Original Research Article

PHYTOCHEMICAL SCREENING AND COMPARATIVE ANTIOXIDANT ACTIVITIES OF FRACTIONS ISOLATED FROM *SONNERATIA CASEOLARIS* (LINN.) BARK EXTRACTS.

ABSTRACT

Aims: Our study was carried out to appraise the phytochemical screening and antioxidant potentials of *Sonneratia caseolaris* (Linn.) bark extracts.

Study design: For the purpose of this experiment the extracts were subjected for an *in-vitro* study.

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

METHODOLOGY : The various fractions of *Sonneratia caseolaris* (Linn.) barks as Ethanolic (ETF), ethyl acetate (EAF), chloroform(CLF) and pet ether (PTF) fractions were obtained after extraction were subjected to preliminary phytochemical screening. The antioxidant capacity of these fractions were evaluated using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay .Total antioxidant activity and total phenolic content of ETF, EAF, CLF and PTF extracts of *S. caseolaris* were determined.

RESULTS: The phytochemical screening showed the presence of flavonoid, steroid, tannin compounds in large amounts. In DPPH scavenging assay among the

extracts, ethanolic fractions exhibited the highest radical scavenging activity with IC_{50} of 4.57 µg /ml .The highest phenolic content was found in EAF extracts (63.00 mg of GAE / g. of dried extract) followed by CLF (36.25 mg of GAE / g. of dried extract) and PTF (26.28 mg of GAE /g. of dried extract). The highest total antioxidant activity was also found in ETF fraction (185 GAE/g of dried sample followed by EAF fraction (99.00GAE/g of dried sample), PTF (84.00 GAE/g of dried sample) and Chloroform (49.00 GAE/g of dried sample).

CONCLUSION: Our result demonstrates that all the extractives of *S. caseolaris* have appreciable antioxidant activities. But, further study is necessary to isolate the active compounds.

KEYWORDS: *Sonneratia caseolaris,* DPPH, Total antioxidant activity, Total phenolic content.

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 20 m height), evergreen tree with elliptic-oblong leaves (5 to 9.5 cm 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, tannin, flavonoid, saponin, phytosterol, and carbohydrate[5-6].*S. caseolaris* is 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].

Oxidative stress cause due to imbalance of oxidizing agents and natural antioxidants in the body induces the brutality of a number of diseases like atherosclerosis, cancer, cardiovascular ailments, neurodegenerative disorders and diabetes [9]. As self-protective measure against such oxidative damages, biological systems have evolved a range of enzymatic machineries and scavengers. These include dietary antioxidants (αtocopherol, β-carotene, ascorbic acid, glutathione, uric acid), hormones (estrogen, angiotensin), enzyme systems (superoxide dismutase, glutathione peroxidase, catalase) [10-11]. A large number of antioxidative agents, both natural (e.g. α-tocopherol) and synthetic (e.g. butylated hydroxyanisole, butylated hydroxytoluene, tert-butyl hydroquinone and propyl gallate) are broadly used in the food industry to lengthen shelf life as they inhibit lipid oxidation [12]. However, the use of these synthetic antioxidants is increasingly getting restricted because of their toxicity and health risks [13].Therefore, discovery of novel antioxidative of natural origin is the urgent need of the hour and plants can be a good source for the purpose[12]. Earlier research focused on methanolic bark extracts to illustrate the antioxidant activity of *S. caseolaris*. However, here we focus on comparative antioxidant activities of different fractions of *Sonneratia caseolaris* (Linn.) barks extracts.

2. METHODS

2.1 Collection, identification and preparation of plant material

The stems 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 active pharmaceutical ingredients. These stems were spread on plastic bags while avoiding their stacking and every day these stems were mixed 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 g. 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 Whatman 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.

2.2.1 Phytochemical screening

Phytochemical screening of the stems extracts of *S.caseolaris* were tested for the presence of active principles such as alkaloids, flavonoids, tannins, reducing sugar. using the standard procedures.

Test for saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. About 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for saponins (Kokate, 1999) The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min and a 2 cm layer of foam indicates the presence of saponins.

Test for Tannins About 2.5 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (Trease and Evans, 1989).

