.9 kcal/mol). It formed four hydrophobic interactions, indicating strong receptor affinity to provide locomotor activity (Supplementary Figure S3). For antipyretic efficacy, 24-noroleana-3,12-diene (−6.3 kcal/mol) exhibited the highest

binding affinity for microsomal prostaglandin E synthase 1 (mPGES-1) (PDB: 4YK5), forming six hydrophobic bonds, followed by epoxylathyrol (−5.8 kcal/mol) and beta-sitosterol (−5.4 kcal/mol) (Supplementary Figure S4). 24-Noroleana-3,12-diene and beta-sitosterol (−8 kcal/mol) showed strong binding affinity to human tPA, suggesting potential thrombolytic activity (Supplementary Figure S5). 4-Noroleana-3,12-diene (−8.8 kcal/mol) showed superior binding to the tubulin–colchicine: stathmin-like domain complex (PDB: 1SA0) compared to albendazole (−6.3 kcal/mol), forming 12 hydrophobic bonds and indicating strong anthelmintic potential (Supplementary Figure S6).

3. Discussion

Anxiolytic effects, manifested as an increase in time spent in the open arm, are the cause of the decrease in aversion to the open arm. Anxiolytic medications lower the primary index, which is spatial, while anxiogenic substances may improve it [27]. In our test, with SIF-ME (400 mg/kg), the open arm duration was longer than the closed arm duration. This implies the significant anxiolytic activity of SIF-ME. The hole board test can be used to determine anxiety in animals; the rise in head dipping actions may indicate an anxious-reducing mood [28,29]. According to our findings, the anxiolytic-like effect of SIF-ME (400 mg/kg) was confirmed by the increased head dipping behavior (46 ± 2.51 times, $p < 0.01$) and the rapid response to the first head dip in comparison to the control. The conflict between the tendency to explore and the initial inclination to avoid unfamiliar situation causes anxiety in the light/dark test [30]. This conflict can be measured by the number of transitions into and the time spent in the light chamber [31,32], with increases in these parameters being interpreted as indicating anxiolytic-like properties. Our findings indicated that the extract (400 mg/kg) had an anxiolytic effect by increasing the amount of time spent in the light chamber (132.86 ± 2.05 s, $p < 0.01$) compared to the control (92.5 ± 1.69 s).

Typically, antidepressant activity is assessed using the forced swimming test in mouse models. A CNS-depression response is shown in prolonged immobility, whereas a decreasing percentage of immobility duration indicates antidepressant activity [33]. Depression is caused by a decrease in the concentrations of neurochemicals, including dopamine, norepinephrine, and serotonin. Any antidepressant medication increases the activity of at least one of these chemical transmitters at the same time [34–36]. SIF-ME (both 200 and 400 mg/kg) reduced the immobility time (137.87 ± 2.77 s and 98.2 ± 3.83 s, respectively) in mice after administration, according to the forced swimming test. This suggests that SIF-ME may increase the activity of at least one of the neurotransmitters involved in depression. The standard treatment, fluoxetine also exhibited central nervous system (CNS) antidepressant effects in the mouse model, potentially acting through the same mechanism. The main basis for the tail suspension test is the observation of animal behavior under the hemodynamic stress of being dragged in an unpredictable position by its tail, an inevitably stressful condition. In comparison to the control group, the immobility time will be greatly reduced if an antidepressant medication is administered effectively [37]. Following the administration of SIF-ME at 200 and 400 mg/kg, the immobility time was significantly lower than that of the control group, which demonstrated significant antidepressant-like activity. These two methods suggest that the extract had significant antidepressant activity.

As a central nervous system depressant, the medication diazepam, which is a member of the benzodiazepine group, is used to treat sleeping disorders like insomnia. The type-ionophore complex of the GABA receptor contains a binding site for benzodiazepines [38]. They relax the recipient, minimize engagement, and reduce activity. Diazepam and other drugs with sedative properties decrease exploratory activity and lengthen the duration of barbiturate-induced sleep [39]. In both the open field and hole cross tests, any sedative med-

ications will decrease the number of locomotor movements, which is interpreted as a lack of curiosity about new surroundings [38]. An indication of mental alertness or wakefulness is locomotor activity, while a decrease in locomotion is a sign of tranquility and drowsiness, which may be interpreted as a decrease in central nervous system excitability [40]. The number of square blocks crossed (25.33 ± 1.45) and holes crossed (7.33 ± 0.33) in 120 min was reduced by SIF-ME (400 mg/kg), which also affected the mice's locomotor activity, indicating its locomotor effect.

