# *Original Research Article*

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




## ABSTRACT

**Aims: Barks of *Sonneratia caseolaris* (Linn.) (Sonneratiaceae) were screened for its analgesic ,anti-inflammatory and CNS activities by using different solvent systems of various methods.**

**Study design: For the purpose of this experiment the extracts were subjected for 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 fractions (ETF), ethyl acetate (EAF), chloroform(CLF) and pet ether (PTF) extracts of barks of *S. caseolaris* were used to evaluate the analgesic activity using Acetic acid induced writhing and Formalin test. The same fractions of extracts were evaluated for anti-inflammatory activity using Carrageenan induced hind paw edema model. The CNS depressant activity was evaluated by Hole cross method.**

**RESULTS: The different fractions of extracts produced significant (*p*<0.05) writhing inhibition in acetic acid induced writhing in mice at dose of 150 and 300mg/kg BW comparable to the standard drug diclofenac sodium at the dose of 10 mg/kg BW and reduced the number of licks induced by formalin in a dose dependent manner. Among these fractions the most potent activity was found in Ethanol fraction of 79.40 % (300 mg/kg) that was almost 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%.**

**In formalin-induced paw licking model, all fractions of *S. caseolaris* showed superior result in the late phase compare to the early phase at 150 and 300 mg/kg p.o. The same ranges of doses of ethanol, pet ether, chloroform and ethyl acetate caused significant (p<0.05) inhibition of carrageenan induced paw edema after1, 2 or 3 h in a dose dependent manner. A statistically significantly (*p*<0.05) decrease in locomotor activity at dose of 150 and 300 mg/kg was also observed.**

**Conclusion: Our result find out that all the extractives of *S. caseolaris* have noticeable analgesic, anti-inflammatory and CNS depressant activities. The activity can be predictable to the phyto-constituents viz phytosterol ,terpenoids, and flavonoids present in the *S. caseolaris* extracts.**

**CONCLUSION: Our result demonstrates that all the extractives of *S. caseolaris* have appreciable antioxidant activities.**

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

## 1. INTRODUCTION

*Sonneratia caseolaris* (L.) (Sonneratiaceae) is such a mangrove plant found widespread in tropical and subtropical tideland. *S. caseolaris* is a medium-size plant (2-20m hight), evergreen tree with elliptic-oblong leaves (5-9.5cm long) [1-2]. *S. caseolaris* is reported to have 24 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 *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* to be 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].

So far our knowledge, previously no reports have been found on analgesic, anti-inflammatory and CNS depressant activities of different fractions of this plant.

Present study was aimed to explore the analgesic, anti-inflammatory and CNS depressant activities of different fractions based on polarities of *Sonneratia caseolaris* barks part

## 2. METHODS

### 2.1 Collection, identification and preparation of plant material

The stems were harvested after identification by an expert taxonomist from the plant growing at 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 so that 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 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 for three times The extract was filtered with Whiteman No. 1. filtered paper and the collected filtrate was evaporated in an oven at 50°C. This extract was weighed so that to determine the yield obtained from the initial powder quantity 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]. Anyway, 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

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.) and also 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:

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

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

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

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

| 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 | Ethanol fraction | 150 | 8± 2.12* | 60.21 |
| IV | | 300 | 5 ± 1.70* | 79.40 |
| V | Ethyl Acetate Fraction | 150 | 7.6 ±1.51* | 68.85 |
| VI | | 300 | 6.2 ±1.63 * | 74.59 |
| VII | Chloroform Fraction | 150 | 9.8± 2.05* | 59.83 |
| VIII | | 300 | 6.6± 1.67* | 72.95 |
| IX | Pet-ether Fraction | 150 | 14.6± 2.35* | 40.16 |
| X | | 300 | 11.6± 1.06* | 52.45 |

