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Comparative Analysis on Antioxidant Activities of Barks from *Sonneratia caseolaris* Grown in Three Saline Zones of Sundarbans

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ABSTRACT

Sonneratia caseolaris is a medicinal mangrove plant whose barks possess a high content of bioactive compounds that exhibit different pharmacological activities viz. antioxidants and antimicrobial activities. High salinity is responsible for ion poisoning and subsequent oxidative stress. Total alkaloids of *S. caseolaris* barks was highest in high saline (HS) zones (6.35±0.32 g/100g extract) followed by moderately saline (MS) and low saline (LS) zones (5.78±0.23 and 5.23±0.36 g/100g extract, respectively). The orders (from highest to lowest value) of total saponins of *S. caseolaris* barks were found in HS, MS and LS zones (10.02±0.4 > 9.18±0.4 > 8.07±0.5 mg/g extract, respectively). Highest, moderate and lowest tannins of the plant barks were observed in HS, MS and LS zones (5.15±0.29, 4.81±0.21 and 4.39±0.24 g TAE/100g extract, respectively). Total phenolics of *S. caseolaris* barks was highest in MS zones (5.75±0.3 g GAE/100g extract) followed by LS zones (4.87±0.19 g GAE/100g extract) and HS zones (4.61±0.25 g GAE/100g extract). Highest, moderate and lowest flavonoid contents of the plant barks were found in MS, LS and HS zones (8.97±0.28, 8.21±0.47 and 8.05±0.39 g QE/100g extract, respectively). In the present study, highest phenolics and flavonoids of *S. caseolaris* barks were found in MS zones while highest alkaloids, saponins and tannins of the plant barks were found in HS zones of Sundarbon.

Keywords: *Sonneratia caseolaris, antioxidant activities, saline zones, Sundarbans, phenolics, flavonoids, alkaloids*

Introduction

The Sundarban is the single largest continuous mangrove forest in the globe and the UNESCO announced it as a World Heritage Site (WHS) in 1997. About 60 % of the Sundarbans forest is located in Bangladesh and the residual parts in India. There are total 334 plants, 87 monocotyledons, 230 dicotyledons, 165 algals, 17 ferns and 13 special orchids in the Sundarbans [1]. Salinity is the environmental stress which can result in a oxidative burst so that it leads to the formation of reactive oxygen species (ROS) that causes biochemical and physiological changes and produce low biomass in plants [2,3]. Mangrove plants can synthesize various secondary metabolites (mostly phenolic compounds) in their body which have antiallergic, anti-inflammatory, cardioprotective, antiviral, anticancer, antioxidant activities and these metabolites serve as an important tool in plant defense system so that plants can survive under salinity stress [4,5]. Natural antioxidants can cure diabetes, ameliorate glucose ejection

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and ameliorate several associated complications and they are also used in food companies and pharmaceuticals as additives while synthetic antioxidants can show carcinogenicity as a side effect [6].

Sonneratia caseolaris L is a small tree belonging to the family *Sonneratiaceae* and its Bengali name is ora, choila, archaka, orcha (Sundarbans); chhaila (Barisal) etc. It is found in Bangladesh (especially Chakaria Sundarbans, a tidal forest of Barisal, Chittagong, Sunderbans), India, Sri Lanka, Myanmar, Malaysia, China, Indonesia, Philippines, Singapore, Thailand, Viet Nam and Northeast Australia. Wood and bark of *S. caseolaris* possess phenolic compounds, colouring agents, emodin, archinin, chrysophanic acid and prousinol [7]. *S. caseolaris* also show high tolerance to salinity stresses and can ameliorate in low inter-tidal regions where other indigenous species survive difficulty [8]. It was reported that *S. caseolaris* indicated the presence of saponins, flavonoids, tannins and reducing sugars [9].

The present study was sketched to determine antioxidant activities (including phenolics, flavonoids, alkaloids, saponins and tannins) of *S. caseolaris* barks grown in different saline zones of the Sundarbans and to recognize the best saline zones where *S. caseolaris* barks have high antioxidant activity.

Materials and Methods

Collection of soil and plant samples

Soil samples and *Sonneratia caseolaris* bark samples were collected from three zones of the Sundarbans in Bangladesh (viz. Bagerhat (Low saline), Khulna (Moderately saline) and Satkhira (High saline) zones.

