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Investigation of the pharmacological characteristics of *Sphaeranthus indicus* flowers using phytochemical analysis

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ABSTRACT

Although the conventional usage of *Sphaeranthus indicus* flowers in medicine exists, there is a lack of research on the precise pharmacological characteristics and comprehensive phytochemical composition of its flowers, resulting in a knowledge gap on their whole therapeutic capabilities. The aim of this work is to identify and describe the phytochemicals found in the flowers of *Sphaeranthus indicus* and assess specific pharmacological effects, such as antioxidant, antibacterial, anti-inflammatory, thrombolytic, antidepressant, and antidiarrheal characteristics. The investigation revealed that the MESIF (methanol extract of *S. indicus* flowers) showed substantial antioxidant performance (IC_{50} = 26.33) accompanied by a moderate phenolic content (93.33 ± 1.76). At 250 and 500 µg/ml, the extract exhibited modest to moderate antibacterial sensitivity against many tested pathogens and significantly reduced inflammation by protein denaturation (41.63 ± 0.11 and 70.52 ± 0.20). Furthermore, MESIF exhibited remarkable thrombolytic activity with a statistically significant (***P<0.001) lysis of clots. The extract showed substantial reduction in immobility in both the Forced Swim Test (FST) and Tail Suspension Test (TST) at doses of 200 mg/kg and 400 mg/kg (***p<0.001). It also a showed significant potent antidiarrheal activity (**p<0.01 at 200 mg/kg and ***p<0.001 at 400 mg/kg), effectively reducing diarrheal episodes in the test models. These findings suggest that the MESIF holds potential for developing treatments with antioxidant, antibacterial, anti-inflammatory, thrombolytic, antidepressant, and antidiarrheal effects. Further research is recommended to isolate specific bioactive compounds and elucidate the mechanisms underlying these pharmacological actions.

INTRODUCTION

Free radicals are the results of various physiological and biological processes that cause oxidative stress in the human body. These processes include reactive oxygen species (ROS) and reactive nitrogen species (RNS). Excess production of these free radicals damages biomolecules such as DNA, proteins, and lipids. They are a major contributor to the development of many chronic diseases [1].

While the link between inflammation and the pathophysiology of arterial thrombosis was elucidated in the previous decade, it remains largely unknown. Recently, researchers have recognized inflammation as a potential mechanism by which certain risk factors trigger the formation of blood clots in veins. The activation of endothelial cells, platelets, and leucocytes results in the formation of a thrombus, which triggers inflammation and the generation of microparticles that activate the coagulation system by means of tissue factor recruitment [2]. Hence, the crucial initiation of venous thrombus formation is likely inflammation of the vein wall. In healthy individuals, C-receptive protein (CRP) is a plasma protein that acts as an acute-stage reactant. Its plasma content is significantly elevated during both acute and chronic inflammation [3]. On the other hand, thrombosis is the cause of several atherothrombotic disorders,

including cerebral or myocardial infarction. A homeostatic imbalance in an artery leads to the formation of blood clots, also known as thrombus. These clots obstruct the vascular organs and result in life-threatening symptoms that ultimately lead to death [4].

Diarrhea is typically characterized by the frequent transit of liquid or watery faecal matter, followed by abdominal pain. According to the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF), there were more than 2 billion cases of diarrhea per year, resulting in almost 370,000 fatalities worldwide among children under the age of five in 2019. The potential application of folk medicine in the therapy of diarrhea produced by common enteric infections needs to be scientifically assessed [5]. Given that pathogenic bacteria are a leading source of sickness and mortality in humans, pharmaceutical industries are working hard to develop novel antibacterial drugs that are becoming important in the fight against infections and causing concern worldwide. A number of clinical microbiologists have an affinity for antimicrobials that originate from plant extracts for a few reasons: phytochemicals may one day supplement doctors' prescription lists of antimicrobial agents, and they can also raise public awareness of the dangers associated with the overuse of conventional antibiotics [6].

The World Health Organization (WHO) estimated that 450 million individuals worldwide suffer from anxiety and various depressive disorders, accounting for around 12.3% of all ailments [7]. Only 60% of medications are effective in treating this disease. Depression is the most common disorder linked to emotional and cognitive disabilities like apathy, low energy, and difficulties thinking and acting [8]. Psychological, environmental, genetic, and biological factors all have a role in the maturation of mental illnesses [9]. Depression is the prevailing condition associated with emotional and cognitive impairments such as apathy, diminished energy, and challenges in cognition and behavior. The goal of current research is to create safer and more effective compounds to treat a number of sicknesses. For that reason, the best treatment for both psychiatric disorders and cardiovascular diseases might be a drug that can fight psychiatric disorders, clean up ROS, and break up clots while also being safe.

In earlier times, people used plants as remedies for a variety of illnesses without understanding the compounds that made them active [10]. According to statistics from the WHO, almost 80% of people on the planet use herbal or plant-derived medications for some form of basic medical treatment [11]. Therefore, studying these traditional medicinal herbs is important for therapeutic purposes as well as to establish scientific evidence of their efficacy. Nature most commonly contains secondary metabolites, which are useful in the search for novel therapeutic compounds. A number of key secondary metabolites such as saponins, phenolics, terpenoids, alkaloids, etc. found in medicinal plants have demonstrated the ability to investigate complex pharmacological targets, making them noteworthy when compared to synthetically generated medications [12, 13].