Test for Flavonoid

The presence of flavonoids in the samples was determined using the methods [14].About 10 ml ethyl acetate was added to 0.2g of the powdered sample and heated in a water bath for 5 min. The mixture was cooled, filtered and the filtrates used for the test. Ammonium test**:** About 4 ml filtrate was shaken with 1 ml of dilute ammonia solution. The layer was allowed to separate and the yellow color in the ammoniacal layer indicates the presence of flavonoids. Aluminum chloride solution test: Approximately 1 ml of 1% aluminum chloride solution was

added to 4 ml of the filtrate and shaken. A yellow coloration indicates the presence of flavonoids.

Test for alkaloids

Mayer's test (Evans, 1997): To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

Test for carbohydrates

In Benedict's test, 0.5 ml of the filtrate and 5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red colored precipitate indicates the presence of sugar. [15]

Test for steroids

Two milliliters of acetic anhydride was added to 0.5 g of extracts of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids. [15]

2.2 Evaluation of Antioxidant Activity

2.2.1 Estimation of total phenolic content

The Folin-Ciocalteu's reagent was used as oxidizing agent and test-using gallic acid as standard, the total phenolic content of extractives of *S.caseolaris* was determined using Singleton *et al.* method [16] with some modifications. The assay mixture consisted of extract (0.5 ml that was adjusted to 1.0 ml with distilled water) and 2.5 ml of Folin-Ciocalteu's reagent. Furthermore, after incubation at room temperature for 15 min, 2.5 ml of (w/w) Na_2CO_3 solution was added into the test tube and the test tube was incubated at the same temperature for 20 minutes. Finally, the absorbance was read at 760 nm against reagent blank. However, the methanol extract and in different fractionates in Gallic acid equivalents (GAE) was calculated by the according to the formula.

$C = (c \times V)/m$

Where,

C = total content of phenolic compounds, mg/g plant extract, in GAE; c= the concentration of Gallic acid established from the calibration curve, mg/ml; V = the volume of extract, ml; m = the weight of different pure plant extracts, gm.

2.2.2 Estimation of total antioxidant capacity

Catechin reagent was used as a standard; the total antioxidant capacity of extractives of *S. caseolaris* was determined by the method of Prieto *et al* [17] with slight modifications. The mixture consisted of extracts (0.5 ml standard or plant extract solution) was taken in a test tube with 3 ml of reaction mixture containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate was added into the test tube. In addition, after incubation at 95ºC for 10 minutes, the absorbance of the solution was read at 695 nm against reagent bank using a spectrophotometer. The experiment was done three times at each concentration.

2.2.3 DPPH Radical scavenging Assay

The 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was evaluated according to the method used by Fresin [18] with modified procedure. Test samples were prepared by dissolving 5 mg of dry extracts in 5 ml of methanol. The assay mixture contained extract (0.5 ml) and DPPH (1.0 ml) which were mixed well and incubated in the dark for 30 minutes. The blank was prepared and made to contain methanol (0.5 ml) and DPPH (1.0 ml). The absorbance was measured at 517 nm on a visible spectrophotometer. All experiments were performed in triplicate. DPPH radical activity was calculated by the following equation.

$$\text{Percentage Inhibition} = \{(A_o - A_1)/A_o\} \times 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. In addition, % inhibitions were plotted against concentration and from the graph IC_{50} was calculated.

3. RESULTS

Table 1. Phytochemical test results of different extractives of *S. caseolaris* bark

Phytochemical tests	Crude methanol extract	Ethanol fraction	Chloroform fraction	Petroleum Ether fraction	Ethyl acetate fraction
Saponin	++	+	-	-	+
Tannin	+++	+++	++	++	++
Flavonoid	+++	+++	++	++	++
Alkaloid	+ +	+	-	-	+
Carbohydrate	+ +	++	-	+	-
Steroid	+++	++	++	+	+

Here, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, - = Not present

3.1 Determination of total phenolics

The results were expressed as mg of gallic acid equivalent (GAE)/g of dried extractives. Among the fractions the highest phenolic content was found in EAO fractions (63.00 mg of GAE / g of dried extract) followed by ETF (60.25 mg of GAE / g of dried extract), CLF (36.25 mg of GAE / g of dried extract) and PET (26.28 mg of GAE / g of dried extract). Comparing the phenolic content of different fractions of *S. caseolaris* it was observed that EAO contains considerable amount of phenolic compounds than the other extracts.However, phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown in Table 2 and in Figure 01.

