

# Original Research Article

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

### 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.

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

UNDER PEER REVIEW

## 14 1. INTRODUCTION

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

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

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

31

## 32 2. METHODS

### 33 2.1 Collection, identification and preparation of plant material

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

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

## 46 **2.2 ANALGESIC ACTIVITY**

### 47 **2.2.1 Acetic Acid-Induced Writhing Method for Peripheral Analgesic Assay**

48 Experiment for the detection of the peripheral analgesic activity of bark extracts of *S.*  
49 *caseolaris* were evaluated by the acetic acid-induced writhing test in mice[8]. The abdominal  
50 writhing was induced by intraperitoneal injection of acetic acid solution (0.7%) at a dose of  
51 0.1 ml/10 g of body weight to each mouse, a model of visceral pain. An analgesic agent like  
52 Diclofenac was used as a standard at an oral dose of 10 mg/kg body weight, and the extract  
53 was administered at 150 mg/kg and 300 mg/kg body weight. The standard drug, control  
54 (Normal saline solution, 1mg/kg), as well as the extract, were orally administered 30 minutes  
55 prior to the injection of acetic acid. Each mouse of all groups were observed individually for  
56 counting the number of writhing they made in 30 minutes beginning just 5 minutes after the  
57 intraperitoneal administration of acetic acid solution. Full writhing was not always  
58 accomplished by the animal, because sometimes the animals started to give writhing but  
59 they did not complete it. This incomplete writhing was considered as half-writhing.  
60 Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each  
61 treated group was compared to that of a control group .The percent inhibition (% analgesic  
62 activity) was calculated by the equation  $\{(A-B) / A\} \times 100$

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

### 65 **2.2.2 Formalin-Induced paw licking Method for Central Analgesic Assay**

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

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

79

## 80 **2.3 ANTI-INFLAMMATORY ACTIVITY**

### 81 **2.3.1 Carrageenan Induced Paw Edema Test in Mice**

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

90

$$\% \text{ inhibition} = (1 - D_t/D_0) \times 100$$

91 Where,  $D_0$  was the average inflammation (hind paw edema) of the control group of mice at a  
92 given time,  $D_t$  was the average inflammation of the drug treated (i.e., extract or reference  
93 indomethacin) mice at the same time [9].

94

## 95 **2.4 CNS DEPRESSION ACTIVITY**

### 96 **2.4.1 Hole cross test**

97 The method used was described by Takagi *et al* [10]. The animals were divided into control,  
98 standard and test groups (n = 4 per group). The control group received vehicle (0.9% saline  
99 in water at the dose of 10 ml/ kg) whereas the test group received extract (at the doses of  
100 150 and 300 mg/kg b.w.) and standard group received diazepam at the dose of 1mg/kg body  
101 weight orally. Each animal was then placed on one side of the chamber and the number of  
102 passages of each animal through the hole from one chamber to the other was recorded for 3  
103 min on 0, 30, 60, 90 and 120 min during the study period.

104

## 105 **STATISTICAL ANALYSIS**

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

## 108 **3. RESULT**

### 109 **3.1 Analgesic activity**

#### 110 **3.1.1 Acetic Acid Induced Writhing Method**

111 The effect of administration of ETF, EAF, CLF and PTF extracts of *S. caseolaris* are shown  
112 in Table 1 by acetic acid induced writhing method. It was found that ETF, EAF, CLF and PTE  
113 extracts of *S. caseolaris* significantly inhibited the nociceptive effects induced by acetic acid  
114 compared to the control group (saline water) at the doses of 150, 300 mg/kg, respectively ( $p$   
115  $<0.05$ ). The percentage inhibition of constrictions was calculated. Among these fractions the  
116 most potent activity was found in Ethanol fraction of 79.40 % (300 mg/kg) that was almost

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

121

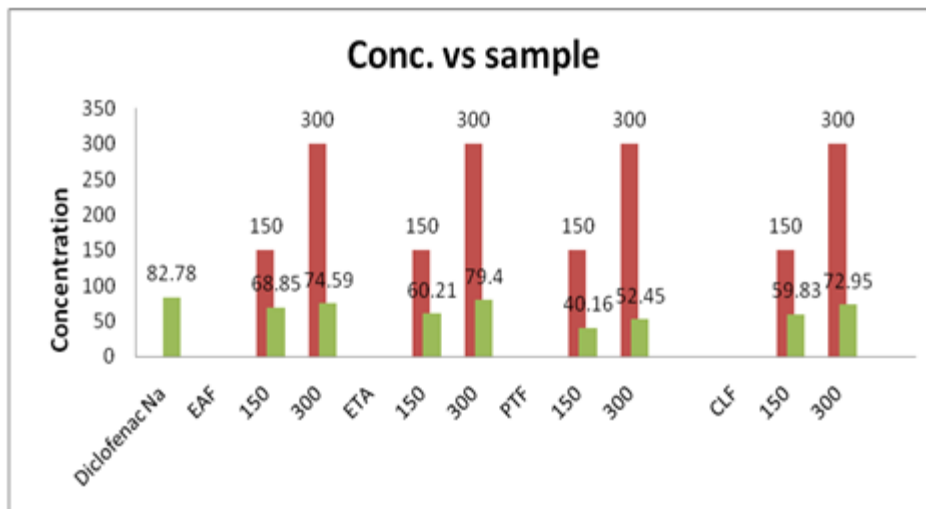
122 **TABLE 1: Antinociceptive effect of ETF, EAF, CLF and PTF extracts of *S. caseolaris* by acetic acid**  
 123 **induced writhing method**