Sphaeranthus indicus is a vital member of the family of Asteraceae with ethnomedical importance [14]. In Bangladesh, Chittagong, Sylhet, and Chittagong Hill tracts are known for their woods and shaded areas. *S. indicus* is a commonly used medicinal plant in Bangladeshi and Indian traditional medicine to heal and diagnosed a variety of illnesses [15]. This plant is said to have hepatoprotective, immunomodulatory, bronchodialator, anxiolytic, neuroleptic, antioxidant, hypolipidemic, and anti-inflammatory qualities [15, 16]. Although the flowers of *S. indicus* are acknowledged for their many therapeutic uses, their precise effects, such as antioxidant, anti-

inflammatory, antibacterial, antidepressant, and thrombolytic capabilities, have not been well investigated. Their whole pharmacological potential has not been thoroughly investigated, thereby requiring more research. To bridge this gap, we methodically carried out a series of studies using both in vitro and in vivo (animal-based) models. The purpose of this study was to comprehensively assess and confirm the many pharmacological effects of *S. indicus* flowers using methanol extract, therefore deepening our knowledge of their therapeutic possibilities and facilitating future advancements in medicine.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were ethanol, acetyl salicylic acid, ascorbic acid, DPPH (2,2-Diphenyl-1-picrylhydrazyl), and BHT (butylated hydroxytoluene), which were purchased from reputable supplier Merck KGaA, Darmstadt in Germany. Diazepam and loperamide HCL obtained from Incepta Pharmaceuticals Ltd. and amoxicillin from Square Pharmaceuticals Ltd. in Dhaka, Bangladesh. The remaining chemicals were sourced from a local vendor via Taj Scientific Ltd. in Chittagong, Bangladesh.

Plant collection, identification and extraction of plants

The flowers of *S. indicus* were collected from seasonal ponds in the hamlet of Chittagong, and the taxonomist from the Forest Research Institute, Bangladesh (BFRIH) identified them. After gathering the *S. indicus* plant, give it a thorough water wash. After that, dry the chosen plant piece (flowers) to turn it into a powder. The methanol extract of *S. indicus* flowers (MESIF) solvent extraction procedure was performed by immersing approximately 520 g of the dehydrated material in 1.5 L of laboratory-grade methanol. The liquid was kept in the jar for two weeks, shaking and stirring it periodically during that time. Using a rotary evaporator, the mixture was filtered by the help of filter paper (Whatman, UK) and condensed to produce concentrated crude methanol extract. Up until it was used for the inquiry, the extract was kept at 4 °C [17].

Experimental animals

The departmental animal research ethics committee ensures acquiescence with the recommendation developed by the Guide for the Care and Use of Laboratory Animals. Jahangirnagar University, Savar, Bangladesh, provided the experimental animals, which were Swiss albino mice with a weight range of 25–30 grams. The mice had adapted to the day-night cycle and maintained adequate ventilation and quiet conditions in their quarters. In a quiet, secluded setting, experiments were conducted. The International Islamic University in Chittagong, Bangladesh's P&D Committee (Pharm-P&D-37/07'12), authorized the study protocol. Before the experiments began, the animals were given ten days to become used to being in the lab.

Qualitative phytochemical screening

One gram of *S. indicus* flowers were dissolved in 100 mL of methanol to create a stock solution. The solution was subsequently analyzed using a standardized procedure to determine the presence of secondary metabolites, particularly flavonoids, alkaloids,

carbohydrates, proteins and amino acids, saponins, glycosides, steroids, tannins, phenols, cholesterols, resins, reducing sugar, and other similar substances [18, 19].

Antioxidant activity analysis

DPPH free radical scavenging assay

The MESIF underwent a free radical scavenging assay. We evaluated free radical scavenging activities of MESIF using slide modification of Rehman *et al.* [20]. Formulated a 0.1 mM DPPH solution by dissolving DPPH in methanol and preserved the solution in darkness at ambient temperature until used. All concentrations of MESIF were exposed to the DPPH reagent and incubated for 30 minutes in a light, restricted environment. Incubated the mixture at room temperature in the dark for 30 minutes to allow the reaction to proceed. Subsequent to the incubation time, we quantified the absorbance at a designated wavelength of 517 nm using a UV spectrophotometer. To establish a baseline, a blank solution was employed as a reference. BHT served as a benchmark. The free radical scavenging test was calculated using the following equation:

$$\% \text{ of Scavenging activity} = [(A_C - A_S)/A_C] \times 100$$

Here, A_C = absorbance of the control, and A_S = absorbance of the sample. Subsequently, the percentage inhibition was plotted against the concentration of the antioxidant sample, and the IC_{50} value was obtained.

