Phytochemical and pharmacological activities of *Pogostemon strigosus* Benth

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Abstract

In the context of drug discovery and development, there is an increasing demand for the utilization of plants and their derived compounds in modern medicinal chemistry. This study aims at the investigation of phytochemical constituents and pharmacological properties of *Pogostemon strigosus* Benth. of the family Lamiaceae. The ethanol extract of the whole plant was fractionated, and then followed by preliminary phytochemical investigation and the evaluations of several pharmacological activities which include anti-atherothrombosis, anti-diarrheal, anti-inflammatory, analgesic, anxiolytic, antipyretic, neuropharmacological activities using models which includes application of human erythrocytes blood clot test), castor oil-induced diarrheal test, egg albumin test, acetic acid-induced writhing model, swing test, open field, light-dark test, and brewer's yeast induced fever test, respectively. Phytochemical investigation suggests that the ethanol extract of *P. strigosus* contains alkaloids, carbohydrates, flavonoids, oleoresin, glycosides, reducing sugar, steroids, tannins, and terpenoids. The pharmacological evaluation suggests that *P. strigosus* has significant anti-atherothrombosis, anti-diarrheal, anti-inflammatory, analgesic, anxiolytic, anti-pyretic, and neuropharmacological effects. The chloroform and n-hexane fractions also showed better anti-inflammatory capacities in the egg albumin (77.51 ± 0.06 and 125.7 ± 0.07 µg/mL, respectively) and human erythrocytes (HRBC) (50.60 ± 0.07 and 30.01 ±0.10 µg/mL, respectively). The ethanol extract of *P. strigosus* showed the above-mentioned pharmacological activities in the mice model. *P. strigosus* may be one of the potential sources of phytotherapeutic lead compounds. Further studies are necessary to characterize the phytochemicals responsible for the observed bioactivities. This report adds credence to the traditional claims about the application of the plant in folkloric medicine.

Key words: *Pogostemon strigosus*. Phytochemicals. Pharmacological Effect. Erythrocytes.

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1. Introduction

As the people are becoming progressively aware of the potency and side effects of synthetic therapeutics, there is increasing attention to the application of natural products as alternatives [1]. Traditional medicinal plants or natural products have been a major source of drug "lead"in the pharmaceutical industry. Plants have been used for medicinal purposes from ancient prior to the prehistoric period.

Due to the increasing price of western medicines, medicinal plants afford cheaper, readily available and less toxic alternatives to the treatment and management of diseases. The decision of to usea medicinal plant is often based on folkloric knowledge about the plant [2]. The understudied *P. strigosus* of the family Lamiaceaeis grossly utilized in ethnomedicine for the treatment of a wide range of human diseases and disorders such as diabetes, cuts or wounds, indigestion problem, cough, asthma, kidney stones, hepatitis, gall bladder stones, urinary tract bleeding, hemorrhoids, constipation, diarrhea, vomiting, blood poisoning, fertility problems, septicemia, skin allergy, rheumatism, high cholesterol levels, and low blood pressure.

About 25 species of *Pogostemon* are reported to occur in India. Patchouli is also known as patchouly, *tamalapatra* in Sanskrit, *patchouli* in Hindi, *patchetene* in Kannada, *pacchilai* in Tamil, *patchilla* in Malayalam, *patchapan* or *patcha* in Marathi and *guanghouxiang* in Chinese, *nilam* in Malaysia and Indonesia, *phimsen* in Thailand. Morphologically, patchouli is a hardy perennial herb adapted to hot and humid climatic conditions. It grows up to 1 to 1.2 m height with an erect stem and broad leaves (0.85 inches). The margins of the leaves are lobed and abundant hairs are present on its dorsal surface [3]. The leaves are known to accumulate essential oil in the glandular trichomes [4]. The plant bears small pale pink-white flowers [5]. *P. strigosus* Benth. (pathchoula (Bengali) synonym: *Dysophylla strigosa* Benth.)

It is a glabrous herb grown as annual and reproducing viaseed. It is a small woody climber with grooved, strigose, branchlets plant. Leaves are sub or bicular, palmately 3-lobed, pilose on both surfaces. Flowers minute campanulate, in a lax much-branched inflorescence. The capsule is globose, size of a large cherry, studded with subulate barbed prickles. Apparently, there is a dearth of information on the plant as it is an endangered species [6].

