Original Research Article

Pharmacological screening for CNS Depression, analgesic and anti-inflammatory potentials of *Sonneratia caseolaris* (Linn.) barks in different solvent fraction

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ABSTRACT

Aims: Bark of different fractions of *Sonneratia caseolaris* (Linn.) (Sonneratiaceae) were screened for its analgesic, anti-inflammatory and CNS activities

Study design: For the purpose of these experiments the extracts were subjected to an *in-vivo* study.

Place and Duration of Study: The study was carried out in August 2014 in the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.

METHODOLOGY : Ethanolic (ETF), ethyl acetate (EAF), chloroform(CLF) and pet ether (PTF) fractions of bark of *S. caseolaris* were used to evaluate the analgesic activity using Acetic acid induced writhing and Formalin test. The same fractions were evaluated for anti-inflammatory activity using Carrageenan induced hind paw edema model. The CNS depressant activity was evaluated by Hole cross method. Two doses of 150mg/kg and 300mg/kg were used.

RESULTS: The different fractions produced significant (p<0.05) writhing inhibition at both doses and reduced the number of linking induced by formalin. Among these fractions the most potent activity was found in ETF about 79.40 % (300 mg/kg) that

was almost similar to standard Diclofenac-Na 82.78% (10mg/kg), then EAF 74.59% followed by CLF 59.03% and PTF 52.45% at dose 300 mg/kg).

In formalin-induced paw licking model, all fractions of *S. caseolaris* showed superior result in the late phase compare to the early phase .The same fractions of extracts caused significant ($p<0.05$) inhibition of carrageenan induced paw edema in a dose dependent manner. A statistically significant ($p<0.05$) locomotor activity was also observed.

CONCLUSION: Our result revealed that all the extractives of *S. caseolaris* have noticeable analgesic, anti-inflammatory and CNS depressant activities.

KEYWORDS: *Sonneratia caseolaris, Analgesic, Anti-inflammatory, CNS activity.*

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1. INTRODUCTION

Sonneratia caseolaris (L.) (Sonneratiaceae) is a mangrove plant found widespread in tropical and subtropical tideland. *S. caseolaris* is a medium-size plant (2 to 20m height), evergreen tree with elliptic-oblong leaves (5 to 9.5cm long) [1-2]. Twenty four compounds such as nine triterpenoids, eight steroids, three flavonoids and four benzene carboxylic derivatives have been isolated from stems and twigs of medicinal mangrove plant of *S. caseolaris* [3]. This plant contains phenolic compound like gallic acid and flavonoids e.g. luteolin and luteolin-7-O-glucoside [4]. It contains alkaloid, tanin, flavonoid, saponin, phytosterol, and carbohydrate[5-6].*S. caseolaris* has been used in traditional medicine systems in several countries, it is used for sprains, swelling helminthiasis, poultices, coughs, hematuria, small pox, astringent, antiseptic, arresting hemorrhage, piles, and also used as remedy to stop blood bleeding [7]. *S. caseolaris* possessed intestinal α-glucosidase inhibitory property [8] and it has also been reported to be toxic against mosquito larvae [7].

Based on available literatures, little or no reports have been found on analgesic, anti-inflammatory and CNS depressant activities of different fractions of this plant.

Therefore, this study is aimed at exploring the analgesic, anti-inflammatory and CNS depressant activities of different fractions based on polarities of *S. caseolaris* barks part .

2. METHODS

2.1 Collection, identification and preparation of plant material

The stems of *S. caseolaris* were harvested after identification by an expert taxonomist from Barisal on August 5, 2014. The stems were dried under shade at room temperature for a period of two weeks in order to avoid solar radiations from altering the API. These stems were spread on plastic bags while avoiding their stacking. Every day we turned these stems upside down in order to to favor a homogenous drying process. The dried leaves were ground in a clean electric grinding machine in such a way to obtain a fined powder, which

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was stored in an airtight container. The total dried powder material was obtained 600 gm. It was divided equally into four portions and was refluxed with ethanol ,ethyl acetate, pet ether and chloroform solvent three times .The extracts were filtered with Whatman No. 1. Filter paper and the recovered filtrate were evaporated in an oven at 50°C. These extracts were weighed in order to determine the yield obtained from the starting material and then stored in an air-tight container for subsequent experimental tests.