Table 2: Determination of total phenolic content of different fractions of *S. caseolaris.*

Fraction	**Conc. (µg/ml)**	**Absorbance**	**GAE/gm of dried sample**
Ethanol	250	0.296	60.25
Chloroform	250	0.25	36.25
Ethylacetate	250	0.324	63.00
Pet-ether	250	0.174	26.28

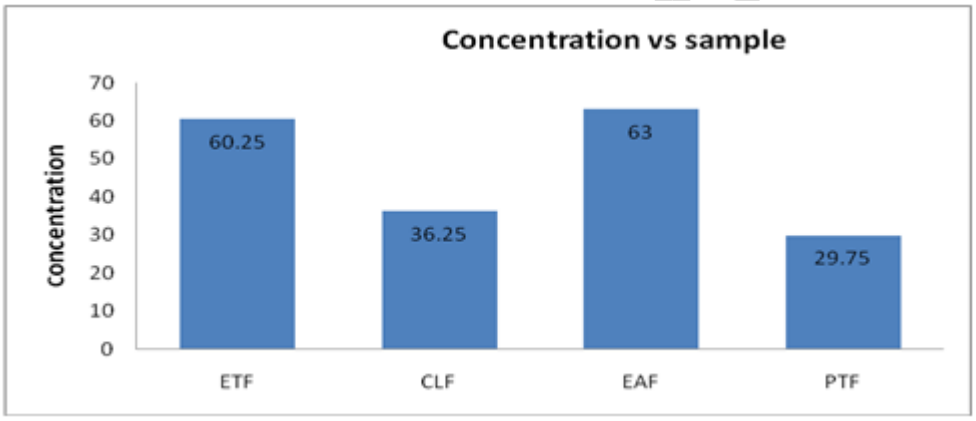

Figure 1: Total phenolic content of different fractions of barks of *S. caseolaris.*
Here, ETF = Ethanol fraction, CLF = Chloroform fraction, EAF = Ethyl acetate fraction, PTF = pet-ether fraction.

3.2 Determination of total antioxidant activity

Total antioxidant activity of four different fractions as ETF, CLF, EAF and PTE were investigated. Among the four different fractions ETF showed the highest total antioxidant activity with absorbance at 200 µg/ml concentration followed by EAF (absorbance of 0.388 at 200 µg/ml), PTF (absorbance of 0.187 at 200 µg/ml) and CLF (absorbance of 0.166 at 200 µg/ml). Our result demonstrates that all the extractives of *S. caseolaris* have appreciable

total antioxidant activity. However, total antioxidant activity of plant extracts and (+)-catechin (standard) were depicted in Table 3 and 4 and in Figure 2 and 3.

Table 3: Absorbance of catechin (standard) at different concentrations for determination of total antioxidant activity.

Name of sample	Concentration (µg/ml)	Absorbance			Absorbance Mean ±STD
		a	b	c	
(+)-Catechin	6.25	0.018	0.019	0.021	0.019± 0.0015
	12.5	0.207	0.211	0.209	0.209 ± 0.002
	25	0.037	0.039	0.035	0.037 ± 0.002
	50	0.118	0.119	0.116	0.117 ± 0.001
	100	0.380	0.383	0.379	0.381 ± 0.002
	200	0.803	0.801	0.805	0.803 ± 0.002