One potential explanation for the activity of antipyretic drugs is the inhibition of prostaglandin synthesis, which occurs when paracetamol blocks cyclooxygenase, hence preventing prostaglandin synthesis [41]. The antipyretic activity is due to the inhibition of several mediators that cause pyrexia [42]. After 180 min of treatment, the rectal temperature was reduced mildly by SIF-ME (400 mg/kg) to 99.3 ± 0.53 °F, while the control group's temperature was 99.53 ± 0.96 °F. As a result, it can be speculated that SIF-ME may have antipyretic properties because it contains bioactive phytochemicals that block prostaglandin formation, just as other analgesic medications, which lower the rectal temperature in mice given yeast injections. Through a reduction in prostaglandin synthesis in the hypothalamus, nonsteroidal anti-inflammatory drugs (NSAIDs) have also been shown to exhibit antipyretic action [43].

According to thrombolytic research, the coagulation process is divided into three stages: the production of the prothrombin activator, thrombin generation, and fibrin development. Thrombolytic or antithrombotic medications can stop a thrombus from forming. Eliminating plasmin fibrin is the main function of the thrombolytic mechanism, and it can be initiated by plasminogen activators that are not inactive. Urokinase (UK) and streptokinase (SK) operate by indirect clot lysis. Through an indirect mechanism, the thrombolytic enzymes successfully remove fibrin, as demonstrated by in vitro thrombolysis investigations [44]. With a blood clot lysis rate of 67.74%, this study demonstrates that SIF-ME can suppress coagulation. This can be a crucial part of the management of thrombolytic treatment without affecting the body's normal clotting process at modest doses.

The results of our current investigation indicate that SIF-ME exhibits notable anthelmintic potential in a dose-dependent manner. A significant number of condensed tannins (4.78 ± 0.34 mg/g TAE) as well as bioactive phytoconstituents such as alkaloids, tannins, flavonoids, phenols, and saponins [25] may be the cause of this action. Alkaloids, tannins, phenols, and other phytochemicals may be the cause of the notable anthelmintic activity [45]. In this case, alkaloids can cause paralysis by affecting the central nervous system (CNS), whereas tannins and polyphenols attach to free proteins in the gastrointestinal tract (GI tract) in a specific way and ultimately result in death. On the other hand, saponins' ability to permeabilize membranes accounts for their anthelmintic effectiveness [46]. One of these phytochemicals alone or in combination may be responsible for SIF-ME's anthelmintic action.

Molecular docking is a type of bioinformatic model in which two or more molecules are combined to form a stable adduct. Molecular docking generates many potential adduct structures, which are then graded and classified using the software's scoring algorithm. The docking technique can be used to estimate the ligand's binding energy, free energy, and stability [47]. Molecular docking studies were used to support the biological testing investigations in this study. It is becoming increasingly important in the process of rational pharmaceutical development. The chemical binding of one molecule (the ligand) to the pocket of another molecule (the receptor), which is often a protein, is known to provide precise pharmacological activity. Protein–ligand docking is the process of finding the exact ligand conformations inside a given protein when the structure of the protein is known [48]. The main compounds extracted from the methanolic extract of *S. indica*

showed good interactions with human monoamine oxidase (PDB: 2Z5X), human serotonin transporter (PDB: 5I6X), human GABA_A receptor alpha1-beta2-gamma2 subtype (PDB: 6X3T), microsomal prostaglandin E synthase 1 (mPGES-1) (PDB: 4YK5), human tissue-type plasminogen activator (tPA) (PDB: 1A5H), and tubulin–colchicine: stathmin-like domain complex regulator (PDB: 1SA0) in the evaluation of anxiolytic, antidepressant, locomotor, antipyretic, thrombolytic, and anthelmintic activities, respectively. Molecular docking scores ranging from 0 to −10.2 kcal/mol indicate that SIF-ME could be an asset for drug design and discovery. The PASS prediction data in Table 4 also supported the above results.

4. Phytochemistry Reported from Previous Works

According to Jiko et al. [25], a GC-MS/MS analysis identified approximately 60 compounds in SIF-ME, with peak areas varying between 0.15% and 8.29%. Further details are demonstrated in Table 6. The primary components included 4-(3-hydroxyprop-1-en-1-yl)-2-methoxyphenol (8.29%), 9-octadecenamide (7.39%), 9,12,15-octadecatrienoic acid, methyl ester (6.02%), 24-noroleana-3,12-diene (5.93%), 9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester (5.34%), sinapyl alcohol (3.68%), beta-sitosterol (3.5%), methyl 5,11,14-eicosatrienoate (3.35%), and retinoic acid (2.57%). The remaining compounds, comprising less than 2% of the total, were present in smaller concentrations.

Table 6. GC-MS/MS analysis of the compounds of SIF-ME.