2.2 ANALGESIC ACTIVITY

2.2.1 Acetic Acid-Induced Writhing Method for Peripheral Analgesic Assay

Experiment for the detection of the peripheral analgesic activity of bark extracts of *S. caseolaris* were evaluated by the acetic acid-induced writhing test in mice[8]. The abdominal writhing was induced by intraperitoneal injection of acetic acid solution (0.7%) at a dose of 0.1 ml/10 g of body weight to each mouse, a model of visceral pain. An analgesic agent like Diclofenac was used as a standard at an oral dose of 10 mg/kg body weight, and the extract was administered at 150 mg/kg and 300 mg/kg body weight. The standard drug, control (Normal saline solution, 1mg/kg), as well as the extract, were orally administered 30 minutes prior to the injection of acetic acid. Each mouse of all groups were observed individually for counting the number of writhing they made in 30 minutes beginning just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group .The percent inhibition (% analgesic activity) was calculated by the equation $\{(A-B)/A\} \times 100$

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

2.2.2 Formalin-Induced paw licking Method for Central Analgesic Assay

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The formalin-induced method is a popular technique to evaluate analgesic activity in mice described by Achinta [9].Swiss albino mice (Experimental animals) were selected by randomly and allocated into six groups designated as group-I, group-II, group-III, group-IV, group-V and group-VI, consisting of 3 mice in each group.

Twenty micro liters (20 µl) of 1% formalin was injected intradermally on the plantar surface of the hind paw of each mouse one hour after administration of the test extracts (150 mg /b. w. and 300mg/b. w.) as well as the controls. The time in seconds spent in paw licking as an index of painful response was determined at 0 – 10 min (Early) and 15– 30 min (late phase) after formalin injection. This represent, neurogenic and inflammatory responses, respectively. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch. The data was presented as Mean ± S.E.M of time(s) spent in pain behaviour. The mean of time (s) spent in pain behaviour for the extracts were compared with that of the control.

2.3 ANTI-INFLAMMATORY ACTIVITY

2.3.1 Carrageenan Induced Paw Edema Test in Mice

Swiss albino mice (25-30g) were divided into six groups of four animals each. The test groups received 150 and 300 mg/kg body weight, p.o. of EA, CLF and PET extracts respectively. The reference group received Indomethacin (10 mg/kg body weight, p. o.) while the control group received 1 ml/kg body weight normal saline. After 30 min, 0.1 ml, 1% carrageenan suspension in normal saline was injected into the subplanatar tissue of the right hind paw. The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

$$\% \text{ inhibition} = (1-D_t/D_o) \times 100$$

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Where, D_o was the average inflammation (hind paw edema) of the control group of mice at a given time, D_t was the average inflammation of the drug treated (i.e., extract or reference indomethacin) mice at the same time [9].

2.4 CNS DEPRESSION ACTIVITY

2.4.1 Hole cross test

The method used was described by Takagi *et al* [10]. The animals were divided into control, standard and test groups (n = 4 per group). The control group received vehicle (0.9% saline in water at the dose of 10 ml/ kg) whereas the test group received extract (at the doses of 150 and 300 mg/kg b.w.) and standard group received diazepam at the dose of 1mg/kg body weight orally. Each animal was then placed on one side of the chamber and the number of passages of each animal through the hole from one chamber to the other was recorded for 3 min on 0, 30, 60, 90 and 120 min during the study period.