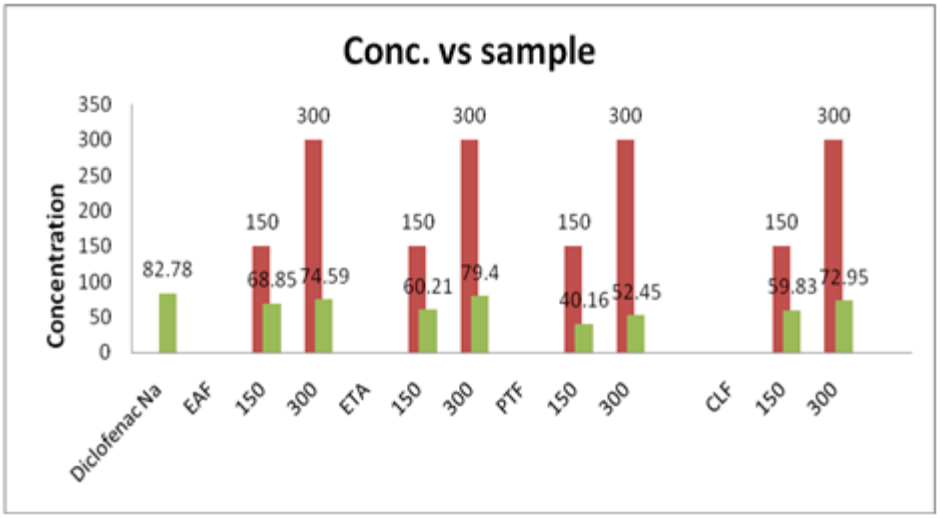


Figure 01: Evalution of analgesic activity of extracts of different solvents fractions of *S. caseolaris* by acetic acid induced writhing method in mice.

### 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 |
| III | Ethanol Eraction | 150 | 10.6 ±1.55* | 40.28 |
| IV | | 300 | 8.4 ± 52.67* | 52.67 |
| V | Ethyl acetate fraction | 150 | 10.8± 1.76* | 43.15 |
| VI | | 300 | 9.8 ± 1.64* | 50.52 |
| VII | Chloroform fraction | 150 | 7.8 ± 1.38* | 58.94 |
| VIII | | 300 | 7.6 ± 1.06* | 60.94 |
| IX | Pet-ether 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.

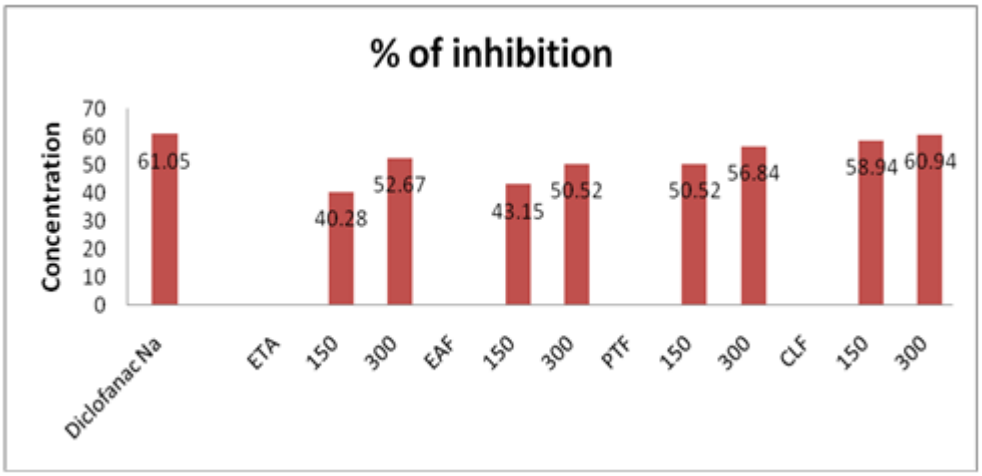


**Figure 02: Evaluation of % of inhibition of different extract of *S. caseolaris* by Formaline Induced writhing Method. (Early Phase).**

**Table 03: 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 | Ethyl Acetate Fraction | 150 | 3.20 ±1.76* | 66.66 |
| IV | | 300 | 2.60 ± 1.64* | 72.91 |
| V | Ethanol Fraction | 150 | 4.00± 1.55* | 58.33 |
| VI | | 300 | 3.20 ± 1.72* | 66.66 |
| VII | Pet-ether Fraction | 150 | 3.4 ± 1.06* | 64.58 |
| VIII | | 300 | 2.8 ± 0.66* | 70.83 |
| IX | Chloroform 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.