Aerial parts Flower

Figure 1. Different parts of *P. strigosus* Benth

In the study, we have investigated phytochemical composition, anti-atherothrombosis, anti-diarrheal, anti-inflammatory, analgesic, anxiolytic, antipyretic, neuropharmacological activities of the ethanol extract of *P. strigosus* by applying some *in vitro* and *in vivo* test methods.

2. Materials and Methods

2.1 Collection, identification, and extraction

The fresh whole plant of *P. strigosus* was collected from Adampur Reserve forest, Kamalganj, Moulovibazar, Bangladesh in January 2019 at the daytime. The fresh whole plants were collected from healthy host plants. During collection, all type of adulteration was strictly prohibited. The plants were mounted on a herbarium sheet and the sample was identified by Specialists at the Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen (with Accession number-46861, DACB) was also deposited at the Ethnobotany Database of Bangladesh. The collected plants were separated from undesirable materials or plant parts and then washed with water. They were shade-dried for one week followed by pulverization into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced. Approximately 350 gm of powdered material was taken in a clean, flat-bottomed Amber glass container and soaked in 1500 mL ethanol (99-100%). The container with its contents was sealed and kept for a period of 21 days with accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white Markin cloth. Thereafter, the extract was finally filtered through filter paper and the resultant extract was concentrated on a rotary evaporator to evaporate ethanol. The concentrated extract was air-dried for several days in a beaker covered with perforated aluminum foil. This afforded a gummy concentrate of greenish-black color which was designated crude extract of Ethanol. The yield was determined and qualitative phytochemical screening to determine the presence of phytoconstituents such as - carbohydrates, alkaloids, glycosides, tannins, gums, flavonoids, and terpenoids were carried out.

2.2 Reagents and chemicals

Ascorbic Acid and acetic acid were purchased from Merch, Germany, while tween-80 from obtained from the Loba chemical Pvt. Ltd., India. Solvents and all the other reagents and chemicals were of analytical grade. Streptokinase (SK, Durakinase) powder for reconstitution; 1500000 unit/vial used in clot lysis test was purchased from Dongkook Pharmaceutical Co. Ltd., South Korea. Loperamide was kindly provided by Square Pharmaceuticals Ltd., Bangladesh.

2.3 Experimental animals

Young Swiss-albino mice aged 4-5 weeks, average weight 20-25 gm acquired from the Department of Pharmacy, Jahangirnagar University were used for the experiment. They were kept in standard environmental condition for one week in the animal house of the Pharmacy Department, Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Bangladesh for adaptation after their purchase. The animals were provided with standard laboratory food and tap water and maintained a natural day-night cycle. All the experiments were conducted in an isolated and noiseless condition.

2.4 Phytochemical investigation

Preliminary phytochemical screenings were carried out to investigate plant secondary metabolites present in the crude extract of the plant by adopting the standard procedure Three types of solution of extract were made first for this test:5% w/v alcoholic solution of extract (1.5 gm was dissolved in 30 mL ethanol), 5% w/v aqueous solution of extract (0.1gm was dissolved in 2 mL distilled water), 5% w/v chloroform extract (0.2gm was dissolved in chloroform) [7-9].

2.5 Anti-inflammatory assay

This test was carried out with a slight modification of [10]. Briefly, the reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of extract so that final concentrations become 0.12 to 0.36% (v/v). A similar volume of distilled water served as a negative control. Then the mixtures were incubated at (37 °C ± 2) in a BOD incubator (Lab-line Technologies) for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using the vehicle as a blank. Acetylsalicylic acid (ASA) at the final concentration 100 µg/mL was used as a reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\%\text{Inhibition} = [(\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Test}}) / \text{Abs}_{\text{Control}}] \times 100$$

2.6 Anti-diarrheal activity assay

Standard procedure was adopted [11,12]. Test samples, control, and loperamide were given orally by means of a feeding needle. The mice were fed with the samples, control, and loperamide 40 minutes prior to the oral administration of castor oil at a dose of 0.3 mL per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour for four hours of study after the castor oil administration. A number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4 hours period and were noted for each mouse.