STATISTICAL ANALYSIS

Data were analyzed by one-way ANOVA followed by Dunnett's test and p value of 0.05 was considered statistically significant.

3. RESULT

3.1 Analgesic activity

3.1.1 Acetic Acid Induced Writhing Method

The effect of administration of ETF, EAF, CLF and PTF extracts of *S. caseolaris* are shown in Table 1 by acetic acid induced writhing method. It was found that ETF, EAF, CLF and PTE extracts of *S. caseolaris* significantly inhibited the nociceptive effects induced by acetic acid compared to the control group (saline water) at the doses of 150, 300 mg/kg, respectively (p <0.05). The percentage inhibition of constrictions was calculated. Among these fractions the most potent activity was found in Ethanol fraction of 79.40 % (300 mg/kg) that was almost

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similar to standard Diclofenac-Na 82.78% (10mg/kg) ,then EAF fraction 74.59% (300 mg/kg) followed by chloroform fraction 59.03% (300 mg/kg) and Pet ether fraction 52.45% .From this result, it is clear that all the extractives of *S. caseolaris* contain considerable analgesic activity.

TABLE 1: Antinociceptive effect of ETF, EAF, CLF and PTF extracts of *S. caseolaris* by acetic acid induced writhing method

Groups	Treatment	Dose	Avg. no. of Writhing	% inhibition
I	Control (Saline)	10ml/kg	24.40 ± 2.13	-
II	Diclofenac-Na	10mg/kg	4.2 ± 1.60*	82.78
III	EAF fraction	150	8± 2.12*	60.21
IV		300	5 ± 1.70*	79.40
V	ETF Fraction	150	7.6 ±1.51*	68.85
VI		300	6.2 ±1.63 *	74.59
VII	CLF Fraction	150	9.8± 2.05*	59.83
VIII		300	6.6± 1.67*	72.95
IX	PTF Fraction	150	14.6± 2.35*	40.16
X		300	11.6± 1.06*	52.45

Values are mean ± SEM, (n = 4), (*) indicates statistically significant compared to vehicle control group (*P<.05) using one way ANOVA followed by Dunnet test.

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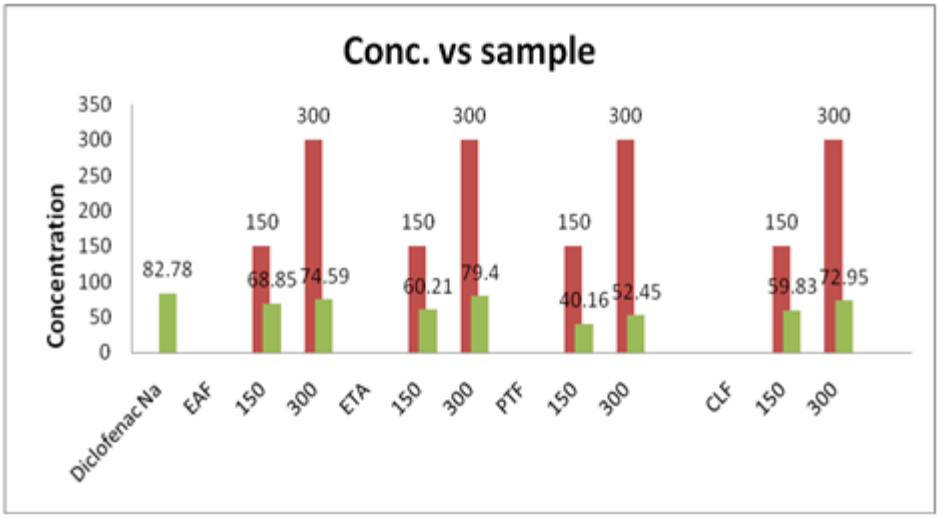

Figure 1: Evalution of analgesic activity of extracts of different solvents fractions of *S. caseolaris* by acetic acid induced writhing method in mice.