SL No.	Compounds	MW	Retention Time	Area %
1	Butanoic acid, 3-methyl	102.1 g/mol	3.904	0.15
2	Phenol, 2-methoxy	124.1 g/mol	6.83	0.37
3	2-Methoxy-4-vinyl phenol	150.1 g/mol	9.289	0.29
4	Phenol, 2,6-dimethoxy	154.1 g/mol	9.632	0.38
5	Vanillin	152.1 g/mol	10.171	0.3
6	endo-1,5,6,7-Tetramethylbicyclo[3.2.0]hept-6-en-3-ol	166.2 g/mol	10.626	0.8
7	Phenol, 2-methoxy-4-propyl	166.2 g/mol	10.695	0.24
8	3(2H)-Benzofuranone, 2,4-dimethyl	162.1 g/mol	10.971	0.2
9	beta.-D-Glucopyranose, 1,6-anhydro	162.1 g/mol	11.031	0.22
10	Guaiacol, 4-butyl	180.2 g/mol	11.35	0.55
11	Phenol, 4-ethenyl-2,6-dimethoxy	180.2 g/mol	11.663	0.33
12	Benzaldehyde, 3-hydroxy-4-methoxy	152.1 g/mol	11.934	0.28
13	5-Methyl-3-phenyl-1,3-oxazolidine	163.2 g/mol	12.375	0.41
14	(3R,3aS,6S,7R)-3,6,8,8-Tetramethyloctahydro-1H3a,7-methanoazulen-6-o	222.3 g/mol	12.425	0.17
15	2,6-Dimethoxyhydroquinone	170.1 g/mol	12.602	0.32
16	Benzaldehyde, 4-hydroxy-3,5-dimethoxy	182.1 g/mol	12.708	0.49
17	Phenol, 2,6-dimethoxy-4-(2-propenyl)	194.2 g/mol	13.18	0.49
18	3-O-Methyl-d-glucose	194.1 g/mol	13.251	0.29
19	2-Propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)	196.2 g/mol	13.566	0.93
20	4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenyl	180.2 g/mol	13.747	8.29
21	Dihydroxy-4-methyldodecahydro-2H-benzo[d]oxecin-2-one	256.3 g/mol	14.882	0.38
22	Sinapyl alcohol	210.2 g/mol	17.386	3.68
23	4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	222.2 g/mol	18.661	0.25
24	9,11-Octadecadienoic acid, methyl ester	294.5 g/mol	18.941	2.56
25	9,12,15-Octadecatrienoic acid, methyl ester	292.5 g/mol	19.046	6.02

Table 6. Cont.

SL No.	Compounds	MW	Retention Time	Area %
26	Phytol	296.5 g/mol	19.197	0.97
27	Methyl stearate	298.5 g/mol	19.449	0.67
28	Hexadecanamide	255.4 g/mol	20.421	0.39
29	9-Octadecenamide	281.5 g/mol	23.448	7.39
30	Z,Z,Z-8,9-Epoxyeicosa-5,11,14-trienoic acid, methyl ester	334.5 g/mol	23.558	1.54
31	Cyclohexanone, 5-ethenyl-5-methyl-4-(1-methylethenyl)-2-(1-methylethylidene)	218.3 g/mol	23.75	0.75
32	4-Cycloocten-1-one, 8-(4-octen-4-yl)	234.3 g/mol	23.876	0.93
33	5.Beta.,7.beta.H,10.alpha.-Eudesm-11-en-1.alpha.-ol	222.3 g/mol	23.99	0.71
34	Bicyclo[4.1.0]heptane, 1-(3-oxo-4-phenylthiobutyl)-2,2,6-trimethyl	316.5 g/mol	25.15	0.58
35	Diethylene glycol dibenzoate	314.3 g/mol	25.397	0.39
36	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	330.5 g/mol	25.849	0.64
37	Bis(2-ethylhexyl) phthalate	390.6 g/mol	26.147	0.31
38	Retinoic acid	300.4 g/mol	26.94	2.57
39	S-Octahydro-9-phenanthrene methanol	216.3 g/mol	27.12	1.03
40	Epoxyathyrol	350.4 g/mol	28.065	0.61
41	3,3'-Dimethoxy-4,4'-dihydroxystilbene	272.2 g/mol	28.195	0.6
42	Oleic Acid	354.6 g/mol	28.38	0.39
43	Retinol	286.5 g/mol	28.44	1.34
44	Methyl 5,11,14-eicosatrienoate	320.5 g/mol	28.636	3.35
45	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	352.5 g/mol	28.755	5.34
46	Benzene, 1-[(4-butyl phenyl)ethynyl]-4-ethoxy-2-methyl	292.4 g/mol	28.83	2.59
47	10,13-Dimethyl-3-oxo-2,3,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl 2,2,2-trifluoroacetate	331.5 g/mol	28.974	3.04
48	Retinal	284.4 g/mol	29.323	1.04
49	(1S,2E,4S,5R,7E,11E)-Cembra-2,7,11-trien-4,5-diol	306.5 g/mol	29.41	0.58
50	Methyl 1,4a-dimethyl-6-methylidene-5-[2-(5-oxo-2Hfuran-4-yl)ethyl]-3,4,5,7,8,8a-hexahydro-2Hnaphthalene-1-carboxylate	346.5 g/mol	29.72	1.73
51	9-(Acetyloxy)-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-9aH-cyclopropa	560.7 g/mol	30.861	1.7
52	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	498.8 g/mol	31.266	1.96
53	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl	492.6 g/mol	31.842	2.44
54	(11.xi.)-4,7-Dihydroxy-12,13-epoxytrichothec-9-en-8-one	280.3 g/mol	33.31	0.22
55	Spirost-5-en-3-ol, acetate, (3.beta.,25R)-	456.7 g/mol	34.002	0.24
56	Nonacosan-10-ol	424.8 g/mol	34.551	0.73
57	Stigmasterol	412.7 g/mol	37.316	1.18
58	22-Desoxycarpesterol	546.8 g/mol	37.815	0.33
59	Beta-sitosterol	414.7 g/mol	38.573	3.5
60	24-Noroleana-3,12-diene	394.7 g/mol	39.627	5.93