2.7 Anti-pyretic assay

Twenty-five albino mice weighing 20-25g were used. They were divided into five groups of five mice each. Group one serves as negative control (n=5) and was given 1mL/kg of normal saline, group two control (n = 5) was treated with brewer yeast alone, and group three positive control (n = 5) was given 100mg/kg of Paracetamol, while groups four and five were treated with 250 mg/kg, and 500 mg/kg (n = 5) of *P. strigosus* respectively. A suspension of brewer's yeast was injected subcutaneously to induce fever in all the experimental animals. After 18 hrs, the rectal temperature was taken and the animals were administered ethanol extract of *P. strigosus* (250 and 500 mg/kg) and paracetamol (standard group, 100 mg/kg) orally.

2.8 Anxiolytic assay

The light-dark test is the sensitive model commonly used to detect activity in anxiety-related disorders. This apparatus consists of an acrylic box (40 × 60 × 20 cm) divided into light and dark chambers. The light chamber (40 × 40 cm) was painted white and connected via an opening (7 cm) at floor level to the dark chamber (40 × 20 cm), which was painted black. A lamp with a 60-W white light was placed 40 cm above the light chamber. Albino mice (20-25 gm) of either sex were divided into four groups of four mice in each group and they were fasted overnight prior to the test but the water was given. Group I - Normal control received vehicle only, Group II - Diazepam (1 mg/kg, i.p.), Group III - EPS 250 mg/kg, p.o., Group IV - EPS 500 mg/kg, p.o.

2.9 Pentobarbital-indued sleeping test

Righting reflex is a useful measure for assessing whether or not animals are asleep. Mice were given a single IP dose of the vehicle, diazepam (2 mg/kg) as the reference drug or different concentrations of the extract (250, 500 mg/kg). These treatments were carried out 30 min before challenging the animal with IP injection of pentobarbital (40 mg/kg). The latency of the loss of the righting reflex and the total sleeping time (the time between the loss and the recovery of the righting reflex) were determined for each mouse.

The mouse was considered as being awake if it could right itself (return to the upright position). Once a mouse righted itself, it was placed on its back once more and allowed to right a second time for confirmation [13].

2.10 Anti-nociceptive test

Test samples, control, and diclofenac sodium were given orally by means of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. Then the writhing-inducing chemical, acetic acid solution (0.7%) was administered intraperitoneal to each animal group. After an interval of 5 minutes, which was given for absorption of acetic acid, a number of squirms (writhing) was counted for 15 minutes.

2.11 Thrombolytic activity test

4 mL venous blood was drawn from healthy volunteers and was distributed in Group I- negative control (distilled water); Group- II: standard (streptokinase) and Group III: for 5 different concentrations of the aqueous crude extract pre-weighed sterile microcentrifuge (Alpin / Ephendorf's) tubes (0.5 ml/tube) and incubated at 37 °C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of the tube alone). To each micro-centrifuge tube containing a pre-weighed clot, 100 µL (5 µg/µL) of crude extract of the plant was added separately. As a positive control, 100 µL of streptokinase and as a negative control, 100 µl of distilled water were separately added to the controls marked tubes. All the tubes were then incubated at 37 °C for 90 min and observed for clot lysis. After incubation, the fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption [14,15].

2.12 Statistical analysis

The result was presented as mean ± SEM (standard error of mean); Student's t-test was used to determine the significant difference between the experimental and control groups. The test results were statistically significant when $p<0.05$ at 95% confidence intervals. Half maximal effective concentrations were calculated by using "GraphPad Prism" software (Version: 6.0, San Diego, California, USA copyright© 1994-1999), considering $p<0.05$ at 95% confidence intervals.

3. Results

3.1 Phytochemical screening results

Phytochemical investigation suggests that the ethanol extract of *P. strigosus* contained alkaloids, carbohydrates, flavonoids, resins, glycosides, reducing sugar, steroids, tannins, and terpenoids (**Table 1**).

Table 1. Phytochemical screening of the ethanolic extract of *P. strigosus*

Phytochemical group	Test result
Alkaloids	+
Tannins	+
Carbohydrates	+
Saponins	-
Combined reducing sugars	-
Gums	+
Terpenoids	+
Steroids	+
Glycoside	+
Flavonoids	+
Reducing sugars	+

+ = Positive (Present); - = Negative (Absent).