3.1.2 Formalin Test

ETF, EAF, CLF and PTF extracts of *S. caseolaris* showed a dose-related inhibition of formalin induced nociception and caused significant inhibition of both neurogenic (0–5 min) and inflammatory (15–30 min) phases of formalin-induced licking test at the doses of 150, 300 mg/kg when compared with control group (Saline water) (Table 2 and Table 3).However, its effect was more pronounced in the second phase of this model of pain. Diclofenac sodium(10 mg/kg, i.p.) significantly reduced formalin induced nociception in both phases ($p < 0.05$). Among these fractions, at 300mg/ kg, the most potent activity was found in EAF and CLF which showed highest % of inhibition (72.91%) after standard Diclofenac-Na (77.08%) in late phase. At 300 mg/kg, % of inhibition of PTF was (70.83%) and ETF (66.66%).

Table 2: Effects of ETF, EAF, CLF AND PTF extracts of *S. caseolaris* in the Hindpaw licking in the formalin test in mice (Early phase)

Groups	Treatment	Dose	Late phase	% of protection
I	Control (Saline)	10ml/kg	17.75 ± 1.30	-
II	Diclofenac-Na	10mg/kg	7.4 ± 1.29*	61.05

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III	EAF Eraction	150	10.6 ±1.55*	40.28
IV		300	8.4 ± 52.67*	52.67
V	ETF fraction	150	10.8± 1.76*	43.15
VI		300	9.8 ± 1.64*	50.52
VII	CLF fraction	150	7.8 ± 1.38*	58.94
VIII		300	7.6 ± 1.06*	60.94
IX	PTF Fraction	150	9.4 ± 1.51*	50.52
X		300	8.2± 1.51*	56.84

Values are mean ± SEM, (n = 4), (*) indicates statistically significant compared to vehicle control group (*P<.05) using one way ANOVA followed by Dunnet test.

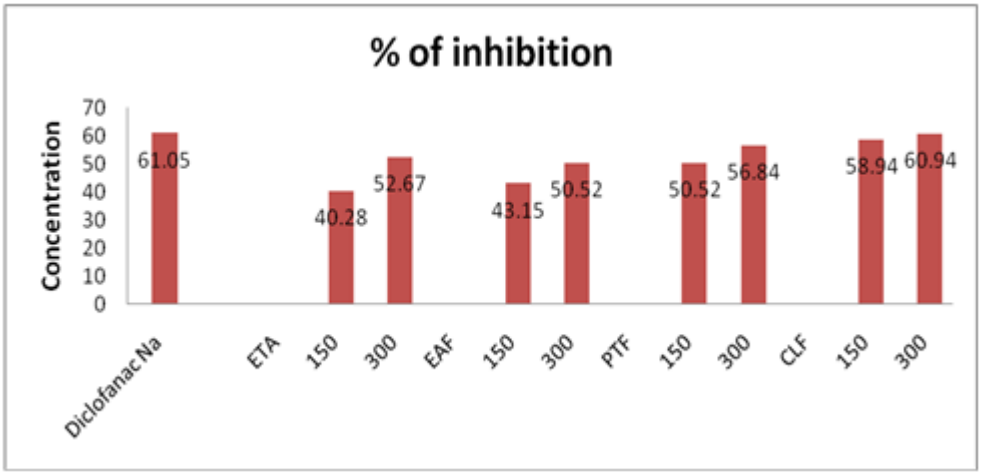

Figure 2: Evaluation of % of inhibition of different extract of *S. caseolaris* by Formaline Induced writhing Method. (Early Phase).