Determination of total phenolic content

The Folin-ciocalteu technique, as modified by Ali Reza *et al.* [21], was used to determine the TPC (Total Phenolic Content). A diluted sample of 200 µL was mixed with 1 mL of Folin-ciocalteu reagent, which had been diluted at a ratio of 1:10. After duration of 4 minutes, we introduced 800 mL of a sodium carbonate solution that was saturated and had a concentration of 75 gm/L. Following a 2-hour incubation period at ambient temperature, the absorbance was measured three times at a wavelength of 765 nm. In order to create a standard curve, we performed a calibration procedure using Gallic acid at concentrations that varied from 0 to 500 mg/L. The measurements were expressed in milligrams of gallic acid equivalent (mg GAE) per gramme of plant material, using its dry weight as the basis.

***In vitro* antimicrobial activity by disc diffusion assay**

The tests included formulating five different media to evaluate how solubilizing agents affect the diffusion of components through agar [22]. This established process inoculates agar plates with a regulated inoculum of the test microorganism. Subsequently, filter paper discs, about 6 mm in diameter, infused with the test drug at a specified concentration, are positioned on the agar surface. The Petri dishes are incubated under optimal circumstances. The antimicrobial agent typically permeates the agar, inhibiting the test microorganism's germination and proliferation, after which the widths of the inhibition zones were determined. Variety of gram-positive and gram-negative strains were examined, including *Lactobacillus casei* (+), *Corynebacterium species* (+), *Bacillus cereus* (+), *Staphylococcus aureus* (+), *Escherichia coli* (-), *Pseudomonas aeruginosa* (-), *Bacillus azotoformans* (+), *Salmonella typhi* (-), *Vibrio cholerae* (-), and *Klebsiella*

pneumoniae (-). The observed results were compared to the established standard for amoxicillin.

Anti-inflammatory assay using protein denaturation method

The anti-inflammatory potential of the MESIF has been investigated through minor alterations by Khan *et al.* [23]. The mixture used for the reaction was generated by mixing 3 ml of a 5% solution of egg albumin with 3 ml of test extract concentrations that were different from each other. The pH of the liquid was modified to 5.6 ± 0.2 by adding 0.1 N HCl. The final concentration levels of the test extract were set to 250, and 500 µg/ml. A control was used with an equivalent volume of methanol. The solutions were regularly put in a biochemical oxygen demand incubator and kept at a temperature of 37 ± 2 °C for 15 minutes. Afterwards, they were subjected to heating for a duration of 20 minutes at a temperature of 57 °C. Subsequently, the absorbance was measured at 660 nm wavelength using a blank vehicle. The quantification of protein denaturation inhibition was assessed using the following equation:

% of inhibition of protein denaturation = $[(A_C - A_S)/A_C] \times 100$

Here, A_C = absorbance of the control and A_S = absorbance of the sample

***In vitro* thrombolytic activity assay**

The effectiveness of MESIF in dissolving blood clots was evaluated using the approach described in the study by Rashid *et al.* [24] with slide modification. A group of ten young individuals (5 males and 5 females), who were in good health and aged between 22 and 25 years old and had no previous use of oral contraceptives or anticoagulant medicine, had 5 mL of blood drawn from a vein. The blood of each participant was distributed into five pre-weighed sterile micro-centrifuge tubes and thereafter incubated at a temperature of 37 °C for a period of 45 minutes. When the clot started to form, we carefully removed the serum without disturbing it, and we weighed the clot in each tube after that. A 10 mg/mL MESIF solution was added to each microcentrifuge tube, holding a clot one after the other. Then, as positive and negative controls, 100 µL of streptokinase (SK) and 100 µL of distilled water were added to the control tube, respectively. Subsequently, we transferred all the tubes to a regulated setting with a temperature of 37 °C and observed the process of clot dissolution for a duration of 90 minutes. After the incubation period, fluid was collected that ejected and weighed the tubes again to determine the precise amount of weight lost due to the clot breaking apart. The weight difference between before and after clot lysis was quantified as the percentage of clot disintegration, as shown below:

% of clot lysis = (weight of released clot / clot weight) ×100

Antidepressant effects of MESIF

Tail suspension test

Five groups of mice, five male and five female, received the following treatments for five days prior to the tail suspension test (TST): the control group received 10 ml/kg of 1% Tween-80 orally, the fluoxetine group received 20 mg/kg orally, and the methanol flower extract group received 200 mg/kg and 400 mg/kg orally. The TST was conducted with slide modification described by Shetu *et al.* [25], which included inducing immobility for the whole period. The tip of the mice's tail was elevated roughly 1 cm

above the floor using adhesive tape. This suspension was done in a way that provided both auditory and visual isolation. Over the course of six minutes, we kept an eye on the length of immobility. Mice were considered stationary, even when they were hanging motionless. The flower extract's ability to shorten the mice's TST immobility duration suggested that it had either an antidepressant or CNS stimulatory effect.

Forced swimming test

In forced swimming test (FST), the animals were kept in a cylindrical glass cage with a diameter of 10 cm and a height of 25 cm, where they were forced to swim alone. The tank was kept at a constant temperature of 25 ± 1 °C and filled with pure water up to a depth of 19 cm. The test recording lasted for a total of 6 minutes, with the final 4 minutes specifically measuring the length of immobility. The first 2 minutes were designated as an adaptation phase. Each animal was deemed immobile after it ceased to struggle and remained suspended without any movement in the water, additional for the necessary actions to keep its head above the water. A reduction in the length of immobility was seen as an antidepressant-like effect [26].