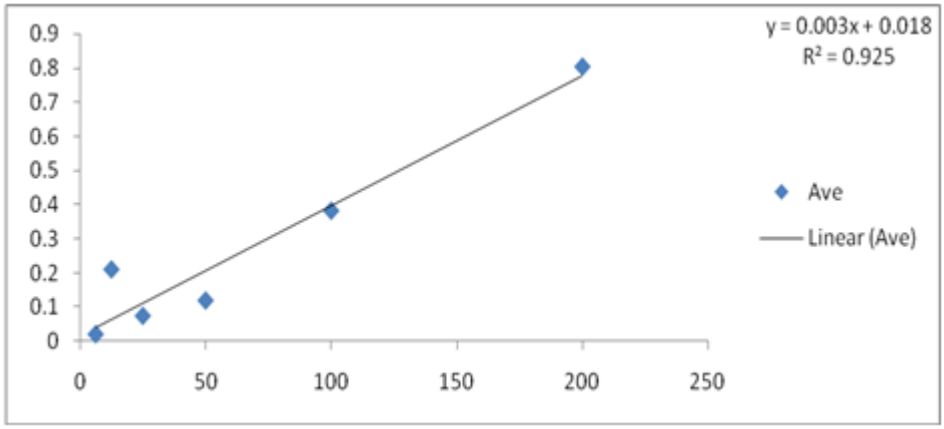

Figure 2: Standard curve of catechin for the determination of total antioxidant capacity.

Table 4: Determination of total antioxidant capacity of different fractions of *S. caseolaris*.

Fraction	Conc. (µg/ml)	Absorbance	GAE/g of dried sample
Ethanol	200	0.388	185
Chloroform	200	0.166	49.00
Ethyl acetate	200	0.216	99.00
Pet-ether	200	0.187	84.00

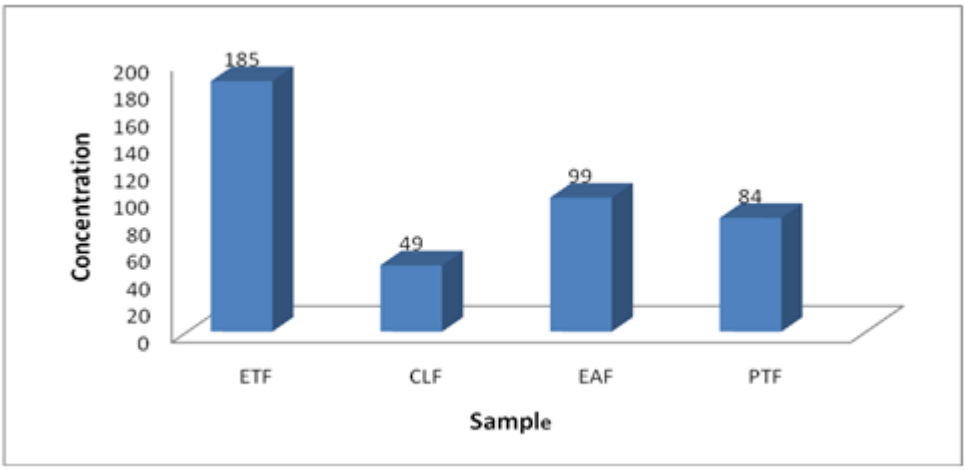

Figure 3: Total antioxidant activity of different solvents fractions of the extracts of *S. caseolaris*.

3.3 Determination of DPPH radical scavenging activity

DPPH radical scavenging activity of different fractions of ETF, CLF, EAF and PTE were investigated. Among all extracts ethanol fraction (EAF) showed the highest DPPH radical scavenging activity with IC_{50} value of 4.57 µg/ml and chloroform fraction ethanol fraction (EAF) showed the lowest DPPH radical scavenging activity with IC_{50} value of 197.27 µg/ml respectively. The results of DPPH radical scavenging assays of plant extracts and butylated hydroxytoluene (BHT) (standard) are given in Table 5 and in Figure 4.

Table 5: DPPH radical scavenging activity of different fractions of extracts of *S. caseolaris* and BHT (Standard) at different concentrations.

Sample	Conc. (µg/ml)	Absorbance	% of scavenging	IC_{50} (µg/ml)
BHT	200	0.073	94.45	3.25 µg/ml
	100	0.071	94.48	
	50	0.079	94.33	
	25	0.085	93.40	

	12.5	0.098	92.39	
	6.25	0.147	88.58	
Ethanol fraction	200	0.085	93.40	4.57 µg/ml
	100	0.073	94.33	
	50	0.071	94.48	
	25	0.080	93.78	
	12.5	0.126	90.21	
	6.25	0.409	68.24	
Chloroform fraction	200	0.635	50.69	197.27 µg/ml
	100	1.038	19.40	
	50	0.675	47.59	
	25	0.707	45.10	
	12.5	0.935	27.40	
	6.25	0.689	46.50	
Ethyl acetate fraction	200	0.061	95.26	13.09 µg/ml
	100	0.228	82.29	
	50	0.432	66.45	
	25	0.555	56.90	
	12.5	0.673	47.74	
	6.25	0.697	45.85	
Pet-ether fraction	200	0.749	41.84	12.32 µg/ml
	100	0.637	51.47	
	50	0.698	45.80	
	25	0.742	42.39	
	12.5	0.635	50.69	
	6.25	0.524	59.31	