Table 3: Effects oF ETF, EAF, CLF and PTF extracts of *S. caseolaris* in the Hindpaw licking in the formalin test in mice (late phase)

Groups	Treatment	Dose	Avg. no. of Writhing	% inhibition
I	Control (Saline)	10ml/kg	9.60 ± 1.30	-
II	Diclofenac-Na	10mg/kg	2.20 ± 1.29*	77.08

III	ETF Fraction	150	3.20 ±1.76*	66.66
IV		300	2.60 ± 1.64*	72.91
V	EAF Fraction	150	4.00± 1.55*	58.33
VI		300	3.20 ± 1.72*	66.66
VII	PTF Fraction	150	3.4 ± 1.06*	64.58
VIII		300	2.8 ± 0.66*	70.83
IX	CLF Fraction	150	3.00 ± 1.38*	68.75
X		300	2.60± 1.06*	72.91

Values are mean ± SEM, (n = 4), (*) indicates statistically significant compared to vehicle control group (*P<.05) using one way ANOVA followed by Dunnet test.

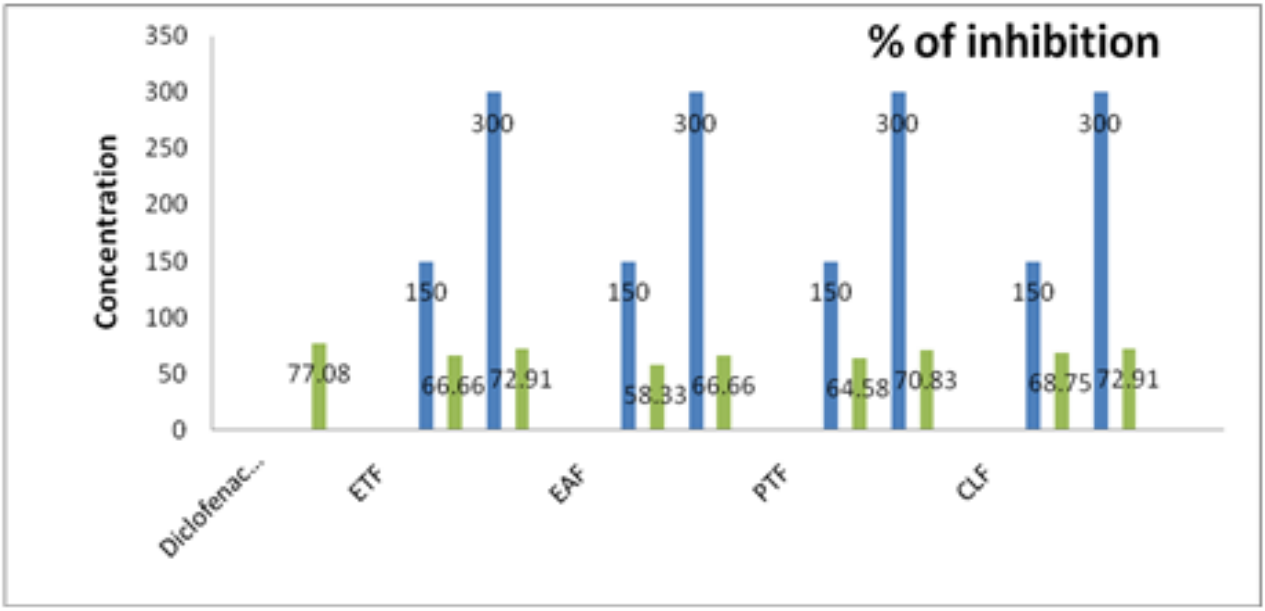

Figure 3: Evaluation of % of inhibition of different extract of *S. caseolaris* by formaline induced writhing method. (Late phase).

3.2 Determination o f Anti-Inflammatory Activity

3.2.1 Carrageenan Induced Paw Edema in Mice

The effect of administration of ETF, EAF, CLF and PTF extracts of *S. caseolaris* are shown in Table 04 and Figure 04 by carrageenan induced paw edema test. It was found that ETF, EAF, CLF and PTF extracts of *S. caseolaris* significantly inhibited oedema diameter compared to the control group (saline water) at the doses of 150, 300 mg/kg, respectively ($p < 0.0001$). Among these fractions the most potent activity was found in pet ether fraction (PTF) showed moderate % of inhibition (37.73%) after standard Indomethacin (62.35%). On the other hand,ETF, EAF, CLF showed slight anti-inflammatory activity is measured by considering the % of inhibition.

