

Original Research Article

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3 **Pharmacological screening for CNS Depression, analgesic and anti-**
4 **inflammatory potentials of *Sonneratia caseolaris* (Linn.) barks in**
5 **different solvent fraction**
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11 **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% (10 mg/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 after 1, 2 or 3 hour 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.

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

14

15 1. INTRODUCTION

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

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

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

32

33 2. METHODS

34 2.1 Collection, identification and preparation of plant material

35 The stems [insert the name of the plant] were harvested after identification by an expert
36 taxonomist from the plant growing at Barisal on August 5, 2014. The stems were air dried
37 under shade at room temperature for a period of two weeks in order to avoid solar radiations
38 from altering the API. These stems were spread on plastic bags while avoiding their
39 stacking. Every day we turned these stems upside down so that to favor a homogenous

40 drying process. The dried leaves were ground in a clean electric grinding machine in such a
41 way to obtain a fined powder, which was stored in an airtight container. The total dried
42 powder material was obtained was 600 gm. It was divided equally into four portions and was
43 refluxed with ethanol ,ethyl acetate, pet ether and chloroform solvent for three times. The
44 extract was filtered with Whiteman No. 1. filtered paper and the collected filtrate was
45 evaporated in an oven at 50°C. This extract was weighed so that to determine the yield
46 obtained from the initial powder quantity and then stored in an air-tight container for
47 subsequent experimental tests.

48 **2.2 ANALGESIC ACTIVITY**

49 **2.2.1 Acetic Acid-Induced Writhing Method for Peripheral Analgesic Assay**

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

67 **2.2.2 Formalin-Induced paw licking Method for Central Analgesic Assay**

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

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

81

82 **2.3 ANTI-INFLAMMATORY ACTIVITY**

83 **2.3.1 Carrageenan Induced Paw Edema Test in Mice**

84 Swiss albino mice (25 – 30 g) were divided into six groups of four animals each. The test
85 groups received 150 and 300 mg/kg body weight, p.o. of EA, CLF and PET extracts
86 respectively. The reference group received Indomethacin (10 mg/kg body weight, p. o.) while
87 the control group received 1 ml/kg body weight normal saline. After 30 min, 0.1 ml, 1%
88 carrageenan suspension in normal saline was injected into the subplanatar tissue of the right
89 hind paw. The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection
90 using a micrometer screw gauge. The percentage inhibition of the inflammation was
91 calculated from the formula: % inhibition = $(1-D_t/D_0) \times 100$

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

95

96 **2.4 CNS DEPRESSION ACTIVITY**

97 **2.4.1 Hole cross test**

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

105

106 **STATISTICAL ANALYSIS**

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

109 **3. RESULT**

110 **3.1 Analgesic activity**

111 **3.1.1 Acetic Acid Induced Writhing Method**

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

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

122

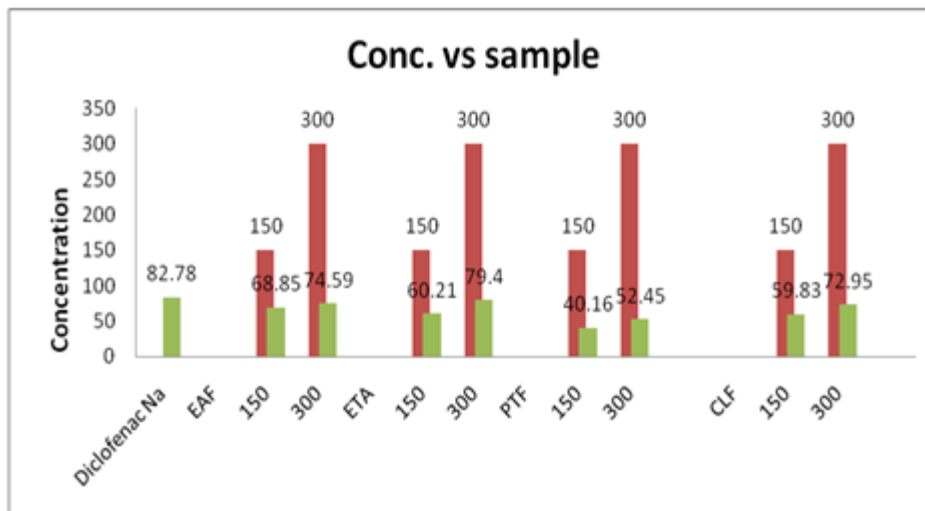
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124 TABLE 1: Antinociceptive effect of ETF, EAF, CLF and PTF extracts of *S. caseolaris* by acetic acid
 125 induced writhing method