Castor oil-induced diarrhea method

The experiment used the diarrhea induction methodology described by Alam *et al.* [27], which included the administration of castor oil. There were four groups in the experiment, each group including five mice. The negative control group (Group 1) was administered a treatment of 1% Tween-80 at a dosage of 10 ml/kg, while the positive control group (Group 2) was given a treatment of loperamide at a dosage of 5 mg/kg. The remaining two groups were administered the plant extract at levels of 200 and 400 mg/kg, respectively. Following the 60-minute process, we delivered castor oil to all groups, with each mouse receiving a dose of 0.5 mL. In order to enhance the process of observation, we placed each mouse in a separate cage on a floor coated with blotting paper. Over the next four-hour duration, we saw and recorded the unique excrement characterized by diarrhea, consisting of both moist and desiccated fecal matter. The control group had a fecal quantity of 100%. At the start of every hour, the aged blotting paper was changed with a new one. The defecation inhibition percentage was calculated by comparing it to the control using the following equation:

Inhibition of defecation (%) = $[(A_c-B_t)/A] \times 100$

Where, A_c is the mean number of defecations in the control group and, B_t is the mean number of defecations caused by test group (standard or plant extracts).

Statistical analysis

The findings of the experiments were investigated using GraphPad Prism (Version 8.4.4). The values were represented as mean ± SEM (standard error mean). The data's statistical significance was defined as *$p< 0.05$, **$p< 0.01$, and ***$p< 0.001$. The one-way analysis of variance (ANOVA) was conducted using Dunnett's test to compare it with the negative control. The experiments were performed five times each to ensure result consistency and statistical accuracy.

RESULTS

Qualitative phytochemical screening

As shown in Table 1, the qualitative phytochemical screening of MESIF found flavonoids, alkaloids, carbohydrates, proteins and amino acids, glycosides, tannins, phenols, cholesterols, and reducing sugars in different amounts.

Table 1. Phytochemical screening of MESIF.

Sl.	Phytochemicals	Name of the tests	Observation
1	Flavonoids	Wagner's reagent test	+
2	Alkaloids	Wagner's reagent test	+
3	Carbohydrates	Benedict test	+
4	Protein & amino acid	Sulphuric acid test	+
5	Saponins	Foam height test	-
6	Glycosides	Keller-killiani test	+
7	Steroids	Salkowski test	-
8	Tannins	Lead acetate test	+
9	Phenols	Iodine test	+
10	Cholesterols	Salkowski test	+
11	Resins		-
12	Reducing sugar	Benedict test	+

(+) means present, (-) means absent, ; MESIF=Methanol extract of *S. indicus* flower.

Antioxidant effects of MESIF

The antioxidant properties of MESIF were investigated using the DPPH free radical scavenging method. The crude extract has possible antioxidant effects, as seen in Figure 1. Most antioxidant power (71.08%) was seen in MESIF at 1000 µg/ml concentration. The standard chemical tert-butyl-1-hydroxytoluene (BHT) had a 75.69% effect at the same concentration. This concentration enhanced the scavenging (SCV) characteristics compared to the higher concentration. The IC_{50} (minimal inhibitory concentration required to inhibit 50% of enzyme activity) values of MESIF and BHT were determined to be 26.33 and 6.88, respectively, using a linear regression model.

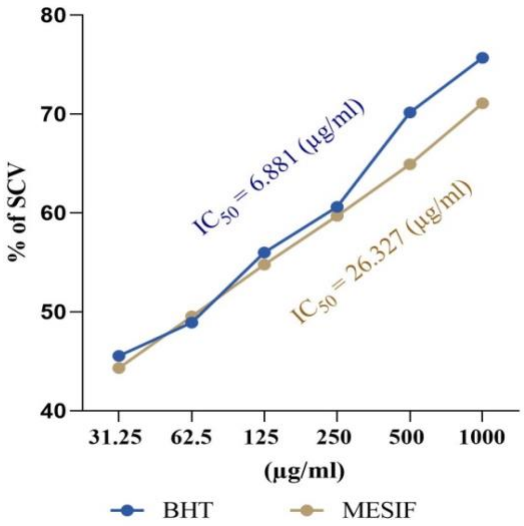

Figure 1. IC_{50} value of BHT and MESIF observed with DPPH. BHT = Tert-butyl-1-hydroxytoluene; MESIF = Methanol extract of *S. indicus* flower and SCV = Scavenging.

A quantitative estimation was conducted to determine the TPC of the MESIF. A concentration of 1000 µg/mL was used to quantify the phenolic content of MESIF, which was found to be 93.33±1.76 mg QE/gm (Table 2). This study conducted the MESIF analysis using a linear regression equation. In particular, for the phenol assay, the equation is represented as y = 0.0045x + 0.8615.

Table 2. Determination of TCP of MESIF.

Concentration (µg/ml)	Weight of dry extract (gm) (m)	Absorbance at 760 nm	GAE conc. C (µg/ml) (x)	GAE conc. C (mg/ml) (x/1000)	Volume of extract (ml)	TPC as GAE C=x×(v/m) (mg /ml)	Mean ± SEM (mg/ml)
500	0.0005	1.073	47	0.047	1	94	
500	0.0005	1.077	47.89	0.048	1	96	93.33±1.76
500	0.0005	1.066	45.44	0.045	1	90	

MESIF =Methanol extract of *S. indicus* flowers and TPC = Total phenolic contents. Gallic acid equivalent, GAE; total phenolic content, TPC.