5. Materials and Methods

5.1. Plant Collection and Identification

Dried fruits of *Shirakiopsis indica* (Willd) were collected from the rural area of Anowara in Chattogram, Bangladesh, in July 2022. Professor Dr. Shaikh Bokhtear Uddin from the University of Chittagong, Bangladesh, taxonomically verified the samples. A voucher specimen was assigned the accession number (cu/mm/3578) and deposited in the herbarium center of the University of Science and Technology, Chittagong. The collected fruits were cleaned and then shade-dried for four weeks at about 25 °C to prepare them for additional processing.

5.2. Extraction Methods

A blender was used to grind 2 kg of dried *S. indica* fruits that had been undersized in a mortar. About 50 g of the powder, diluted as 1 part powder to 30 parts methanol, was put in a clean glass beaker and submerged in 1.5 L of methanol. The beaker was then sonicated for 30 min at 40 °C after being covered with foil that had holes in it. After the entire mixture was filtered through Whatman No. 1 filter paper (Bibby RE200; Sterilin Ltd., Newport, UK), the solvent was extracted using a Buchii Rota evaporator (Sigma Aldrich Co., St. Louis, MO, USA) under controlled heating set at 60 °C and 80 rpm. Using this method, methanol was fully evaporated from the extract. The yield of the extract was measured at 12.50 percent. SIF-ME was kept in a glass vial at 4 °C until the experiment was carried out [49].

5.3. Chemicals and Reagents

Streptokinase, methanol, and chloroform were supplied by Mamun Pharma, located in Chittagong, Bangladesh, our local scientific supply store. General Pharmaceuticals Ltd. in Bangladesh provided us with diclofenac sodium. The University of Science and Technology Chittagong provided a variety of chemicals, including diazepam, fluoxetine, paracetamol, and albendazole. For all other compounds in this experiment, analytical-grade chemicals were used.

5.4. Experimental Animals and Ethics Statement

The 22–30 g Swiss albino mice used in the experiment were supplied by Comilla University's animal research department in Comilla, Bangladesh. Animals were housed in polycarbonate cages under standard circumstances (25 ± 2 °C, 55–60% humidity) and a 12 h/daylight cycle. The animals had ample access to food and water. The research protocols were approved by the Institutional Animal Ethics Committee of the University of Science and Technology Chittagong, Bangladesh (approval number USTMEBBC/18/03/24).

5.5. Experimental Design

Swiss albino mice, both male and female, were used in the test, control, and standard sets. Each group contained five mice. The control group received 10 mL/kg of body weight of a 1% Tween 80 solution in water. SIF-ME was given orally to the test groups at 200 and 400 mg/kg of total body weight, respectively, using oral gavage. Diazepam (1 mg/kg/kg, BW, IP) was the standard medicine for the open field test, hole board test, elevated plus maze test, light–dark box test, and hole cross test. To evaluate the effects of the antidepressant in the tail suspension and forced swimming tests, fluoxetine was given PO at a dose of 10 mg/kg, depending on body weight. Finally, paracetamol, streptokinase, and albendazole were used as references for the antipyretic, thrombolytic, and anthelmintic activities.

5.6. Neuropharmacological Activity

5.6.1. Anxiolytic Activity

Elevated Plus Maze Method

The elevated plus maze is an essential instrument for researching the neuroprotective and anxiolytic properties of test drugs [50]. Rodents spend a disproportionate amount of time in restricted spaces because they dread heights. Animals frequently become terrified and immobile when they enter an environment with open arms [51]. The primary advantages of this testing methodology are (a) its rapidity, ease of use, and shortened testing duration; (b) the lack of unpleasant stimuli or training; and (c) its accuracy and consistency in evaluating the anxiolytic behaviors of mice and anxiolytic drug characteristics. The elevated plus maze is a plus-shaped device positioned 40 cm above the floor. It has two perpendicularly oriented open arms measuring 25×5 cm and two closed arms measuring 25×5 cm and 16 cm long. The stopwatch was started after positioning each test subject in the middle of the maze and facing an open arm. In five minutes, several elements were noted. The mice initially preferred the arms that were either closed or open. A mouse was considered to have entered if all four paws were within an arm. The number of entries into both open and closed arms was recorded. Next, the animals were given a variety of drugs, such as saline for the control group, diazepam for the standard group, and two test samples of *S. indica* (200 and 400 mg/kg bw). Each animal was returned to the maze's center after a 30 min break from therapy. How long each animal spent in the open and closed arms was recorded in the final five minutes [52].

