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Research Article

STUDY OF ANALGESIC AND ANTIDIARRHOEAL ACTIVITIES OF *Sonneratia caseolaris* (LINN.) LEAF AND STEM USING DIFFERENT SOLVENT SYSTEM.

Bishwajit Bokshi[1]*, Md.Nazmul Hasan Zilani[1], Aparajita Malakar[1], Debendra Nath Roy[2], Jamil Ahmad Shilpi[1], Samir Kumar Sadhu[1]

[1]Pharmacy Discipline, Khulna University, Khulna, Khulna-9208 Bangladesh.
[2]Pharmacy, Jessore Science and Technology University, Independence Road), 7408- Bangladesh

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*Corresponding author
Bishwajit Bokshi

Email :
bokshi06@yahoo.com

ABSTRACT

The different fractions of crude ethanol extract of leaf and stem of *S. caseolaris* (Linn.) (*Sonneratiaceae*) were screened for its analgesic and antidiarrhoeal activities. The different fraction of crude extract was obtained by using four different solvent systems. The different fractions of crude extract produced significant writhing inhibition in acetic acid induced writhing in mice at dose of 250 and 500mg/kg BW comparable to the standard drug diclofenac sodium at the dose of 25mg/kg BW. When tested for its antidiarrhoeal effects on castor oil induced diarrhea in mice, it increased mean latent period and decreased the frequency of defecation significantly at the dose of 250 and 500mg/kg BW comparable to the standard drug loperamide at the dose of 50mg/kg BW. The overall results tend to suggest the analgesic and antidiarrhoeal activities of the different fractions of crude extract. Both ethyl acetate fraction of stem and chloroform fraction of leaf have significant analgesic activity. Again between the two fractions of crude ethanol extract ethyl acetate fraction of *S.caseolaris* stem have most significant antidiarrhoeal activity.

Key words: analgesic, antidiarrhoeal, *S.caseolaris,* diclofenac sodium, loperamide.

INTRODUCTION

The history of medicinal plants in remedy of different diseases is well established. Various species of different family of plant and other sources contribute in the development of present therapeutic processes. *Sonneratia caseolaris* Linn. (Sonneratiaceae), locally known as Ora, Orali, Choila, Archaka, Shoila; Orcha, etc., is a small tree distributed in the tidal creek and mangrove swamps of Bangladesh, India, Ceylon, Malay, etc. Its fruit is traditionally used in sprains and swellings, hemorrhage and in the treatment of piles (Kirtikar and Basu, 1987). Based on the traditional usage of this plant, the crude ethanol extract was tested for antioxidant activity using DPPH-radical scavenging effect both quantitatively and qualitatively on TLC in which the extract showed potent antioxidant activity (Ahmed *et al.,* 2006). Although fatty acids (lauric acid), hydrocarbons (squalene), steroids (sitosterol, stigmasterol), pectin and sugars were previously isolated from this plant (Rollet, 1981; Hogg and Gillan, 1984) by taking DPPH-radical scavenging effect as the isolation guide, (Sadhu *et al.,* 2006) isolated a flavone, luteolin and its 7-*O*-β-glucoside (cynaroside) form the crude extract. However, no other biological activity has yet been reported. The objective of the present study was to investigate the analgesic and antidiarrhoeal activity of the various fractions of crude extracts of leaves and stem of *S.caseolaris.*

MATERIAL AND METHODS

Plant material collection and extraction

Collection and Identification

For this present investigation, leaves and stem of the plant *S.caseolaris* (L.) Engl. (family: Sonneratiaceae) were collected from Mangrove Forest of Mongla, Bangladesh in December, 2011 on the day time and was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No.: DACB-36540) and a voucher specimen was also deposited there.

Preparation of crude extract

Drying and grinding

The collected plant parts were separated from undesirable materials or plants or plant

parts and then were washed with water. They were shade-dried for one week. The plant parts (leaves and stem) were ground into a coarse powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Cold extraction (Ethanol extraction)

About 600g of the powdered stem and 500g of powder of leaf was taken in a clean, round bottomed flask (5L) and soaked in 2.0L and 1.5L of ethanol respectively. The containers with its content were sealed by cotton plug and aluminum foil and kept for a period of 14days accompanying routine shaking and stirring. The whole mixtures were then filtered through cotton followed by Whatman No.1 filter paper and the filtrates thus obtained were then air dried to solid residue in different beakers. The weight of the crude extract obtained from stem was 8.7g and 7.5g for leaf.