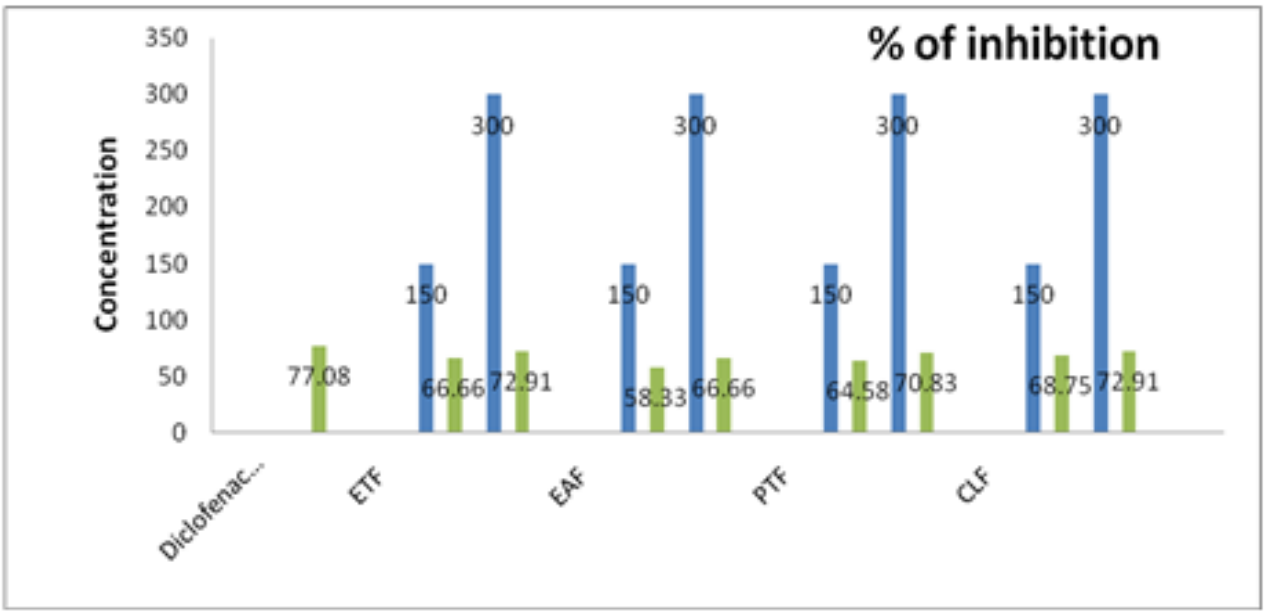

**Figure 03: Evaluation of % of inhibition of different extract of *S. caseolaris* by formaline induced writhing method. (Late phase).**

### 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 04: 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 | Chloroform Fraction | 150 | 30.13 | 31.25 | 32.22 | 24.52 |
| VIII | | 300 | 37.24 | 35.71 | 36.49 | 32.41 |
| IX | Pet-ether Fraction | 150 | 33.05 | 33.93 | 41.70 | 33.94 |
| X | | 300 | 35.66 | 39.73 | 48.34 | 37.73 |

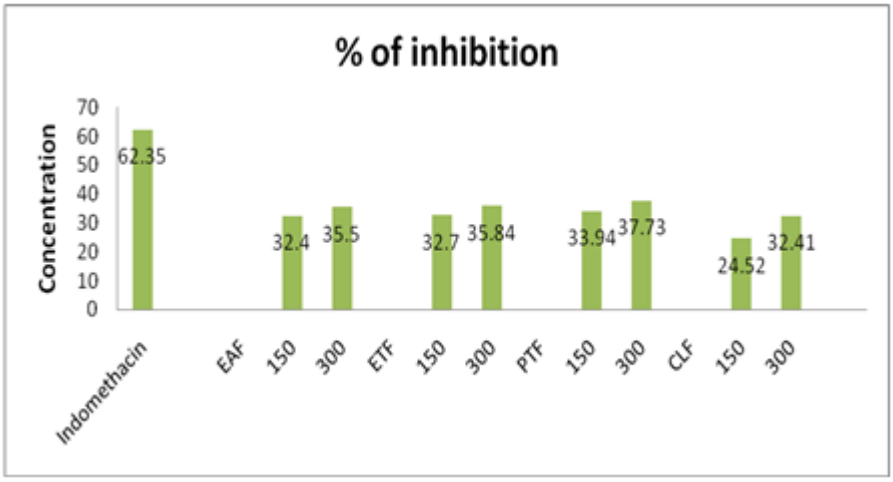


**Figure 04: % of inhibition of different extractives of *S.caseolaris* by carrageenan induced mice paw edema method.**

### 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 05: 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* |
| 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 | Chloroform 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 | Pet-ether Fraction | 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.