Hole Board Test (HBT)

A piece of “hole board equipment” has openings on the floor through which an animal can stick its head. It is known as “head-dipping”. Neophilia, or “directed exploration”, is quantified by the duration and regularity of head dipping. This demonstrates the animal's independent mobility [53]. Low degrees of head dipping are frequently the result of a lack of neophilia or indicate that the animals get more agitated. Elevated head dipping is typically indicative of neophilia. Therefore, as head dipping decreases, anxiety levels rise, and vice versa. Before administering the control, standard, and test samples (200 and 400 mg/kg bw), each mouse was placed on the hole board device for 30 min. After that, throughout a five-minute trial period, we counted how many times each mouse dipped its head into an eye-level hole using a timer [54].

Light–Dark Box Test (LDT)

The light–dark test is a useful method for predicting anxiolytic-like or anxiogenic-like activity in mice. It measures activity and exploration through transitions, which reflect habituation over time, and aversion through the time spent in each compartment. The apparatus consists of a fully automated box monitored by an observer. It features an open-topped rectangular box ($46 \times 27 \times 30$ cm high) divided into two compartments: a small (18×27 cm) dark area painted black and a large (27×27 cm) illuminated area painted white and brightly lit by a 60 W (400 lx) light source placed at the center of the white compartment. A central opening (7.5×7.5 cm) connects the two compartments at floor level. The extract (200 or 400 mg/kg, p.o.), distilled water (10 mL/kg, p.o.), and diazepam (3 mg/kg, i.p.) were given to four groups of five mice each. During the 5 min test conducted in a dark environment, parameters such as the time spent in the illuminated and dark compartments, the number of transitions between compartments, and the latency of the first crossing were recorded. These data were directly collected by the observer. This test exploits the conflict between the animal's innate tendency to explore novel environments and its avoidance of bright light, providing insights into anxiety-related behaviors [55].

5.6.2. Antidepressant Activity

Forced Swimming Test (FST)

Using the previously described methodology, the forced swimming test was used to assess SIF's antidepressant impact on mice. To acclimate the animal models to the experimental setup, preparatory research was carried out the day before the final study. The experimental swimming gear was a transparent glass tank measuring $25 \pm 15 \pm 25$ cm and filled with water up to 15 cm (25 ± 1 °C). The mice were divided into four groups of three each. The vehicle was supplied to Group I, the test sample (SIF 200 and 400 mg/kg b.w.; p.o.) was given to Groups III–IV, and Group II received the traditional drug (diazepam: 1 mg/kg b.w.; p.o.). Thirty minutes later, each mouse was placed in the tank for six minutes, during which the first few minutes were regarded as the adjustment time and the next four minutes as the immobilization period [56]. The calculation of immobility % is as follows:

$$\% \text{ Immobility} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Tail Suspension Test (TST)

The tail suspension test is a quick and easy method of gauging the antidepressant effects. There are four classifications in which mice are categorized, with three mice in each class. The test sample (SIF 200 and 400 mg/kg b.w.; p.o.) was administered to Groups III–IV, whereas Group II received the conventional treatment (diazepam: 1 mg/kg b.w.; i.p.). Group I was given the vehicle. Once the test samples were applied, the mice were placed in an immobile position, with adhesive tape holding them there at the end of their tails, about 1 cm from the tip. Each mouse in every group was examined for a total of six minutes, but only the final four minutes of the immobility time were recorded [57]. The calculation of immobility (%) is as follows:

$$\% \text{ Immobility} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

5.6.3. Locomotor Activity

Open Field Method

The open field test is commonly used to assess locomotor activity and emotional behavior in rodents [58]. Animals were divided into control, positive control, and test groups (SIF 200 and 400 mg/kg bw). The apparatus, made of plywood ($72 \text{ cm} \times 72 \text{ cm} \times 36 \text{ cm}$), featured a half-square-meter open field divided into a series of squares, alternately colored black and white. The floor, constructed of cardboard, was divided into 16 smaller squares ($18 \text{ cm} \times 18 \text{ cm}$). Observations were conducted at room temperature under normal lighting conditions. The number of squares crossed by the animal was counted for 3 min at intervals of 0, 30, 60, 90, and 120 min after the oral administration of the test drugs.

Hole Cross Method

The method was performed as described by Takagi et al. [59]. A steel partition was fixed in the middle of a cage measuring $30 \times 20 \times 14$ cm, with a 3 cm diameter hole placed at a height of 7.5 cm in the center of the partition. The animals were divided into control, positive control, and test groups, each containing five mice. The test groups received *S. indica* orally at doses of 200 mg/kg and 400 mg/kg body weight, while the control group received the vehicle (1% Tween 80 in water). The number of passages of each mouse through the hole from one chamber to the other was counted for 3 min at intervals of 0, 30, 60, 90, and 120 min after the oral administration of the test drugs.