Solvent-solvent partition of crude extract

Modified Kupchan Partition (Beckett and Stanlake, 1986), partitioning is a process in which liquid-liquid extraction is done to separate the components of a mixture between two immiscible solvent phases of different densities (Leo *et al.,* 1971). Typically, one of the phases will be aqueous, and the other a non-polar lipophilic organic solvent such as carbon tetrachloride, chloroform or ethyl acetate.

To perform partitioning, the two phases and the plant extract to be separated in solution are added to a separating funnel through the top with the stopcock at the bottom closed. The funnel is then closed and shaken gently by inverting the funnel multiple times; if the two solutions are mixed together too vigorously emulsions will form. The funnel is then inverted and the tap carefully opened to release excess vapor pressure. The separating funnel is set aside to allow for the complete separation of the phases. The top and the bottom tap are then opened and the two phases are released by gravitation. Any plant extract may contain hundreds of compounds of various polarities. Partitioning of plant extract (Stem and leaf) of *S. caseolaris* was subjected to separate the polar, moderately polar and nonpolar compounds.

Selection of solvent system

The first requirement in the extraction process is to select two immiscible solvents. Water was chosen as one solvent. So, second solvent should be nonpolar hydrocarbon which is immiscible with water. Considering the polarity four solvents were chosen and they were taken one after another. The solvents are CCl_4, $CHCl_3$, ethyl acetate, water

Separation process

Eight point seven gram of ethanol extract of stem was obtained by cold extraction and evaporation of solvent. This extract was dissolved into 200mL water and this solution was transferred to the separating funnel. A 200mL of carbontetra chloride solvent was added to the funnel. The two solutions were mixed together to vigorously. The funnel was then inverted and the tap was carefully opened to release excess vapor pressure. The separating funnel was set aside to allow for the complete separation of the phases. The nonpolar compounds are more soluble in carbon tetrachloride than in the water. So, they would be dissolved in carbon tetrachloride. The top and the bottom tap are then opened and the two phases are released by gravitation.

Water part was again taken to the separating funnel and this time 200mL chloroform was added. The same procedure was followed for chloroform and water mixture and according to polarity some compounds would be separated to the chloroform part. Here the bottom part is chloroform fraction. At last ethyl acetate was taken and same procedure was followed as was followed for carbon tetrachloride and chloroform. The same procedure was done for 6.5g of ethanol leaf extract.

The solvents were evaporated to obtain the separated plant extract according to the polarity of the compound. Water was evaporated by using a water bath. After evaporation the following amount of different compounds were obtained shown in table I.

Diclofenac sodium (Opsonin Chemical Industries Ltd, Bangladesh), Loperamide (Square Pharmaceuticals Ltd, Bangladesh)

Young Swiss-albino mice of either sex, weighing 25-35g, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the tests. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0±2.0°C and 12h light: dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water.

Pharmacological studies

Analgesic activity

Analgesic activity of the different fractions of crude ethanol extract of *S. caseolaris* was tested using the model of acetic acid induced writhing in mice (Whittle, 1964; Ahmed *et al.,* 2004). The experimental animals were randomly divided into four groups, each consisting of five animals. Group I was treated as' control group' which received 1%(v/v) Tween-80 in water by per oral route at the dose of 10mL/kg BW; group II was treated as 'positive control' and was given the standard drug diclofenac sodium at dose of 25mg/kg BW; group III and group IV were test groups and were treated with the extracts at dose of 250 and 500mg/kg BW respectively. Control vehicle, standard drug and extracts were administered orally, 30 min prior to acetic acid (0.7%) injection. Then after an interval of 15min, the number of writhes (squirms) was counted for 5min.