3.2 Anti-inflammatory (egg albumin) and clot lysis test

In the egg albumin (*in vitro*) test, the crude extract showed an anti-inflammatory effect in a concentration-dependent manner. The extract exhibited the highest inhibition of albumin denaturation at 0.36% (v/v). The standard (ASA) at 200 µg/mL exhibited a better anti-inflammatory effect than the test sample. The EC_{50} calculated for the three fractions of the extract of EPS, CPS, HPS was 99.65 ± 0.06%, 77.51 ± 0.06% and 125 ± 0.07% (v/v), respectively. Furthermore, ASA exerted a significant anti-inflammatory effect when compared to all the test concentrations which are given in **Table 2**. The extract exhibited the highest (67.53 %) inhibition of albumin denaturation at 200 µg/mL concentration, while the standard (ASA) at 93.86 ± 0.08 µg/mL exhibited a better anti-inflammatory effect than the test sample.

3.3 Anti-diarrheal activity

Castor oil exhibited cathartic effect in the first hour following its oral administration to mice. When the doses of decoction were administered orally 30 minutes before the administration of castor oil, the time in the excretion of the first diarrheic stool was significantly increased compared to the control group. Likewise, the decoction significantly reduced both the total number of stool and the number of diarrheic stool excreted in the following four hours after the administration of the cathartic agent. The percentage of wet faeces decreased from 81.4% in the control group to 59.8% and 64.3% at doses of 250 mg/kg and 500 mg/kg of lyophilized decoction, respectively (**Table 3, 4**). Four hours after the administration of castor oil, there was no more excretion of diarrheic faeces in any of the groups of animals. The extract of *P. strigosus* increase the latent time and reduce the stool count significantly.

Table 3. Latent periods observed in the treatment groups

Table 2. Anti-inflammatory and membrane stabilization activities of aqueous crude extract of *P. strigosus*

Parameters		**%IPD**				**%IHL**			
		EPS	CPS	HPS	ASA	EPS	CPS	HPS	ASA
Conc. (µg/mL)	25 (µg/mL)	20.5± 0.03	25.3± 0.02	15.6± 0.01	25.5± 0.01	32.5±0.02	35.5± 0.03	50.7±0.02	22.5± 0.01
	50 (µg/mL)	25.3± 0.03	30.2± 0.02	22.3± 0.01	35.2± 0.01	42.5±0.02	45.5± 0.02	56.0±0.01	27.6± 0.02
	100 (µg/mL)	40.6± 0.03	55.8± 0.02	38.8± 0.01	50.3± 0.01	57.3±0.01	65.5± 0.01	72.9±0.03	39.7± 0.03
	150 (µg/mL)	65.8± 0.03	65.9± 0.02	45.7± 0.01	65.6± 0.01	62.3±0.02	75.2± 0.02	78.3±0.01	62.9± 0.01
	200 (µg/mL)	75.3± 0.03	85.2± 0.02	65.6± 0.01	89.4± 0.01	86.9±0.01	84.5± 0.03	87.5±0.01	87.5± 0.02
EC_{50}		99.65 ±0.06	77.51 ±0.06	125±0 .07	75.66 ±0.07	61.06± 0.09	50.62 ±0.07	30.00± 0.10	93.86 ±0.08
CI		67.76 ~146. 6	52.52 ~114. 4	81.01 ~195	47.56 ~120. 4	30.362 ~118.8	33.13 ~77.2 6	15.63~ 50.18	57.45 ~153. 4
R^2		0.91	0.92	0.87	0.90	0.83	0.92	0.87	0.86

Values are mean ± SEM (n = 3); EPS: Ethanolic extract of P. strigosus*; CPS: Chlorofom extraxt of P.* strigosus*; HPS: n-Hexane extract of P.* strigosus*; ASA: Acetyl salicylic acid*

Treatment groups	***Dose and Route of administration***	***Latency (min)***
NC	10 mL/kg (p.o)	15 ± 0.01
LOP	3 mg/kg (p.o.)	30 ± 0.01

EPS	250 mg/kg (p.o.)	20 ± 0.01
	500 mg/kg (p.o.)	25 ± 0.01

Values are mean ± SEM (n = 5); EPS: Ethanolic extract of P. strigosus*; LOP: Loperamide*