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

124

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

127



128

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

131

### 132 **3.1.2 Formalin Test**

133 ETF, EAF, CLF and PTF extracts of *S. caseolaris* showed a dose-related inhibition of  
 134 formalin induced nociception and caused significant inhibition of both neurogenic (0–5 min)  
 135 and inflammatory (15–30 min) phases of formalin-induced licking test at the doses of 150,  
 136 300 mg/kg when compared with control group (Saline water) (Table 2 and Table 3). However,  
 137 its effect was more pronounced in the second phase of this model of pain. Diclofenac  
 138 sodium (10 mg/kg, i.p.) significantly reduced formalin induced nociception in both phases ( $p <$   
 139  $0.05$ ). Among these fractions, at 300mg/ kg, the most potent activity was found in EAF and  
 140 CLF which showed highest % of inhibition (72.91%) after standard Diclofenac-Na (77.08%)  
 141 in late phase. At 300 mg/kg, % of inhibition of PTF was (70.83%) and ETF (66.66%).

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

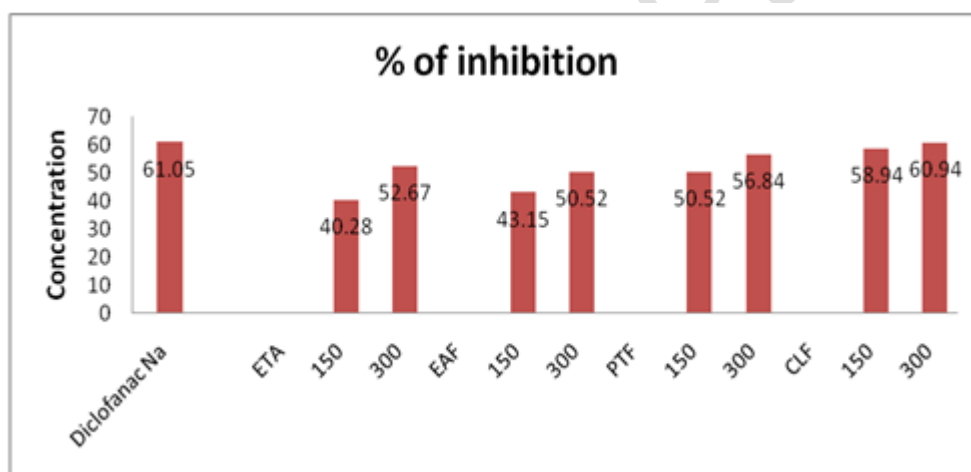
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Groups	Treatment	Dose	Late phase	% of protection
I	Control (Saline)	10ml/kg	17.75 ± 1.30	-
II	Diclofenac-Na	10mg/kg	7.4 ± 1.29*	61.05



III	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

145 Values are mean ± SEM, (n = 4), (\*) indicates statistically significant compared to vehicle  
 146 control group (\*P<.05) using one way ANOVA followed by Dunnet test.  
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149  
 150 **Figure 2: Evaluation of % of inhibition of different extract of *S. caseolaris* by Formaline**  
 151 **Induced writhing Method. (Early Phase).**