126

127 Values are mean \pm SEM, (n = 4), (*) indicates statistically significant compared to vehicle
 128 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 \pm 2.13	-
II	Diclofenac-Na	10mg/kg	4.2 \pm 1.60*	82.78
III	Ethanol fraction	150	8 \pm 2.12*	60.21
IV		300	5 \pm 1.70*	79.40
V	Ethyl Acetate Fraction	150	7.6 \pm 1.51*	68.85
VI		300	6.2 \pm 1.63 *	74.59
VII	Chloroform Fraction	150	9.8 \pm 2.05*	59.83
VIII		300	6.6 \pm 1.67*	72.95
IX	Pet-ether Fraction	150	14.6 \pm 2.35*	40.16
X		300	11.6 \pm 1.06*	52.45



129

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

132

133 **3.1.2 Formalin Test**

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

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

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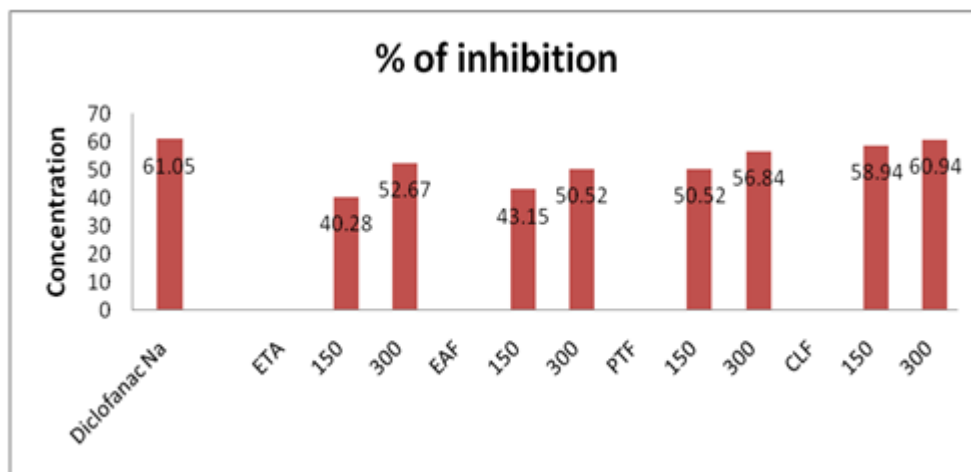
Groups	Treatment	Dose	Late phase	% of protection
I	Control (Saline)	10ml/kg	17.75 ± 1.30	-

II	Diclofenac-Na	10mg/kg	7.4 ± 1.29*	61.05
III	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

146

147 Values are mean ± SEM, (n = 4), (*) indicates statistically significant compared to vehicle

148 control group (*P<.05) using one way ANOVA followed by Dunnet test.