Antidiarrhoeal activity

Antidiarrhoeal activity of the different fractions of ethanol extract of *S. caseolaris* was tested using the model by castor oil induced diarrhoea in mice (Chatterjee, 1993). The mice were all screened initially by giving 0.5mL of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into four groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10mL/kg BW; group II was treated as 'positive control' and was given the standard drug loperamide at dose of 50mg/kg BW; group III and group IV were test group and were treated with the extract at dose of 250 and 500mg/kg BW. Control vehicle, standard drug and the extracts were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5mL per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment (5h). The latent period of each mouse was also counted. At the beginning of each hour new papers were placed for the old ones.

Statistical analysis

Student's *t*-test was used to determine a significant difference between the control group and experimental groups.

RESULTS AND DISCUSSION

Analgesic activity

The ethyl acetate soluble fraction of stem (EACS) and chloroform soluble fraction of leaf (CFCL) were subjected to determine the analgesic activity by acetic acid induced writhing method on mice. The standard group showed analgesic activity with writhing inhibition of 67% compared to the control group. The ethyl acetate fractions of stem (EACS) and chloroform soluble fraction of leaf (CFCL) showed analgesic activity with writhing inhibition of 33%, and 17% at 250mg/kg dose respectively and 50%, and 54% at 500mg/kg dose respectively (TableII and III).

From this experiment, it may be assumed that both ethyl acetate fraction of stem and chloroform fraction of leaf have significant analgesic activity.

Antidiarrhoeal activity

Table IV showed EACS of *S.caseolaris* stem and CFCL of *S.caseolaris* leaf on the basis of mean latent of castor oil induced diarrhoeal episode activity in the dose of 250mg/kg and 500mg/kg BW on mice. The standard group showed mean latent period of 2.35h compared to the control group 0.6h.

Table I. After evaporation obtained weight of different fractions

Plant part	Fraction	Weight
S. caseolaris stem extract 8.7 g.	Ethyl acetate fraction	1.44 g
	Chloroform soluble fraction	0.43 g
	Carbon tetrachloride fraction	0.35 g
S. caseolaris leaf extract 7.5 g.	Ethyl acetate fraction	0.30 g
	Chloroform soluble fraction	1.88 g
	Carbon tetrachloride fraction	0.40 g

Table II. Analgesic activity of different fraction of *S. caseolaris* stem and leaf on acetic acid induced writhing of mice.

Animal group	Writhing Count					Mean writhing	% of writhing	% of inhibition
	M-1	M-2	M-3	M-4	M-5			
Control	22	23	25	26	25	24	100	0
Standard	6	7	8	8	10	8	33	67
EACS(250mg/kg)	14	19	15	16	18	16	67	33
EACS(500mg/kg)	10	11	12	13	13	12	50	50
CFCL(250mg/kg)	18	19	19	20	22	20	83	17
CFCL(500mg/kg)	9	10	12	12	11	11	46	54

EACS=Ethyl acetate fraction of *S. caseolaris* stem
CFCL=Chloroform fraction of *S. caseolaris* leaf

Table III. Statistical evaluation

Animal group	SD	SE	t-test (value of p)
Control	1.34	0.67	
Standard	1.34	0.67	16.8 P<0.001
EACS (250mg/kg)	1.90	0.95	6.9 P<0.001
EACS (500mg/kg)	2.24	1.12	9.1P<0.001
CFCL (250mg/kg)	1.41	0.71	4.12P<0.01
CFCL (500mg/kg)	1.18	0.59	9.6P<0.001

SD = Standard deviation; SE = Standard error.

Table IV. Effect of ethyl acetate fraction of *S.caseolaris* stem and chloroform fraction of *S.caseolaris* leaf on the latent period of castor oil induced diarrhoeal episode in mice.

Animal group	Latent period (hr)					MLP (hr)	SD	SE	t-test (p-value)
	M-1	M-2	M-3	M-4	M-5				
Control	0.6	0.7	0.8	0.5	0.5	0.60	0.13	0.07	
Standard	2.34	2.32	2.37	2.38	2.35	2.35	0.02	0.01	25 p <.001
EACS(250mg/kg)	0.7	0.9	0.9	0.8	1.0	0.90	0.11	0.06	3.26 p <.05
EACS(500mg/kg)	1.3	1.35	1.4	1.45	1.4	1.40	0.06	.03	11.42 p<.001
CFCL(250mg/kg)	0.62	0.65	0.63	0.67	0.68	0.65	0.03	0.02	0.2.14 p <.1
CFCL(500mg/kg)	0.90	0.94	0.96	0.98	0.99	0.95	0.04	0.02	5.00 p <.01

MLP= Mean Latent Period; SD= Standard deviation; SE= Standard error

Table V. Effect of ethyl acetate fraction of *S.caseolaris* stem and chloroform fraction of *S.caseolaris* leaf on the basis of mean stool count of castor oil induced diarrhoeal episode in mice.