Effect of MESIF on antimicrobial activity

The MESIF demonstrate a moderate zone of inhibition against bacterial multiplication, with a scale of zone of inhibition measuring from 14 mm to 24 mm. The methanol extract of *S. indicus* flowers showed a maximal inhibition zone of 24 mm against *B. cereus* and *L. casei* and 23 mm against *P. aeruginosa*, respectively. In addition, amoxicillin, when administered as a standard medicine, had rapid antibacterial action ranging from 28 to 34 mm against *C. species* and *B. cereus*. Table 3 shows the suppression of the proliferation of the microbes.

Table 3. Antimicrobial assay by disc diffusion method of MESIF.

Test microorganisms	Diameter of zone of inhibition (mm)			
	MESIF 200 µg /mL	MESIF 400 µg /disc	MESIF 800 µg /disc	Amoxicillin 50 µg/disc
Lactobacillus casei(+)	18	20	24	32
Corynebacterium species(+)	15	19	21	28
Bacillus cereus(+)	17	23	24	34
Staphylococcus aureus (+)	14	18	22	32
Escherichia coli (-)	16	20	22	28
Psedomonas aeruginosa(-)	18	21	23	31
Bacillus azotoformans(+)	15	19	22	32
Salmonella typhi(-)	15	17	21	30
Vibrio cholera(-)	14	16	20	30
Klebsiella pneumoniae(-)	15	16	21	32

MESIF = Methanol extracts of *S. indicus* flower.

Effect of MESIF on inflammatory activity using protein denaturation method

Table 4 provides data demonstrating the anti-inflammatory effects of MESIF on protein denaturation. The plant extract exhibited inhibitory efficacy that varied according to the dosage in comparison to diclofenac sodium. The inhibition percentages were 41.63 ± 0.11 and 70.52 ± 0.20 for MESIF, and 76.39 ± 0.45 and 88.45 ± 0.63 for diclofenac sodium, at doses of 250 and 500 µg/mL, respectively.

Table 4. Effects of MESIF on inflammatory activity using protein denaturation method.

Concentration (µg/mL)	% of Inhibition (Mean ± SEM)	
	Diclofenac sodium	MESIF
250	76.39 ± 0.45	41.63± 0.11
500	88.45 ± 0.63	70.52 ± 0.20

Values are represented in mean ± SEM (n = 3). MESIF = Methanol extracts of *S. indicus* flowers.

Effect of MESIF on *in vitro* thrombolytic activity

Figure 2 demonstrates the thrombolytic effect of MESIF. The test results indicate that MESIF has a significant (***p<0.001) clot lysis ability of 31.47 ± 1.04% compared to the negative controls, while streptokinase demonstrates an even higher clot-breaking ability of 74.45 ± 1.10%.

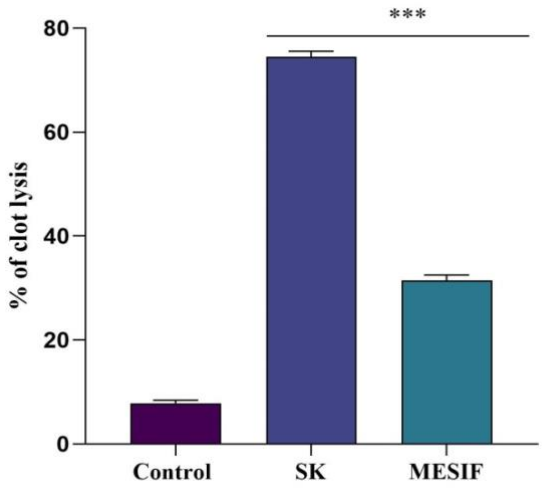

Figure 2. The clot lysis activity of MESIS and SK. Values are represented as mean ± SEM of six consecutive experiments. One-way analysis of variance (ANOVA) followed by Dunnett's test was employed where p<0.05 were considered statistically significant compared to the control. SK = Streptokinase; *** = Highly significant and MESIF = Methanol extracts of *S. indicus* flowers.

Antidepressant effects of MESIF

After being given the MESIF solution by mouth, Figures 3A and B show the lengths of time that the animals were immobile in the FST (forced swimming test) and TST (tail suspension test). According to the data, there was a notable reduction of immobility period in both the FST and TST that depended on the dosage as comparison to the negative control (***p<0.001).