149

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

152

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)**

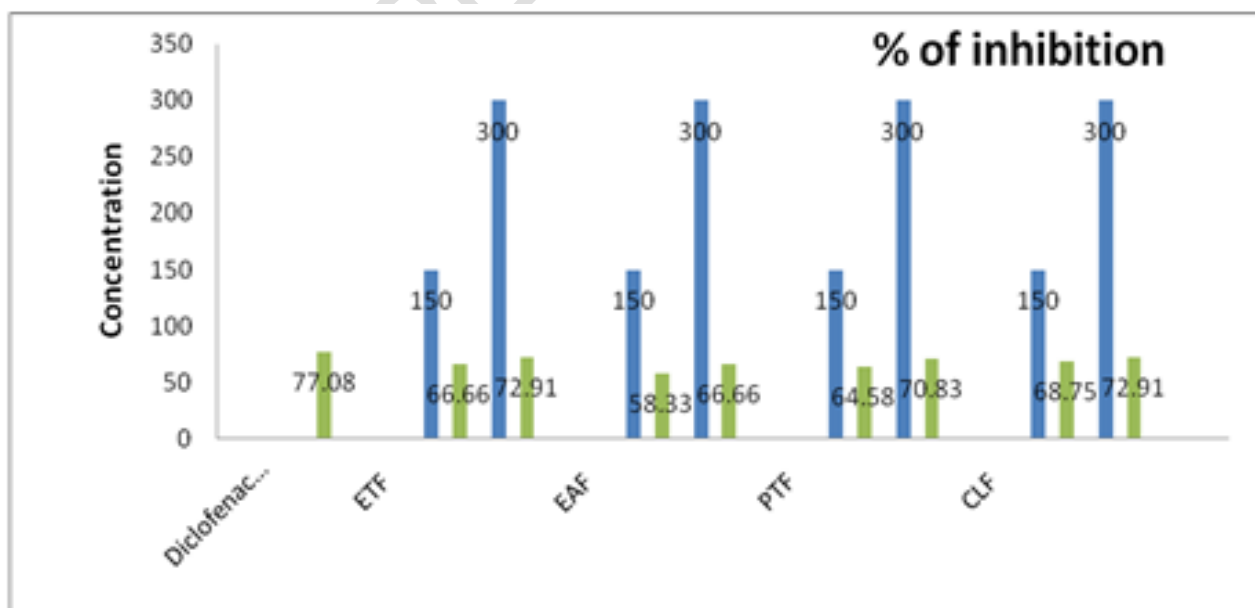
Groups	Treatment	Dose	Avg. no. of Writhing	% inhibition
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I	Control (Saline)	10ml/kg	9.60 ± 1.30	-
II	Diclofenac-Na	10mg/kg	2.20 ± 1.29*	77.08
III	Ethyl Acetate Fraction	150	3.20 ± 1.76*	66.66
IV		300	2.60 ± 1.64*	72.91
V	Ethanol Fraction	150	4.00 ± 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

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 03: Evaluation of % of inhibition of different extract of *S. caseolaris* by**
 161 **formaline 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.

173

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**

176

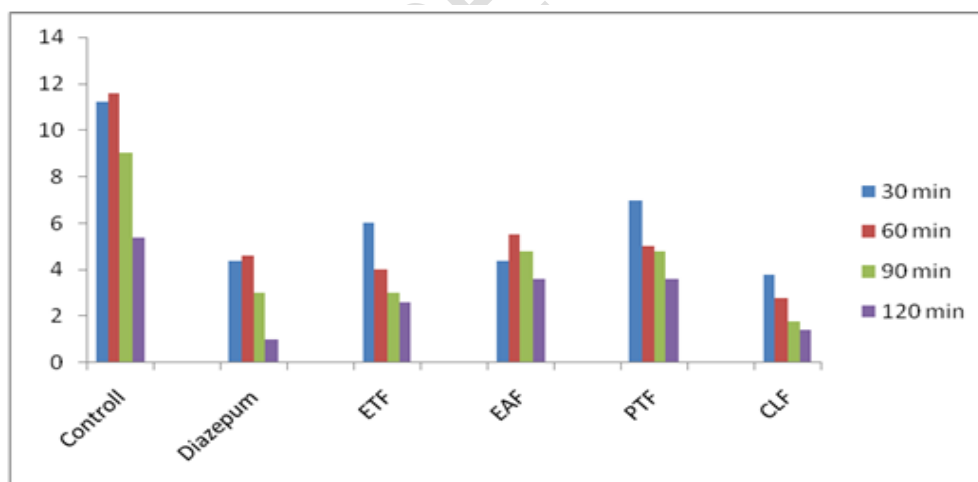
Group	Treatment	Dose	Inhibition (%)			
			1h	2h	3h	4h
I	Control (Saline)	10ml/kg	4.70±0.11	4.40±0.09	4.17±0.11	3.75±0.14
II	Indomethacin	10mg	47.69	51.45	54.76	62.35
III	ETF Fraction	150	29.29	39.29	41.70	32.70
IV		300	35.98	43.30	43.12	35.84
V	EAF Fraction	150	32.22	28.57	30.47	32.40
VI		300	38.08	31.69	36.19	35.50