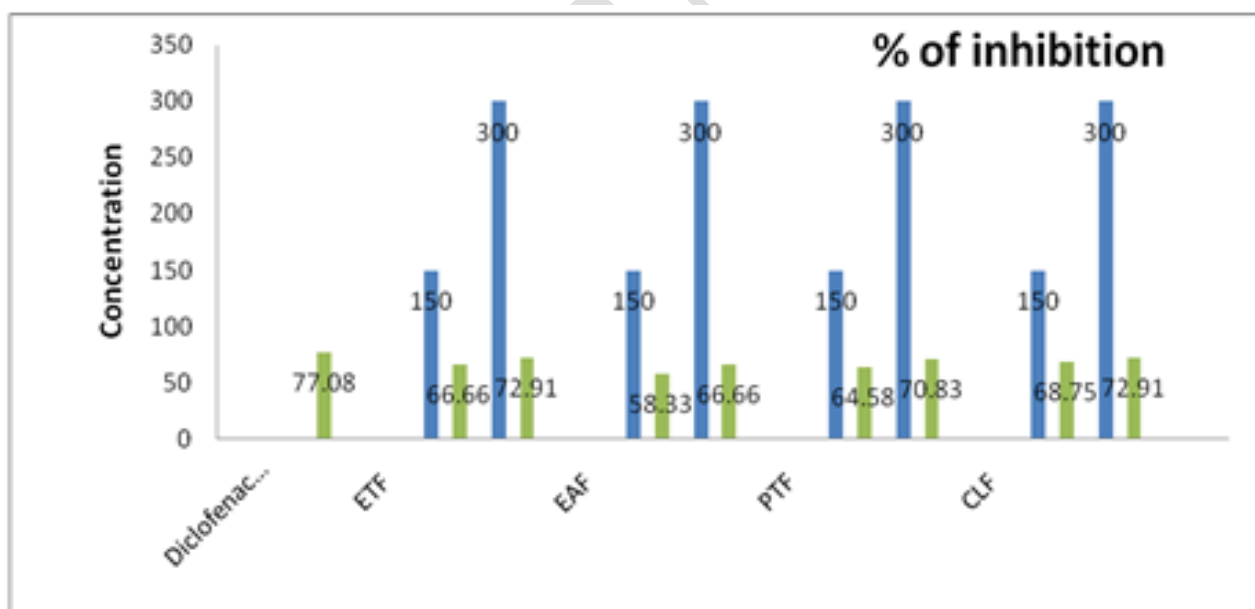
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 153 **Table 3: Effects of ETF, EAF, CLF and PTF extracts of *S. caseolaris* in the Hindpaw**  
 154 **licking in the formalin test in mice (late phase)**

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

155 Values are mean ± SEM, (n = 4), (\*) indicates statistically significant compared to vehicle  
 156 control group (\*P<.05) using one way ANOVA followed by Dunnet test.  
 157

158



159

160 Figure 3: Evaluation of % of inhibition of different extract of *S. caseolaris* by formaline  
 161 induced writhing method. (Late phase).  
 162

### 163 3.2 Determination of Anti-Inflammatory Activity

#### 164 3.2.1 Carrageenan Induced Paw Edema in Mice

165 The effect of administration of ETF, EAF, CLF and PTF extracts of *S. caseolaris* are shown  
 166 in Table 04 and Figure 04 by carrageenan induced paw edema test. It was found that ETF,  
 167 EAF, CLF and PTF extracts of *S. caseolaris* significantly inhibited oedema diameter  
 168 compared to the control group (saline water) at the doses of 150, 300 mg/kg, respectively ( $p$   
 169  $<0.0001$ ). Among these fractions the most potent activity was found in pet ether fraction  
 170 (PTF) showed moderate % of inhibition (37.73%) after standard Indomethacin (62.35%). On  
 171 the other hand,ETF, EAF, CLF showed slight anti-inflammatory activity is measured by  
 172 considering the % of inhibition.

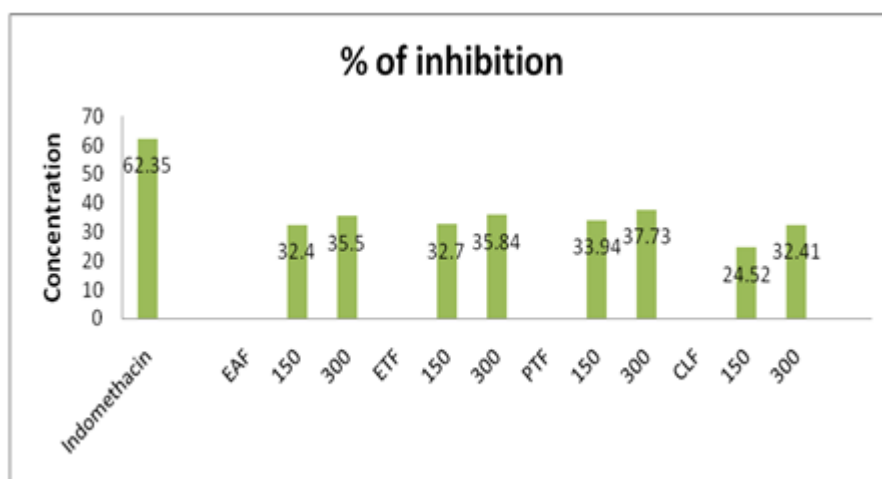
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174 **Table 4: Tables are shown of %inhibition of ETF, EAF , CLF AND PTF extracts of *S.***  
 175 ***caseolaris*. on carrageenan induced paw edema test**

Group	Treatment	Dose	Inhibition (%)			
			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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179 Figure 4: % of inhibition of different extractives of *S.caseolaris* by carrageenan induced mice  
 180 paw edema method.