Table 4: Tables are shown of %inhibition of ETF, EAF , CLF AND PTF extracts of *S. caseolaris.* on carrageenan induced paw edema test

Group			Inhibition (%)			
	Treatment	Dose	1h	2h	3h	4h
I	Control (Saline)	10ml/kg	4.70±0.11	4.40± 0.09	4.17±0.11	3.75±0.14
II	Indomethacin	10mg	47.69	51.45	54.76	62.35
III	ETF Fraction	150	29.29	39.29	41.70	32.70
IV		300	35.98	43.30	43.12	35.84
V	EAF Fraction	150	32.22	28.57	30.47	32.40
VI		300	38.08	31.69	36.19	35.50
VII	CLF Fraction	150	30.13	31.25	32.22	24.52
VIII		300	37.24	35.71	36.49	32.41
IX	PTF Fraction	150	33.05	33.93	41.70	33.94
X		300	35.66	39.73	48.34	37.73

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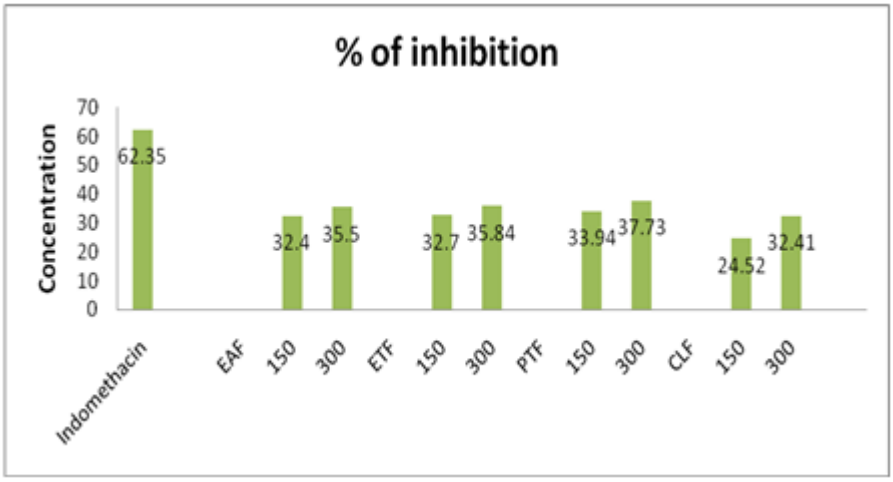

Figure 4: % of inhibition of different extractives of *S.caseolaris* by carrageenan induced mice paw edema method.

3.3 Determination of CNS Depressant Activity

In the hole cross test, extracts of different solvents of *S. caseolaris* doses significantly decreased the number of hole crossed compared to the control group. Extracts of different fractions of *S.caseolari s*exhibited a decrease in the movements of the test animals at all dose levels tested. The depressing effect was moderately intense during the 3rd (90 min) and 4th (120 min) observation periods. The results are shown in table 05 and in figure 05.

Table 5: Determination of volume of CNS depression of mice at different time for different fractions of *S. caseolaris*.

Group	Treatment	Dose	Number of Movements				
			0 min	30 min	60 min	90 min	120 min
Group-I	Control (Saline)	10ml/kg	16.80 ± 0.962	11.20 ± 2.043	11.60± 2.280	9.02 ± 0.962	5. 40± 0.447
II	Diazepum	10	16.00 ± 0.707	4.40± 0.570*	4.60± 0.274*	3.00 ± 1.612*	1.00 ± 0.097*
III	ETF Fraction	150	10.80± 0.962*	6.00 ± 1.173*	4.00 ± 0.612*	3.00 ± 1.173*	2.60± 0.908*
IV		300	4.40 ± 0.570*	5.00 ± 0.935*	2.80 ± 0.418*	1.80 ± 0.0.418*	1.40 ± 0.274*