5.7. Study of Antipyretic Activity

Yeast-Induced Pyrexia in Mice

The antipyretic effect of SIF-ME was tested using the Brewer's yeast-induced pyrexia method in mouse models, wherein a subcutaneous injection of a yeast suspension was administered at a dosage of 10 mL/kg body weight. In this investigation, animal models were given unlimited access to water but were restricted in their food consumption the night before the experiments began. An Ellab thermometer was used to record the rectal temperatures at first [14]. Mice were selected for the assessment of their antipyretic properties only if at 18 h following the subcutaneous injection of yeast solution, their rectal temperature rose by 0.3 to 0.5 °C. The standard treatment for the SIF-ME group was paracetamol (100 mg/kg body weight orally) combined with two different doses of the plant (200 and 400 mg/kg body weight), while the control group received merely distilled water (10 mL/kg). In conclusion, the duration of the rectal temperature tracking was 3 h, with a 1 h break [60].

5.8. Thrombolytic Activity

5.8.1. Blood Clot Lysis Method

Streptokinase (SK) Solution Preparation

A total of 5 ml of pure distilled water was added and thoroughly mixed with the commercially available SK-1500000 I.U. (Polamin-Werk GmbH, Herdecke, Germany) obtained from BCSIR, Chattogram, Bangladesh. For the in vitro thrombolysis experiment, 100 µL (30,000 I.U.) of the suspension was taken [61].

5.8.2. Specimen for the Thrombolytic Test

The experiment was carried out with a previously created procedure, with a few small changes. For this investigation, 5 mL of blood was drawn from ten physically fit human volunteers (n = 10) who had not taken any NSAIDs or anticoagulants in the two days prior. Using an early Eppendorf measurement, 500 µL of blood was drawn and kept in an incubator for 45 min at 37 °C. After the onset of coagulation, the serum was ultimately extracted from the Eppendorf tube. Every tube that only contained coagulation was further weighed to ascertain the precise weight of the coagulation. Subsequently, 100 µL of plant extract was added to the tubes. The test tube containing the plant extract was suspended in the incubator, and a temperature of 37 °C was set for 90 min [15]. Following clot lysis, the blood serum was extracted, and the tube was weighed once more to track any weight changes that coincided with the clot lysis progression. The following formula was used to determine the clot lysis percentage:

$$\% \text{ clot lysis} = \frac{\text{Weight of the lysis clot}}{\text{Weight of clot before lysis}} \times 100$$

5.9. Anthelmintic Activity

The methanolic extract of *Shirakiopsis indica* fruit from Ajaiyeoba et al. exhibits anthelmintic action after modest modifications [62]. The present study employed the aquarium worm *Tubifex tubifex*, which is a member of the annelid family of intestinal worms, due to its anatomical similarities. The average size of the worms, which were taken from the Chittagong local market, was 2–2.5 cm, and they were being studied. For the investigation of anthelmintic action, three distinct concentrations of newly generated methanol extracts (10, 25, 50, and 100 µg/mL) in double-distilled water were combined with the conventional medication albendazole. One group was made up entirely of water and was regarded as a control group. The anthelmintic activity was assessed during the worms' "time of paralysis" and "time of death". When there was no movement at all, except for the worms

shaking violently, it was noted that the time of paralysis had arrived. When the worms lost their ability to move and eventually lost their body colors, their deaths were complete. By submerging the worms in gently heated water, death was further verified. When there were no longer any indications of movement, the parasite was thought to have died.

5.10. *In Silico* Molecular Docking Study

5.10.1. Toxicity Prediction by AdmetSAR

The toxicological properties of the previously identified compounds from SIF-ME were assessed using the admetSAR online tool (<http://lmmd.ecust.edu.cn/admetsar1/predict/>, accessed on 11 September 2024) and the pKCSM online tool (<http://biosig.unimelb.edu.au/pkcsml/>, accessed on 11 September), since toxicity is a major problem in the development of novel medications.

5.10.2. Prediction of Activity Spectra for Substances (PASS)

The pass prediction was explored utilizing the PASS online tools (<http://www.pharmaexpert.ru/passonline/predict.php>, accessed on 11 September 2024) to figure out the probable biological effects of the selected compounds. The values of Pa and Pi ranged between 0.000 and 1.000. A substance is thought to have biological potential when its Pa value is greater than its Pi value. Low pharmaceutical activity is indicated by $Pa < 0.5$, moderate therapeutic potential is suggested by $Pa < 0.7$, and significant medicinal activity is indicated by $Pa > 0.7$ [63,64].

5.10.3. Software Tools

The following resources and technologies were used in the analysis: PubChem, MGL instruments, Swiss pdb-viewer, AutodockVina, Drug Banking, Discovery Studio Visualizer 2021 (BIOVIA), and Protein Data Bank (PDB).