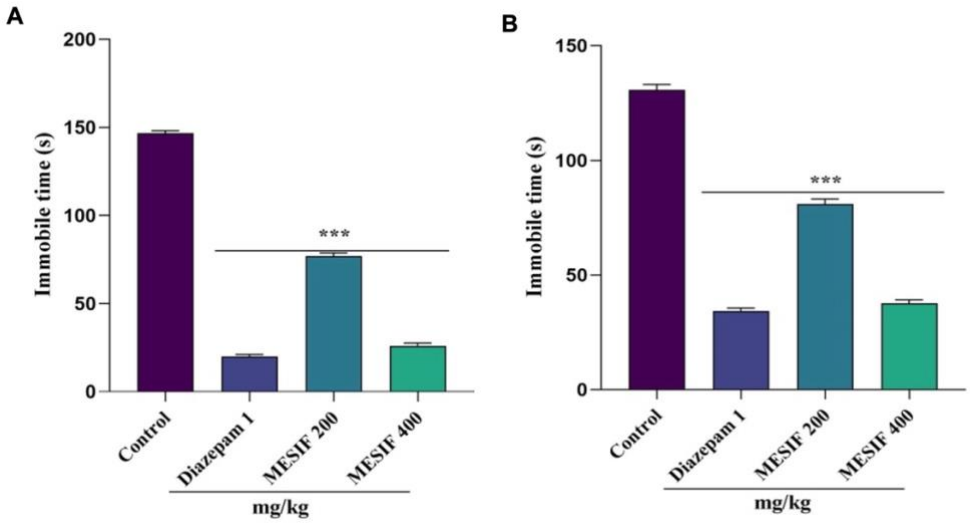

Figure 3. Effects of MESIF on the (A) force swimming test and (B) tail suspension test. Each column represents the mean ± SEM (n = 5). One-way analysis of variance (ANOVA) followed by Dunnett's test was employed where $p<0.05$ were considered statistically significant compared to the control. *** = Highly significant; (MESIF) = Methanol extracts of *S. indicus* flower.

Anti-diarrheal effects of MESIF using castor oil-induced diarrhea method

In comparison to the control group, the MESIF demonstrated good anti-diarrheal properties, resulting in a notable decrease in diarrhea when delivered at dosages of 200 mg/kg and 400 mg/kg. The reduction was 9.8 ± 1.16 ($^{**}p < 0.01$) and 6.5 ± 0.51 ($^{***}p < 0.001$), respectively. The standard loperamide had the highest reduction in defecation (5.25 ± 0.40) ($^{***}p < 0.001$), indicating its significant anti-diarrheal activity. The results are shown in Figure 4.

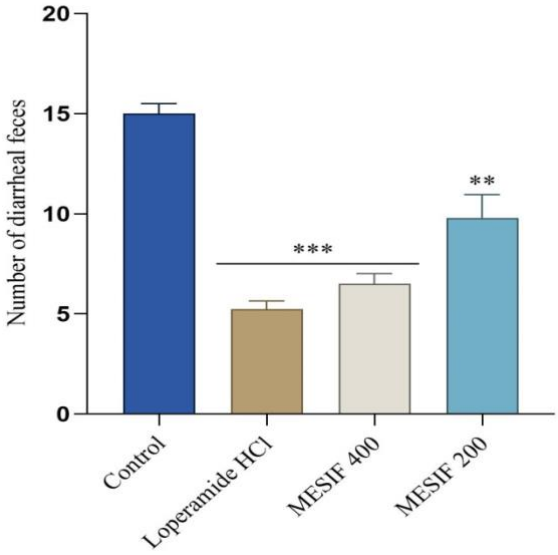

Figure 4. Effect of MESIF and loperamide HCl on castor oil induced diarrhea in mice. Values are expressed as mean ± SEM (n = 5). One-way analysis of variance (ANOVA) followed by Dunnett's test was employed where **p<0.05 were considered statistically significant compared to the control. ** = Moderate significant; *** = Highly significant; HCl = Hydrochloric acid and (MESIF) = Methanol extracts of *S. indicus* flower.

DISCUSSION

Nature is often regarded as the optimal reservoir of therapeutic compounds because it contains a diverse array of natural chemicals with significant therapeutic properties. This study focuses on the ethnomedicinal study of *S. indicus* [28]. To evaluate the therapeutic potential of medicinal plants, which is necessary to undertake many stages, such as detecting and characterizing bioactive compounds. This has captivated researchers eager to investigate the medicinal potential of these plants using an efficient solvent extraction method. Several bioactive chemicals, such as flavonoids, polyphenols, alkaloids, tannins, and terpenoids, have a high solubility in methanol owing to its high polarity index of (5:1) [29, 30]. The justification for the widespread use of methanol in medicinal plant extraction processes, including its application in the investigation of *S. indicus* flower, is well-founded. The ongoing phytochemical screening with qualitative test in this study has also identified the existence of several ingredients, such as flavonoids, glycosides, alkaloids, carbohydrates, tannins, cholesterols, phenols, and reducing sugars and proteins and amino acids.

Plant extracts containing significant polyphenols may reveal redox resources by absorbing and counteracting free radicals via the scavenging capacities of their hydroxyl groups, thereby displaying antioxidant activity. Flavonoids have a high efficacy in reducing unfavorable substances that induce oxidation, including peroxides, single and triplet oxygen, and other free radicals. A number of illnesses recognize these compounds for their role in their progression [31, 32]. The IC_{50} value for MESIF against the DPPH free radical was 26.33.01 µg/mL, showing that its antiradical activity changed with concentration. The total phenolic compound was found to be 93.33±1.76 mg of GAE/gm of methanol extract. The results show that MESIF acts as a notable antioxidant source and has variable hydrogen-donating functions depending on dose. After considering all the results, we concluded that alkaloids, phenols, flavonoids, terpenoids, tannins, and other similar compounds are likely the main factors responsible for the antioxidant, antidiarrheal, depressive, thrombolytic, and antibacterial effects of the MESIF.