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V	EAFfraction	150	10.80± 0.962	6.00 ± 1.173*	4.00 ± 0.612*	3.00 ± 1.173*	2.60± 0.908*
VI		300	5.00 ± 0.791	2.40 ± 0.274*	1.40 ± 0.274*	1.4o ± 0.247*	1.00 ± 0.224*
VII	CLF Fraction	150	5.80 ± 0.742	5.60 ± o.447*	4.60 ± 0.274*	3.60 ± 0.274*	2.00 ± 0.354*
VIII		300	4.20 ± 0.418	3.80 ± 0.418*	2.80 ± 0.224*	1.80 ± 0.418*	1.40 ± 0.274*
IX	PTF	150	8.40 ± 0.570	7.00 ± 0.418*	3.80 ± 0.418*	3.00 ± 0.791*	1.40 ± 0.274*
X		300	6.80 ± 0.418	6.00 ± 0354*	2.60± 0.274*	1.80± 0.418*	3.75 ± 2.428*

Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle control group (*P<.05) using one way ANOVA followed by Dunnet test.

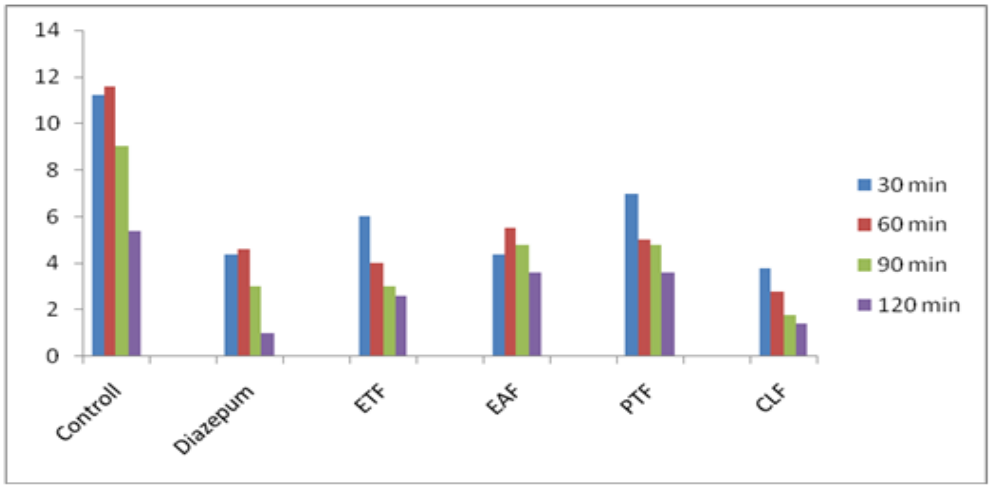

Figure 5: Effect of extract of different solvent fractions of the *S. caseolaris barks* on open field test in mice.

4. DISCUSSION

In this investigation, we have reported the effect of ethanolic and different fractions of *S. caseolaris* on several experimental animal models of pain, inflammation and analgesic as well as CNS activity. In acetic acid induced writhing test, after oral administration of *S. caseolaris*, a dose dependent antinociceptive effect was observed (Table1 and Figure 1). From the table it has been observed that, all fractions showed significant antinociceptive effect. However, EAF (79.40%) and ETF fractions (74.59 %) exhibited better activity.

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Peripheral analgesic activity is done with the help of writhing test in mice. [11] In general, endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins, IL-1β, IL-8,TNF-α and substance P are liberated by intra peritoneal administration of acetic acid and these mediators are responsible for pain.

These mediators stimulate primary afferent nociceptors entering dorsal horn of the central nervous system [12] and are thought to contribute to increased blood-brain barrier (BBB) permeabilization or interruption [13]. Moreover, acetic acid enhance vasodilation and vascular fluid permeability [14].