5.10.4. Selection of Ligands

In all, 18 compounds previously identified from SIF-ME were selected following a thorough analysis of the literature. The retrieved molecules were butanoic acid, 3-methyl (PID: 10430); phenol, 2-methoxy (PID: 460); 2-methoxy-4-vinylphenol (PID: 332); phenol, 2,6-dimethoxy (PID: 7041); vanillin (PID: 1183); phenol, 2-methoxy-4-propyl (PID: 17739); phenol, 4-ethenyl-2,6-dimethoxy (PID: 35960); benzaldehyde, 3-hydroxy-4-methoxy (PID: 12127); 5-methyl-3-phenyl-1,3-oxazolidine (PID: 319003); 2,6-dimethoxyhydroquinone (PID: 96038); benzaldehyde, 4-hydroxy-3,5-dimethoxy (PID: 8655); phenol, 2,6-dimethoxy-4-(2-propenyl) (PID: 226486); 2-propanone, 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl) (PID: 586459); retinoic acid (PID: 444795); octahydro-9-phenanthrene methanol (PID: 607779); epoxylathyrol (PID: 56841080), beta-sitosterol (PID: 222284); and 24-noroleana-3,12-diene (PID: 15427754) [25].

*PID = PubChem ID

5.10.5. Validation of the Ligands as Potential Therapeutic Agents

The selection of these substances as potential treatments was greatly influenced by their physical and molecular characteristics, as well as pharmacokinetic factors such as ADME/T (absorption, distribution, metabolism, excretion, and toxicity). To verify the listed compounds' potential as ligands for therapeutic targets, the pKCSM online tool (<http://biosig.unimelb.edu.au/pkcsml/>) was accessed on 31 July 2024 [65]. Next, using the SwissADME online server, the compounds were assessed for drug potential using Lipinski's rule of five [66].

5.10.6. Protein Preparation and Active Site Determination

The crystal structures of the target proteins human monoamine oxidase (PDB: 2Z5X), human serotonin transporter (PDB: 5I6X), human GABA_A receptor alpha1-beta2-gamma2 subtype (PDB: 6X3T), microsomal prostaglandin E synthase 1 (mPGES-1) (PDB: 4YK5), human tissue-type plasminogen activator (tPA) (PDB: 1A5H), and tubulin–colchicine: stathmin-like domain complex regulator (PDB: 1SA0) were made available by the RCSB protein data bank. Kurumbail et al.'s previously published data were used to determine the enzymes' active sites [67]. To perform the necessary cleaning and preparations, which included removing heteroatoms, cofactors, and water, Swiss-PdbViewer (v4.1) and the BIOVIA Discovery Studio 4.5 Client were implemented. A force field of MMFF94 and the PyRx virtual screening tool were used to reduce the target proteins [68] after hydrogen atoms were added to their structures. Docking studies were made easier by maintaining the target proteins in pdb format.

5.10.7. Validation of the Proteins as Potential Therapeutic Targets

Human monoamine oxidase (MAO, PDB: 2Z5X) is used in molecular docking studies of anxiolytic activity because it regulates neurotransmitters like serotonin, dopamine, and norepinephrine, which influence mood and anxiety. Inhibiting MAO-A increases these neurotransmitters, potentially reducing anxiety. The crystal structure of MAO-A provides insights into its active site, helping identify compounds with strong binding affinity as potential anxiolytic agents. The docking results were correlated with the behavioral studies to validate efficacy. The human serotonin transporter (PDB: 5I6X) is used in docking studies of antidepressant activity because it regulates serotonin reuptake, a key factor in depression. Inhibiting SERT boosts serotonin levels, enhancing mood. The crystal structure of PDB: 5I6X helps identify potential inhibitors, aiding in the discovery of new antidepressants. The human GABA_A receptor alpha1-beta2-gamma2 subtype (PDB: 6X3T) [69] is a critical target for compounds with sedative activity due to its role in inhibitory neurotransmission and its association with calming effects. Docking studies using this structure help identify compounds that can modulate GABAergic signaling, offering insights into their sedative potential. Microsomal prostaglandin E synthase 1 (mPGES-1) (PDB: 4YK5) is used in docking studies of antipyretic activity as a model enzyme to study inhibitory interactions. Its detailed structure helps predict how compounds may inhibit enzyme activity, aiding in identifying potential antipyretic agents for further testing. Human tissue-type plasminogen activator (PDB: 1A5H) [9] is crucial for breaking down blood clots by converting plasminogen to plasmin. Its structure aids in identifying compounds that enhance its activity, guiding the discovery of novel thrombolytic agents. Tubulin (PDB: 1SA0) [70] is vital for helminth survival, with its colchicine binding site disrupting microtubule assembly. Docking studies help identify compounds inhibiting tubulin, aiding in anthelmintic drug discovery.