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	Diazepam	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 ± 0.418*	1.40 ± 0.274*
V	EAFraction	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	Chloroform 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	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 ± 0.354*	2.60 ± 0.274*	1.80 ± 0.418*	3.75 ± 2.428*

190 Values are mean ± SEM, (n = 5), (*) indicates statistically significant compared to vehicle

191 control group (*P<.05) using one way ANOVA followed by Dunnet test.

192



193

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

196

197 **4. DISCUSSION**

198 In this investigation, we **made** reported the effect of ethanolic extract and different fractions
199 of *S. caseolaris* on several experimental animal models of pain, inflammation and analgesic
200 as well as CNS activity. The extracts showed remarkably inhibited the nociception produced
201 by Formaline induced writhing and writhing induced by acetic acid; the extracts also crucially
202 attenuated carrageenan-induced mice right hind paw edema. Estimation suggested that the
203 use of acetic acid induced writhing and Formaline induced writhing for the evaluation of
204 peripherally and centrally acting analgesic drugs respectively [11-12]. *S. caseolaris* extracts
205 to prolong the reaction latency to thermally-induced pain in mice as observed in the
206 Formaline induced writhing test suggest central analgesic activity, on the contrary, the acetic
207 acid-induced abdominal constriction method is widespread used for the investigation of
208 peripheral antinociceptive activity [13]. Commonly, stimulating and production of
209 prostaglandins by acetic acid causes writhing or nociception [14]. GDP and AGE receptors
210 are suggested to be partly connected in the abdominal constriction response .Increased
211 levels of PGE₂ and PGF₂ α in peritoneal fluids as well as lipoxygenase products have
212 associated with prostanoids in general by the method [15-16].As results of the acetic acid-
213 induced writhing strongly recommend that the action of this extracts is linked partially to
214 LOXs (lipoxygenases) and/or COXs (cyclo-oxygenases) pathways. The *S. caseolaris*
215 extracts at the doses tested revealed analgesia in both the nociceptive pain models further
216 demonstrates that the extract possesses both central and peripherally mediated analgesic
217 activities. Carrageenan induced rat paw edema was commonly used as an experimental
218 animal model for acute inflammation and is believed to be biphasic and Carrageenan model
219 of inflammation is also said to be biphasic. Typically, in the early phase (1-2h), histamine,
220 serotonin and kinninsare released and increased synthesis of prostaglandins in the
221 damaged tissue surroundings in the first or two hours. While the late phase sustained to the
222 release of prostaglandin and mediated by bradykinin, leukotrienes, polymorphonuclear cells,
223 and prostaglandins produced by tissue macrophages [17-18] and release of lysosome
224 enzymes in the second to the third hour [19-20]. The present study, indicates that the

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

233

234 **5. CONCLUSION**

235 Our study investigation brings out the scientific rationale for the folkloric uses of the plant in
236 the management of inflammation and pain. Even so, further research is needed towards
237 isolation and ascertaining the active principles present in the extracts, which could possibly
238 be explored for pharmaceutical use.

239 **COMPETING INTERESTS**

240 There are no competing interests.

241 **CONSENT: NOT APPLICABLE**

242 **ETHICAL APPROVAL:**

243 All the experimental mice were treated following the Ethical principles and guidelines for
244 scientific experiments on animals (1995) formulated by the Swiss Academy of Medical
245 Sciences and the Swiss academy of sciences. The institutional Animal Ethical Committee
246 (SEU /IAEC /17-25) of Southeast University Bangladesh approved all experimental rules.

247 **Consent for publication:** Not applicable

248

249

250 **COMPETING INTERESTS DISCLAIMER:**

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

258

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UNDER PEER REVIEW