The antimicrobial effect of an extract is influenced by the phytochemical composition, extraction solvent, solubility and miscibility of the therapeutic ingredient in the test medium, susceptibility of the test organisms, and evaluation technique [33, 34]. Previous investigations indicate that several phytochemical substances, including alkaloids, saponins, tannins, flavonoids, and steroids, possess biological activity and contribute to the antibacterial properties of plants. Consequently, traditional medicine employs these chemicals [35]. In gram-positive bacteria, antibacterial substances have the ability to disturb the cell wall of the bacterial structure, leading to the release of cytoplasm and the coagulation process [36]. The phytocompounds in the MESIF may work in a similar way to other substances to stop bacteria from making biosynthetic pathways, such as those that make cell walls, DNA, lipids, and proteins [37]. The purpose of this study was to assess the antibacterial capabilities of MESIF. The examination revealed that *S. indicus* exhibited a mild to moderate susceptibility to the tested strains. Therefore, we might consider MESIF as a potential reservoir of antimicrobial compounds for further investigation.

Researchers examined the plant's anti-inflammatory properties by examining its capacity to prevent protein denaturation. When the secondary and tertiary structures of proteins are lost due to external stimuli like heat, stress, organic solvents, strong acids, or bases, the process is known as protein denaturation [38]. Differences in hydrophobic, hydrogen disulphide bonding, and electrostatic bonding are all part of the denaturation process [39]. Proteins may be denatured by alkalis, acids, oxidising agents, reducing

agents, and certain organic solvents. Of all the denaturing agents, the ones that have an impact on the secondary and tertiary structures but do not influence the main structure are particularly fascinating [40]. The current study indicated that MESIF had a strong inhibitory effect on protein denaturation, comparable to the conventional medication diclofenac sodium. Phytochemicals are naturally occurring bioactive chemical substances found in plant-based meals. These substances possess antioxidant characteristics, enabling them to counteract detrimental free radicals. There are numerous biological components, such as the proteins, cell membrane, and DNA, that may be damaged by free radicals, which are highly reactive molecules [41]. This corresponds to research that indicates flavonoids, terpenoids, saponins, tannins, and alkaloids are crucial in the management of inflammatory illnesses [42]. Several flavonoids have potent inhibitory effects on an extensive variety of enzymes, including protein kinase C, phosphodiesterases, protein tyrosine kinases, phospholipase A2, and prostaglandin production enzymes [43]. Polyphenols exert their anti-inflammatory properties through a numbers of mechanisms, including chelating metal ions, scavenging free radicals, inhibiting NOX activity, modulating the mitochondrial respiratory chain, inhibiting specific enzymes involved in the generation of ROS, such as xanthine oxidase, and boosting the activity of endogenous antioxidant enzymes [44]. Significant levels of secondary metabolites, such as flavonoids, alkaloids, tannins, saponins, steroids, glycosides, and phenols, may contribute to potent anti-inflammatory properties OF the plant extract. Further studies on the phytochemicals included in the extract and its fractions are highly recommended. Isolating and identifying the bioactive metabolites ought to be the next step. This will make it easier to identify with certainty the precise substances causing the aforementioned pharmacological properties.

In addition, this study evaluated and compared the thrombolytic activity of MESIF using streptokinase as the positive control and sterile distilled water as the negative control. Several thrombolytic drugs activate the plasminogen enzyme, which in turn disassembles the fibrin network. Because of this process, the blood clot becomes soluble and can be further broken down by other enzymes [45]. This process restores blood flow in previously blocked blood vessels. Deep vein thrombosis, myocardial infarction, pulmonary embolism, and thromboembolic strokes can all be effectively treated with this technique. It successfully unblocks the blocked artery and lessens the risk of long-term tissue damage. The results of the present study demonstrated a significant thrombolytic impact of MESIF. The antithrombotic activity of total saponins, flavonoids, and polyphenols may be due to their combined presence [46]. Multiple investigations have demonstrated that polyphenols target certain thrombogenic pathways, including the COX-1-thromboxane, glycoprotein VI (GPVI)-collagen, protease-activated receptor 1 (PAR1)-thrombin, and P2Y1/P2Y12-ADP pathways [47]. These methods can influence the activation, attachment, release of granules, and clumping together of platelets. However, the extract exhibited a smaller percentage of clot dissolution compared to the commercially available medication streptokinase.