Table 4. Diarrheal secretions of mice in different treatment groups at 1st, 2nd, 3rd and 4th hours

Treatment groups	Dose and Route of administration	1st hr	2nd hr	3rd hr	4th hr
NC	10 mL/kg (p.o)	6±0.02	4±0.02	4±0.02	2±0.02
LOP	3 mg/kg (p.o.)	2±0.02	2±0.02	3±0.02	1±0.02
EPS	250 mg/kg (p.o.)	3±0.02	4±0.02	`3±0.02	3±0.02
	500 mg/kg (p.o.)	3±0.02	2±0.02	4±0.02	1±0.02

Values are mean ± SEM (n = 5); EPS: Ethanolic extract of *P. strigosus*; LOP: Loperamide

3.4 Anti-Pyretic (Brewer's yeast-induced pyrexia) test

The results of the antipyretic effect of the different doses of the test crude extract (250 and 500 mg/kg), standard (paracetamol, 100 mg/kg), negative control, and control are depicted in **Table 5**. Paracetamol and the ethanol extract at a dose of 500 mg/kg started showing effective antipyretic activity after 1h of post-dosing; while at a dose of 250 mg/kg the effect was showing mild but significant activity as that of paracetamol when compared with the control. Comparing all other groups with the negative control, there was a significant reduction in mice's body temperature. The antipyretic activity was observed up to 4 h after paracetamol and test extract administration.

Table 5. Antipyretic effect of *P. strigosus* and controls in Brewer's yeast-induced pyrexia in mice

Treatment and dose	***Rectal temperature (°F)***					
	Basal temp.	**0 hr (after 18 hr)**	**1 hr**	**2 hr**	**3 hr**	**4 hr**
Control(10mL/kg, normal saline)	34.99±0.02	35.99±0.02	36.11±0.02	36.35±0.01	36.45±0.02	36.89±0.05
Control(20ml/kg, brewer's yeast)	35.55±0.03	36.99±0.01	37.20±0.02	37.55±0.06	37.65±0.05	37.88±0.04
PARA (100 mg/kg)	35.35±0.01	36.88±0.02	35.55±0.07	35.45±0.08	35.35±0.02	35.35±0.03
EPS (250 mg/kg)	35.44±0.02	36.88±0.02	36.08±0.03	35.82±0.04	35.81±0.02	35.65±0.04
EPS (500 mg/kg)	35.66±0.07	36.95±0.05	36.55±0.03	36.01±0.06	35.99±0.05	35.78±0.06

Values are mean ± SEM (n = 4); EPS: Ethanolic extract of P. strigosus PARA: Paracetamol

3.5 Anxiolytic test

The effects of the different doses of EPS on sleeping time and sleep latency induced by pentobarbital are shown in **Table 7**. EPS at the doses of 250 and 500 mg/kg significantly increased sleeping time compared to the vehicle ($p < 0.05$ and $p < 0.01$), respectively, and this effect is nearly equivalent to the reference drug (diazepam, 2 mg/kg, i.p.), while EPS at dose 250 mg/kg moderately increased sleeping time significantly ($p < 0.05$) compared to diazepam induced anxiolytic test in *swiss* albino mice. Based on our preliminary investigations it can be deduced that the crude extract of *P. strigosus*, some fractions displayed significant anxiolytic activity. The extract of EPS at 500 mg/kg showed significant anxiolytic activity.

Table 6. Pentobarbital induced sleeping test the vehicle

Treatment groups	Dose	OS (min)	DS (min)
NC	10mg/kg (p.o)	5.33±0.01	87.33±0.03
PC (DZP)	2mg/kg (i.p)	3.00±0.01	115.00±0.02
EPS	250mg/kg (p.o)	6.33±0.01	91.00±0.02
	500mg/kg (p.o)	4.00±0.01	105.00±0.02

Values are mean ± SEM (n = 3); EPS: Ethanolic extract of P. *strigosus* ; DZP: Diazepam

Table 7. Diazipum induced anxiolytic test in *swiss* albino mice

Treatment and dose	OFT			HCT	SwT	D/LT
	NFC	Grooming	Rearing	NHC	NSw	DR
Control (10 mL/kg, p.o.)	145±0.01	46±0.02	48±0.01	25±0.02	17±0.01	125±0.02
DZP (2 mg/kg, i.p.)	111±0.01	19±0.02	15±0.01	12±0.02	10±0.01	155±0.02
EPS (250 mg/kg, p.o.)	120±0.01	20±0.02	25±0.01	18±0.02	25±0.01	145±0.02
EPS (500 mg/kg, p.o.)	128±0.01	15±0.02	22±0.01	22±0.02	20±0.01	150±0.02

Values are mean ± SEM (n = 5); EPS: Ethanolic extract of P. *strigosus*; DZP: Diazepam.