181

182 **3.3 Determination of CNS Depressant Activity**

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

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

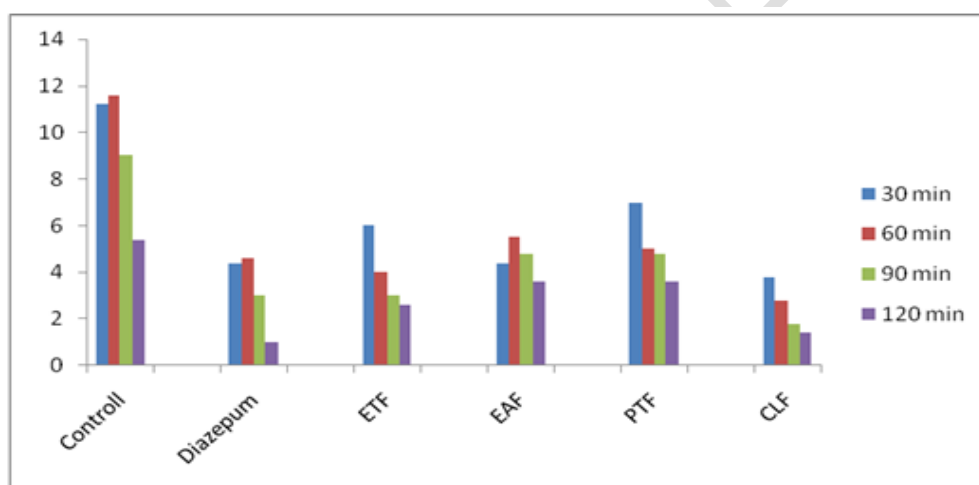
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Group	Treatment	Dose	Number of Movements				
			0 min	30 min	60 min	90 min	120 min
Group-I	Control (Saline)	10ml/kg	16.80 ± 0.962	11.20 ± 2.043	11.60± 2.280	9.02 ± 0.962	5.40± 0.447
II	Diazepum	10	16.00 ± 0.707	4.40± 0.570*	4.60± 0.274*	3.00 ± 1.612*	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*	1.40 ± 0.247*	1.00 ± 0.224*
VII	CLF Fraction	150	5.80 ± 0.742	5.60 ± 0.447*	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 ± 0.354*	2.60± 0.274*	1.80± 0.418*	3.75 ± 2.428*

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

193



194

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

197

#### 198 4. DISCUSSION

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

205 Peripheral analgesic activity is done with the help of writhing test in mice. [11] In general,  
206 endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins, IL-  
207 1 $\beta$ , IL-8, TNF- $\alpha$  and substance P are liberated by intra peritoneal administration of acetic acid  
208 and these mediators are responsible for pain.

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

213 The formalin test is a widely used model of constant nociception [15, 16]. The tests  
214 demonstrate a biphasic response. The first phase begins immediately after the formalin  
215 injection represents neurogenic pain and is caused by direct action on the local sensory C-  
216 fibers, resulting in the release of calcitonin gene-related peptide (CGRP) and substance P  
217 [17,18]. The second phase (15–30 min after injection) is associated with inflammatory pain  
218 of the peripheral tissues due to the release of inflammatory mediators, such as  
219 prostaglandins and nitric oxide, and is responsive to non-steroidal anti-inflammatory drugs  
220 (NSAIDs) [17,19,20,21].

221 Our present results showed that the number of paw licking was significantly reduced by  
222 different fractions of *S. caseolaris* in both neurogenic and inflammatory pain responses ( $p$   
223  $<0.05$ ) in a dose dependant manner (Table 2 ,3 and figure 2 ,3). Ethyl acetate extract  
224 (72.91%), chloroform (72.91%) and pet-ether fraction(70.82%) show better protection than  
225 ethanol fraction. However, the effect of all extracts was more emphasized in the late phase.  
226 Centrally acting analgesic drugs inhibit both the phases of formalin test, while peripherally  
227 acting analgesics restrict only the late phase responses [22]. The late phase response as the  
228 antinociceptive effect observed in formalin test is due to this inhibition of the inflammatory  
229 mediators [23].

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

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

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

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

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

256

257

258 **5. CONCLUSION**

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

263 **COMPETING INTERESTS**

264 There are no competing interests.

265 **CONSENT: NOT APPLICABLE**

266 **ETHICAL APPROVAL:**

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

272 **Consent for publication:** Not applicable

273 **COMPETING INTERESTS DISCLAIMER:**

274 There are no competing interests.

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