Animal group	Stool count (in 4hr)					MNS (4hr)	SD	SE	t-test (p-value)
	M-1	M-2	M-3	M-4	M-5				
Control	10	11	12	13	14	12	1.6	0.8	
Standard	4	3	3	5	4	04	0.84	0.42	8.88 p<.001
EACS(250mg/kg)	8	7	9	8	9	08	0.84	0.42	4.44 p <.01
EACS(500mg/kg)	4	5	6	7	8	06	1.58	0.79	5.37 p <.01
CFCL(250mg/kg)	10	9	8	10	11	10	1.14	0.57	2.04 p <.1
CFCL(500mg/kg)	05	06	07	07	08	07	1.14	0.57	5.1 p <.01

MNS = Mean No. of Stool; M-1= Mice No.1; M-2= Mice No.2; M-3= Mice No.3; M-4= Mice No.4; M-5= Mice No.5

The ethyl acetate (EACS) of *S.caseolaris* stem and chloroform fraction (CFCL) of *S.caseolaris* leaf showed antidiarrhoeal activity with mean latent period of 0.9h, 0.65h at 250mg/kg dose respectively and 1.4h and 0.95h at 500mg/kg dose respectively.

Again table V showed ethyl acetate fraction (EACS) of *S.caseolaris* stem and chloroform fraction (CFCL) of *S.caseolaris* leaf on the basis of mean stool count of castor oil induced Diarrhoeal episode activity in the dose of 250mg/kg and 500mg/kg BW. On mice the standard group showed mean stool count of 4 compared to the control group 12. The Ethyl acetate (EACS) of *S.caseolaris* stem and chloroform fraction (CFCL) of *S.caseolaris* leaf showed antidiarrhoeal activity with mean stool count of 8, 10 at 250mg/kg dose respectively and 6 and 7 at 500mg/kg dose respectively.

From this experiment, it may be assumed that ethyl acetate fraction (EACS) of *S.caseolaris* stem have most significant antidiarrhoeal activity.

To avoid any solvent effect on the experimental animals, the solvent was evaporated completely to dryness. Analgesic activity of the different fractions of ethanol extract of *S.caseolaris* was tested by acetic acid induced writhing model in mice. Acetic acid induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taesotikul et al.,2003). Increased levels of PGE_2 and $PGF_2\alpha$ in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid (Derardt *et al.,*1980). The different fractions of ethanol extract of *S.caseolaris* produced significant writhing inhibition comparable to the standard drug diclofenac sodium (Table II and III). On the basis of this result it can be concluded that the both ethyl acetate fraction of stem and chloroform fraction of leaf have significant analgesic activity. Antidiarrhoeal activity of the different fractions of the ethanol extract of *S.caseolaris* were tested by using the model of castor oil induced diarrhoea in mice (Chatterjee, 1993) (Table IV and V). Castor oil, which is used to induce diarrhea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Most agreed view is that ricinoleate salts stimulates the intestinal epithelial cell's adenyl cyclase (Recusen *et al.,* 1979) or release prostaglandin (Beubler *et al.,* 1979). The extract caused an increase in latent period i.e. delayed the onset of diarrhoeal episode and decreased the frequency of defecation as well as the number of stool. On the basis of the result of castor oil induced diarrhoea, it can be concluded that ethyl acetate fraction (EACS) of *S.caseolaris* stem have most significant antidiarrhoeal activity.

CONCLUSION

In conclusion, it could be suggested that the various fractions of crude extract of *S.caseolaris* possesses analgesic and antidiarrhoeal effect. However, further studies are necessary to find out the active principles responsible for these activities.

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