3.6 Analgesic test

Each mouse of all groups was observed carefully to count the number of writhing that they had made in 15 minutes. The animal do not always perform full writhing, because sometimes the animals begin to produce writing but they do not complete it. Such incomplete writhing was taken as half-writhing, hence, two half-writhing were taken as one full writhing. EPS extract at 250 and 500 mg/kg (p.o.) significantly inhibits writhing in comparison with control.

Table 8. Mean writhing and percentage protection in the treatment groups

Treatment groups	Dose and Route of administration	% Writhing	%Protection
NC	10 mL/kg (p.o)	36.00 ±0.01	-
Diclofenac-Na	25 mg/kg (p.o.)	6.67 ±0.01	81.47±0.01
EPS	250 mg/kg (p.o.)	25.00 ±0.01	30.56±0.01
	500 mg/kg (p.o.)	12.67 ±0.01	64.81±0.01

Values are mean ± SEM (n = 5); EPS: Ethanolic extract of P. *strigosus*;

3.7 Anti-atherothrombosis activity

The extract of the plant showed clot lysis capacity in a dose-dependent manner. The highest clot lysis (58.85±2.98%) was observed in 200 µg/mL of EPS extract and the other two extracts of CPS and HPS showed (56.23 ± 2.45%) and (54.45 ± 2.15%) respectively. The standard (streptokinase) produced clot lysis (82.63 ± 1.23%), while the NC group exhibited a negligible clot lysis capacity (3.56 ± 2.26%). The clot lysis capacity, when compared between the test groups and the SK group, suggests that the EPS, CPS, HPS extract, and SK groups produced significantly ($p < 0.05$, $p < 0.01$, $p < 0.001$) clot lysis effects in comparison to the NC group.

Table 9. Anti-atherothrombosis activity of *P. strigosus*

Parameters		***% of clot lysis***			
		EPS	**CPS**	**HPS**	**SK (100 I.U.)**
Conc. (µg/mL)	25	8.79 ± 2.26	9.56 ± 2.56	7.23 ± 3.25	15.23 ± 1.56
	50	25.89 ± 2.56	22.61 ± 2.69	15.23 ± 2.56	26.56 ± 1.23
	100	32.65 ± 1.69	38.23 ± 3.45	34.65 ± 3.45	46.89 ± 1.25
	150	45.36 ± 3.25	48.23 ± 2.89	42.72 ± 3.42	68.78 ± 1.65
	200	58.85 ± 2.98	56.23 ± 2.45	54.45 ± 2.15	82.63 ± 1.23
VEH	100	3.56 ± 2.26 (0.05% Tween 80 + 0.9% NaCl solution)			

Values are Mean ± SEM (n = 3), EPS: Ethenolic extract of P. stigosus, CPS: Chlorofom extract of P. strigosus, HPS: n-Hexane extract of P. strigosus, SK: Streptokinase

4. Discussion

Erythrocyte membrane stabilization of lysosomal has a great contribution to decreasing inflammatory responses by preventing the release of lysosomal chemicals such as bactericidal enzyme proteases, histamine, and prostaglandin [16,17]. This study indicates a concentration-graded anti-inflammatory and membrane-stabilizing capacity of *P. strigosus*. The results of the present work suggest that the anti-inflammatory activities of plant extracts could be explained in part, by their antioxidant properties [18]. The low concentration-mediated protective effects and the high concentration-mediated toxic effects may be linked to the presence of variation of active constituents in the crude extract.