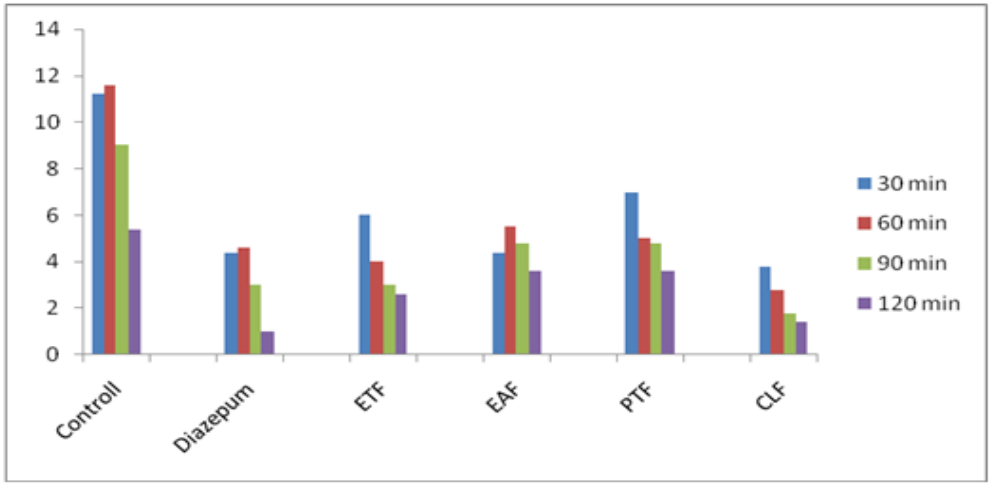


**Figure 05: Effect of extract of different solvent fractions of the *S. caseolaris barks* on open field test in mice.**

## 4. DISCUSSION

In this investigation, we made reported the effect of ethanolic extract and different fractions of *S. caseolaris* on several experimental animal models of pain, inflammation and analgesic as well as CNS activity. The extracts showed remarkably inhibited the nociception produced by Formaline induced writhing and writhing induced by acetic acid; the extracts also crucially attenuated carrageenan-induced mice right hind paw edema. Estimation suggested that the use of acetic acid induced writing and Formaline induced writhing for the evaluation of peripherally and centrally acting analgesic drugs respectively [11-12]. *S. caseolaris* extracts to prolong the reaction latency to thermally-induced pain in mice as observed in the Formaline induced writhing test suggest central analgesic activity, on the contrary, the acetic acid-induced abdominal constriction method is widespread used for the investigation of peripheral antinociceptive activity [13]. Commonly, stimulating and production of prostaglandins by acetic acid causes writhing or nociception [14]. GDP and AGE receptors are suggested to be partly connected in the abdominal constriction response .Increased levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products have associated with prostanoids in general by the method [15-16].As results of the acetic acid-induced writhing strongly recommend that the action of this extracts is linked partially to LOXs (lipoxygenases) and/or COXs (cyclo-oxygenases) pathways. The *S. caseolaris* extracts at the doses tested revealed analgesia in both the nociceptive pain models further demonstrates that the extract possesses both central and peripherally mediated analgesic activities. Carrageenan induced rat paw edema was commonly used as an experimental animal model for acute inflammation and is believed to be biphasic and Carrageenan model of inflammation is also said to be biphasic. Typically, in the early phase (1-2h), histamine, serotonin and kinninsare released and increased synthesis of prostaglandins in the damaged tissue surroundings in the first or two hours. While the late phase sustained to the release of prostaglandin and mediated by bradykinin, leukotrienes, polymorphonuclear cells, and prostaglandins produced by tissue macrophages [17-18] and release of lysosome enzymes in the second to the third hour [19-20]. At the present study, indicate that the

extracts showed outstandingly inhibited the carrageenan-induced acute inflammation in the 4th h of investigation and the discovering was comparable to that of the standard Indomethacin. Therefore, the anti-inflammatory effect of *S. caseolaris* extracts may be due to its suppressive action on protease or lysosome synthesis or prostaglandin synthesis activity. The different fractions of *S. caseolaris* were exhibited crucial antioxidant activity, which was determined by standard method, catechin was chosen as the reference antioxidant in this investigation. CNS study demonstrated that the different extracts of *S. caseolaris*possess potent CNS depressant activity in Diazepam induced sleeping time open field models.

## 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 active principles 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 institutional Animal Ethical Committee (SEU /IAEC /17-25) of Southeast University Bangladesh approved all experimental rules.

**Consent for publication:** Not applicable

**COMPETING INTERESTS DISCLAIMER:**

**Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.**

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