The formalin test is a widely used model of constant nociception [15, 16]. The tests demonstrate a biphasic response. The first phase begins immediately after the formalin injection represents neurogenic pain and is caused by direct action on the local sensory C-fibers, resulting in the release of calcitonin gene-related peptide (CGRP) and substance P [17,18]. The second phase (15–30 min after injection) is associated with inflammatory pain of the peripheral tissues due to the release of inflammatory mediators, such as prostaglandins and nitric oxide, and is responsive to non-steroidal anti-inflammatory drugs (NSAIDs) [17,19,20,21].

Our present results showed that the number of paw licking was significantly reduced by different fractions of *S. caseolaris* in both neurogenic and inflammatory pain responses ($p < 0.05$) in a dose dependant manner (Table 2 ,3 and figure 2 ,3). Ethyl acetate extract (72.91%),chloroform (72.91%) and pet-ether fraction(70.82%) show better protection than ethanol fraction. However, the effect of all extracts was more emphasized in the late phase. Centrally acting analgesic drugs inhibit both the phases of formalin test, while peripherally acting analgesics restrict only the late phase responses [22]. The late phase response as the antinociceptive effect observed in formalin test is due to this inhibition of the inflammatory mediators [23].

The present study also investigated the anti-inflammatory activity of *S. caseolaris* extracts *in* experimental animal models. Carrageenan-induced paw edema in mice as an *in vivo*

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model of inflammation has been frequently used. Carrageenan induced paw edema is a useful replica in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. Edema formation in the carrageenan-induced paw edema model is a biphasic response. In the early hyperemia, 0-2 hrs after carrageenan injection, there is a release of histamine, serotonin, and bradykinin in affecting vascular permeability. The inflammatory edema reached its maximum level at the third hour and after that it started declining. In our study, test extracts of different solvent system in both doses and indomethacin showed anti-inflammatory effects in carrageenan-induced rat paw edema. In our study, PTFextracts showed good activity.

In CNS depression activity, on Hole cross method, CLFfraction has good activity compare to other fractions. It may possible that the mechanism of anxiolytic action of *S. caseolaris* extract could be due to the binding of any of the phyto-constituents to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor [24]. The results were also dose dependent and statistically significant.

Literature review find that *S. caseolaris* posseses two flavonoid compound, luteolin and luteolin 7-O-b-glucoside compounds [25]. Flavonoids have the capability to inhibit ecosanoid biosynthesis such as prostaglandin [26].]. Further-more Phytochemical analyses of methanolic bark extracts revealed the presence of high amounts of phenolics, flavonoids, tannins, alkaloids and saponins [27].

It can be suggest that *S. caseolaris* showed significant and dose dependant analgesic, anti-inflammatory and CNS depressant activity due to the presence of flavonoid, phenolic and tannin like compounds. However, further investigations are required to understand the mechanisms of action of *S. caseolaris* and to identify the active constituents that may be used as a lead compound foe new drug development.

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5. CONCLUSION

Our study investigation brings out the scientific rationale for the folkloric uses of the plant in the management of inflammation and pain. Even so, further research is needed towards isolation and ascertainment of bioactive constituents present in the extracts, which could possibly be explored for pharmaceutical use.

COMPETING INTERESTS

There are no competing interests.

CONSENT: NOT APPLICABLE

ETHICAL APPROVAL:

All the experimental mice were treated following the Ethical principles and guidelines for scientific experiments on animals (1995) formulated by the Swiss Academy of Medical Sciences and the Swiss academy of sciences. The Committee on Ethical Compliance in Research(SEU /Pharm /CECR/101/2019) of Southeast University Bangladesh approved all experimental rules.

Consent for publication: Not applicable

COMPETING INTERESTS DISCLAIMER:

There are no competing interests.

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