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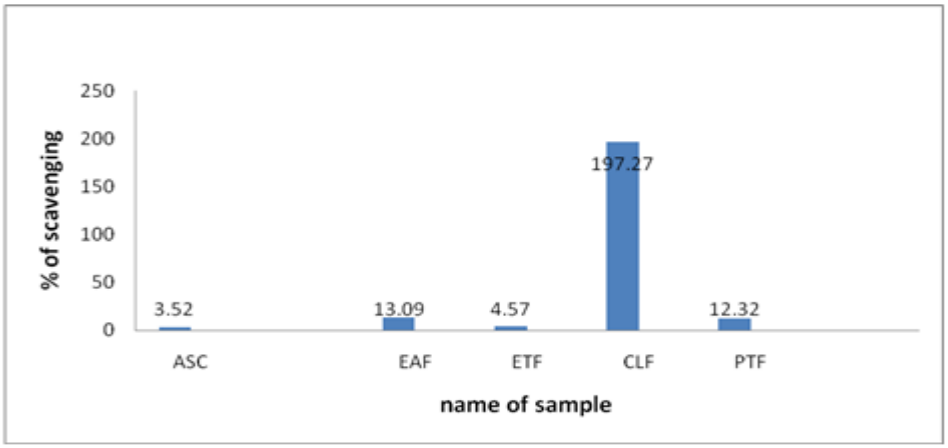

Figure 4: IC_{50} (µg/ml) values of different extractives of *S. caseolaris for* DPPH radical scavenging activity.

4. DISCUSSION

At maximum wavelength at 517 nm, The DPPH free radical can easily accept an electron or hydrogen from antioxidant molecules to develop into a stable diamagnetic molecule .Due to the DPPH radical's ability to bind hydrogen, it is considered to have a radical scavenging property. Discoloration occurs due to the decreasing quantity of DPPH radicals in the environment. The discoloration of the DPPH therefore reflects the radical scavenging activity of the analyzed extracts [19].Based on the data obtained from this study, DPPH radical scavenging activity of *S. caseolaris* extract of chloroform fraction (IC_{50} 4.57µg/ml) was similar to that standard BHT ((IC_{50} 3.25 µg/ml).

Phenolic compounds have redox properties, which let them to act as antioxidants. [19]. Free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Among the fractions the highest phenolic content was found in EAF(63 mg of GAE / gm. of dried extract) and then ETF (60.25 of GAE / gm. of dried extract) ,CLF(36.25 mg of GAE / gm. of dried extract) and PTF(29.75 mg of GAE / gm. of dried extract) . Comparing the phenolic content of ETF, EAF, CLF and PTF extracts of *S. caseolaris* it was observed that ETF contains considerable amount of phenolic compounds than the other extracts.

The total antioxidant capacity (TAC) was based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of greenphosphate/Mo(V)complex at acid pH . It evaluates both water-soluble and fat-soluble antioxidants. Among the different extracts, Ethanol fraction showed the highest total antioxidant activity (185 GAE/g of dried sample).

5. CONCLUSION

Our study investigation brings out the scientific rationale for the folkloric uses of the plant in the management of oxidative stress associated disorders. Previously it has been reported that leaf extracts of *S. caseolaris* posseses two flavonoid compound, luteolin and luteolin 7-O-b-glucoside those hold antioxidant activity [20]. Further-more Phytochemical analyses of methanolic bark extracts revealed the presence of high amounts of phenolics, flavonoids, tannins, alkaloids and saponins which comply with our results [12].The study suggests *Sonneratia caseolaris* bark as a potential source of bioactive compounds with antioxidative properties which contributed by flavonoid, Phenolic and tannin compounds and can be used as natural antioxidative agents in clinical, pharmaceutical and food processing industries.

COMPETING INTERESTS

There are no competing interests.

CONSENT: NOT APPLICABLE

ETHICAL APPROVAL: NOT APPLICABLE

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