In the community, anxiety and depression are exceedingly common and strongly correlated with a significant burden of illness. Therefore, it is crucial to acknowledge and resolve these issues by implementing effective solutions. The two most commonly used animal models for evaluating antidepressant medications are the FST and TST [26]. In both experiments, researchers' subject animals to an inevitable scenario, observing the antidepressant effect as a decrease in the amount of time they remain motionless. Many medicinal substances that effectively treat depression in people can alleviate immobility in the TST, a clear indication of hopelessness. Likewise, the Forced Swim Test (FST) pushes mice to swim inside a limited space from which they are unable to

get away. This induces a state of behavioral despair in animals, believed to mimic the symptoms of depression in humans. The results indicated that MEPI administration contributed to a decline in the duration of immobility time exhibited by the mice during the FST and TST. When animals have lost hope of escaping, immobility is considered to be a posture that reflects a state of behavior despair. The mice in this investigation received oral dosages of 200 and 400 mg/kg of methanol extract. The MESIF exhibited a noteworthy amount (***$p<0.001$) of antidepressant-like effects in both the TST and FST assessments. The diazepam, which served as the positive control, was administered orally at a dosage of 10 mg/kg. The findings demonstrated that the oral administration of the extract had a distinct antidepressant-like effect on the MESIF. This effect is not due to a psychostimulant impact, as evidenced by the decrease in immobility time [48]. TCAs (tricyclic antidepressants), SSRIs (selective serotonin reuptake inhibitors), selective RIMAs (reversible inhibitors of monoamine oxidase A), and specific SNRIs (serotonin-noradrenaline reuptake inhibitors) are often prescribed for a wide range of neurodegenerative health problems. In spite of that, these medications might potentially lead to many negative effects, including heart toxicity, low blood pressure, sexual dysfunction, weight gain, and sleep difficulties [49, 50]. The phytochemical analysis revealed the presence of flavonoids and phenolic compounds, known for their numerous biological activities, including their potential to treat disorders of the CNS (central nervous system) [51].

The research showed that the MESIF significantly decreased the number of diarrheal stools, with statistical significance level of $p < 0.01$ and $p < 0.001$ levels. A lack of coordination between the movement of the smooth muscles in the intestines and/or the absorption process in the gastrointestinal tract may initiate diarrhea [49]. People have widely acknowledged the efficacy of castor oil as a treatment for diarrhea [50]. Castor oil is a potent laxative that is known for its ability to induce diarrhea. It works by promoting the breakdown of fats in the upper portion of the small intestine, which leads to the production of ricinoleic acid. This acid has several effects on the body, including stimulating fluid secretion, inhibiting the water and electrolytes absorption, reducing the absorption of sodium and potassium ions, and decreasing the activity of a specific enzyme in the small intestine and colon named Na+, K+, −ATPase [52]. When the oil comes into contact with pancreatic acid, it releases ricinoleic acid, which causes these effects [53]. Ricinoleic acid may induce the production of prostaglandins, which play a fundamental role in diarrhea development. These prostaglandins regulate the function of the gastrointestinal tract, promoting increased motility and secretion, ultimately leading to diarrhea [54]. A new molecular mechanism shows that ricinoleic acid turns on the EP_3 prostanoid receptor. This receptor controls the drug-like effects of castor oil. EP_3 prostanoid receptors are activated by ricinoleic acid, stimulating intestinal and uterine-muscle cells, shedding light on the cellular and molecular processes by which castor oil induces a laxative effect. MESIF may demonstrate its antidiarrheal effect in many ways, including by decreasing the release of prostaglandins. Plant extracts contain flavonoids and alkaloids that alter the production of COX-1 and COX-2 (cyclooxygenases 1 and 2) and LOX (lipooxygenases). This inhibits the synthesis of prostaglandins and autacoids [55, 56]. Flavonoids, a diverse collection of polyphenolic chemicals, have a range of biological actions, including antioxidant, antispasmodic, anti-inflammatory, and antidiarrheal properties. They may also exhibit antidiarrheal activity by reducing intestinal movement and regulating the fluids and electrolytes release [57, 58]. The claim indicates that the bioactive effects seen in certain substances, such as plant extracts, are probably attributable to the presence of several phytochemical groups, including alkaloids, flavonoids, tannins, saponins, and glycosides, among others. These chemicals are recognized for their many

biological actions, including antioxidant, anti-inflammatory, antibacterial, thrombolytic, antidepressant, and antidiarrheal properties. Nevertheless, while these phytochemicals are thought to account for the observed benefits, more assessments are required to substantiate these theories, pinpoint the precise active compounds, elucidate their methods of action, and ascertain their practical applicability. Such investigations validate the significance and applicability of these discoveries, guaranteeing their proper implementation in disciplines such as medicine, nutrition, and pharmacology.

CONCLUSIONS

The current study shows that the methanol extract from *S. indicus* flowers may have properties that help with depression, diarrhea, blood clotting, inflammation, bacteria, and antioxidants. Secondary metabolites, which have promising pharmacological properties, are likely responsible for these actions. In order to determine the specific bioactive molecules that are responsible for the mentioned pharmacological activity, we highly recommend performing a comprehensive analysis of the plant extract, followed by the identification and isolation of its secondary metabolites. It is recommended to do a comprehensive study to find out the probable molecular mechanism of action in the animal model and, subsequently, in humans to ascertain therapeutic effectiveness.

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AUTHOR CONTRIBUTIONS

AK conducted the study & developed the design of this experiment. MEH & MNI set up the facilities for the experimental investigation and oversaw it. AK, TSR, AF, MJS, & NNA prepared the extract preparation, data collection, investigational work & literature review. AK, TSR, worked on statistical analysis with the study design and interpretation of the findings with MA, & MNI, AK, TSR, AF, MJS, NNA & MA performed the initial drafting. MA & MNI reviewed, correspondence and updated the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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