In the small intestinal transit test, the extract was able to inhibit intestinal motility. A rising tendency of the inhibitory effect on gastrointestinal motility was observed when the dose was increased. During the

experiment, the charcoal meal method was selected to follow the displacement of the gastrointestinal content because the reduction of gastrointestinal motility is a mechanism by which many antidiarrheal activities are measured [19-20]. A decrease in the motility of gut muscles increases the stay of substances in the intestine. This allows a greater time for absorption [21,19]. Thus, the reduction in the intestinal propulsive movement in the charcoal meal model may be due to the anti-motility property of the extract. This assumption is further corroborated by the anti-motility effect of the ethanol extract of *P. strigosusas observed.*

Flavonoids belong to the polyphenol family and are found in most plant materials. Some of the activities attributed to flavonoids include anti-allergic, anti-cancer, antioxidant, anti-inflammatory, and anti-viral [22]. A variety of flavonoids have also been found to inhibit prostaglandin synthase (e.g., COX-2) production and transcription [23,24]. Plants such as *P. hirsute, K. Schum* and *J. Trichotomum* are known to contain flavonoids and have been found to possess antipyretic properties [25,26]. Hence, the presence of flavonoids might account for the antipyretic effect that was observed in *P. strigosus*. The efficacy of the antipyretic effect of *P. strigosus* extract was observed to have increased with increased concentration (dose-dependent manner). This can be said to be due to the increased concentration of the component of the extract exhibiting antipyretic effects.

The elevated hole cross after treatment with ethanol extract of *P. strigosus* revealed the anxiolytic activity. Since significant attenuation of anxiety-like behavior which is the most representative indices of anxiolytic activity [27]. The OFT is used to evaluate the animal's emotional state. The open field model examines anxiety-related behavior characterized by the normal aversion of the animal to an open, bright area [28]. The anxiolytic activity was also observed in the light/dark test. LDT is an ethological-based approach-avoidance conflict test. Earlier reports on the chemical constituents of plants and their pharmacology suggest that plants containing flavonoids, alkaloids, phenolic compounds, and tannins possess activity against many CNS disorders [29]. Investigations on the phytochemical composition of *P. strigosus* revealed the presence of alkaloids, glycosides, steroids, tannins, phenolic compounds, and flavonoids. Diazepam which belongs to the benzodiazepine group is a central nervous system depressant used in the management of sleep disorders such as insomnia. Benzodiazepines have a binding site on the GABA receptor type-ionophore complex ($GABA_A$) [30,31].

The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [32]. The agent reducing the number of writhing will render an analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [33,34]. The significant pain reduction by EPS might be due to the presence of analgesic principles acting through the prostaglandin pathways. The abdominal writhing induced by acetic acid was also reported to be less selective [35]. and proposed to act indirectly by releasing endogenous mediators stimulating neurons that are sensitive to other drugs such as narcotics and centrally acting agents [36].

Atherosclerosis may start early in life and progress asymptomatically through adult life. Clinically it is manifested as coronary artery disease, stroke, transient ischemic attack, and peripheral arterial disease [37]. The atherosclerotic plaque is prone to disruption, thus leading to local platelet activation and aggregation, which is a major consequence of thrombus formation [38]. Traditional anti-atherosclerotic therapy is mainly focused on improving blood lipid profile and does not target various stages of plaque progression. The retention of cholesterol precedes plaque formation. Therefore, targeting the latter pathway may be beneficial for preventing further atherogenic progression. In this regard, herbal preparations can be used, due to their good tolerability and suitability for prolonged treatment [39].

5. Conclusion

The aqueous ethanol crude extracts of the *P. strigosus* have significant *in vitro* anti-inflammatory and membrane stabilizing activity. Antidiarrheal activities of the extract may be attributed to the synergistic effect of phytochemicals which includes tannins, alkaloids, saponins, flavonoids, and phytosterols. The ethanol extracts of *P. strigosus* exhibited antipyretic properties and the inhibition of the biosynthesis and/or release of inflammatory mediators. Based on our preliminary neuropharmacological screening, it can be deduced that the crude aqueous extract of *P. strigosus* displayed significant anxiolytic activity. For the first time, we have demonstrated that *P. strigosus* potentiates pentobarbital-induced sleeping behaviors in mice. The peripheral analgesic property of the plant was dose-dependent the aqueous extract exhibited significant thrombolytic properties.

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Conflict of interest

The authors declared no conflict of interest.

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