5.10.8. Molecular Docking and Post-Docking Analysis

AutoDock, version 4.2, and PyRx 0.3 (<https://pyrx.sourceforge.io/>) (accessed 31 July 2021) were used for the docking computations [71]. AutoGrid was used to create a grid box with a grid spacing of 0.375 Å and the following measurements: X, 47.0589; Y, 29.4311; and Z, 36.9696 Å points. The outcomes of the docking were examined using PyMOL. The type of interaction—hydrogen bond, π – π , or cation– π interactions, for instance—and its contribution to ligand binding can be determined using these technologies. More data about the interactions between ligands and receptors were gathered using PyMOL (Protein–ligand docking: Current state and future challenges).

5.11. Statistical Analysis

The results are presented as the means $\pm$ SEMs, or standard errors of the means. The statistical analysis was performed using the statistical program “Statistical Package for the Social Sciences” (SPSS, Version 16.0, IBM Corporation, New York). For comparisons after a one-way analysis of variance (ANOVA), a post hoc Dunnett test was applied. The following criteria were used to establish the significance levels: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. The figures show statistical significance when compared to the study group.

6. Conclusions

The results indicate that the mangrove species *Shirakiopsis indica* fruit methanolic extract (SIF-ME) has therapeutic efficacy for treating anxiety, depression, insomnia, fever, blood coagulation disorders, and helminthiasis, which were evident in in vivo animal models and other related in vitro models. Furthermore, in silico investigations of bioactive substances revealed potential binding affinities to several receptors during a molecular docking study. The potential pharmacological activities, safety, and toxicological features of the bioactive substances support the use of this plant’s fruit as a prospective medicinal source. As a result, *Shirakiopsis indica* fruit is a promising choice for therapeutic development. However, more research is still required to extract and purify novel bioactive leads to determine the biological activities and mechanisms of the observed pharmacological effects, as well as to ascertain their safety and efficacy profiles.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ddc4030031/s1>, **Table S1:** Evaluation of Anxiolytic Activity through Elevated Plus Maze Test, **Table S2:** Evaluation of Antidepressant Activity through Forced Swimming Test, **Table S3:** Evaluation of Locomotor Activity through Open Field Test, **Table S4:** Molecular docking scores for anxiolytic, antidepressant, locomotor, antipyretic, thrombolytic, and anthelmintic activities, **Table S5:** In silico binding affinity and non-bonding interaction of selected phytochemicals of SIF-ME for Anxiolytic, Antidepressant, Locomotor, Antipyretic, Thrombolytic, and Anthelmintic activities, respectively, **Figure S1:** Molecular docking interaction of compounds against the human monoamine oxidase (PDB: 2Z5X). Where A1 is Retinoic acid, A2, is Octahydro-9-phenanthrene methanol, A3 is Beta-sitosterol, and A4 is Diazepam (Standard), **Figure S2:** Molecular docking interaction of compounds against the human serotonin transporter (PDB: 5I6X). Where B1 is Beta-sitosterol, B2 is 24-Noroleana-3,12-diene, B3 is Retinoic acid, and B4 is Fluoxetine (Standard), **Figure S3:** Molecular docking interaction of compounds against the human GABAA receptor alpha1-beta2-gamma2 subtype (PDB: 6X3T). Where C1 is 24-Noroleana-3,12-diene, C2 is Beta-Sitosterol, C3 is Epoxylathyrol, and C4 is Diazepam (Standard), **Figure S4:** Molecular docking interaction of compounds against the microsomal prostaglandin E synthase 1 (mPGES-1) (PDB: 4YK5). Where D1 is 24-Noroleana-3,12-diene, D2 is Epoxylathyrol, D3 is Beta-sitosterol, and D4 is Paracetamol (Standard), **Figure S5:** Molecular docking interaction of compounds against the human tissue-type plasminogen activator (tPA) (PDB: 1A5H). Where E1 is 24-Noroleana-3,12-diene, E2 is Beta-sitosterol, E3 is Retinoic acid, and E4 is Estreptoquinasa (Standard), **Figure S6:** Molecular docking interaction of compounds against the tubulin-colchicine: stathmin-like domain complex regulator (PDB: 1SA0). Where F1 is 24-Noroleana-3,12-diene, F2 is Retinoic acid, F3 is Beta-sitosterol, and F4 is Albendazole (Standard).

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Institutional Review Board Statement: This study was approved by the Institutional Review Board of the University of Science & Technology, Chittagong, Bangladesh (approval number UST-MEBBC/18/03/24) on animal experiments and human sample tests. All biological activity testing was carried out following the ethical standards outlined in the 2013 Declaration of Helsinki. The 2013 edition of the rules for animal euthanasia was followed in the processing and testing of the animals, and the Swiss Academy of Medical Sciences and the Swiss Academy of Science approved euthanasia. Dried fruits of *Shirakiopsis indica* (Willd) were collected from the rural area of Anowara in Chattogram, Bangladesh, in July 2022. Professor Dr. Shaikh Bokhtear Uddin from the University of Chittagong, Bangladesh, taxonomically verified the samples. The use of plants in the present study complies with international, national, and/or institutional guidelines and the plant's fruits were collected with permission.

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