Determination of Electrical Conductivity (EC) of soil sample

Electrical Conductivity (EC) of soil samples was estimated by EC meter in soil maintaining the ratio of soil to water of 1:5 as suggested by USDA [10].

At first, 10 g of soil sample was taken in each conical flask. Then, 50 mL of distilled water was added to the conical flask as the ratio soil and distilled water is 1.5. The mixture was mixed completely by stirring for 30 min and filtered through Whatman filter paper no.1. The filtrate was taken in a test tube. Finally electrical conductivity was measured by EC meter.

Say EC reading of the meter is A.

1. Multiply reading (1:5 ratio) by 5 to get the equivalent EC value for saturated extract (1:1 ratio). Therefore, the EC value is 5A.
2. Then, calculate EC with any of the following formula (straight-line equation).

If 5A is <2, EC= [(5A× 1.323) + 0.122]
If 5A is between 2-14, EC= [(5A× 1.267) + 1.269]
If 5A is >14, EC = [(5A× 0.895) + 8.31].

Finally, EC value is multiplied by 0.064 for measuring salt percentage.

Extraction

Ten grams (10 g) of powder of each three bark sample was taken in a separate airtight bottle. Then, 100 ml of methanol was added to each bottle and the mixture was shaked vigorously and kept for overnight. After 24 hours, the mixtures were filtered through Whatman filter paper no.1. The filtrates were then air dried. The solid/crude extracts were weighed separately and stored in a refrigerator (4ºC).

Determination of total alkaloid contents

Quantification of the alkaloids was performed according to the method of Harborne [11]. 5 g of each powdered sample was taken in a conical flask. Chemicals viz. acetic acid, ethanol and ammonium hydroxide are used in the method.

Determination of total saponin contents

Saponins are quantified according to the method of Obadoni and Ochuko [12]. Ethanol, diethyl ether and n-butanol are the chemicals used in different steps of the method for the assurance of saponins.

Determination of total tannin contents

Tannin determination was performed following the method of Van-Burden and Robinson with slight modifications [13]. 50 ml distilled water was taken in a 500 ml flask and 500 mg of the each sample was added to the flask. It was kept in shaker for 1 h. Then it was filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filtrate was taken into a test tube and mixed with 2 ml (10 fold diluted) of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. Lastly, the absorbance was estimated at 605 nm within 10 min.

Determination of total phenolics

According to Folin-Ciocalteu method [14], total phenolics of bark extracts were quantitatively determined. 1 ml of each diluted bark extract was taken in a test tube. Then, 1 ml of Folin-Ciocalteu's reagent was added to it and vortexed vigorously. The mixture was kept for 3 minutes. The mixture was incubated at room temperature for 1 hour after adding 1ml of a 10% (w/v) sodium carbonate aqueous solution and then the colorimetric measurements were made at 700 nm against distilled water. Gallic acid was used as the standard and expressed (g) as Gallic Acid Equivalents (GAE)/100g of extract.

Determination of total flavonoid contents

Total flavonoid contents were measured by using a colorimetric method according to the Zhishen *et al* with some modification [15]. Quercetin was used as the standard and expressed (g) as Quercetin Equivalents (QE)/100g of extract. 1.5 ml of diluted bark extract was added to each test tube.

Then, 75 µl of 5% (w/v) $NaNO_2$ solution was mixed with the extract and vortexed vigorously. After 6 min, 150 µl of a 10% (w/v) $AlCl_3.6H_2O$ solution was added to the mixture and waited for further 5 min. 0.5 ml of 1 M NaOH solution and 275 µl of distilled water were added to the mixture and mixed well. The absorbance was estimated at 510 nm against distilled water using a spectrophotometer.

Statistical analysis

Samples are prepared in three replicates and analyzed statistically using the Statistical Package for Social Sciences (SPSS) database, version 16.0, submitting the data to a simple one factor Analysis of Variance (ANOVA). ANOVA was performed and mean separation was done by SPSS database ($p<0.05$).

Results

Electrical Conductivity (EC) and salt percentage of soil sample

Highest EC value and salt percentage were determined in the soil sample of HS zone and lowest EC value and salt percentage were in the soil of LS zone (**Table 1**).

Total alkaloid contents

Graph 1 showed the total alkaloid contents (TAC) of *S. caseolaris* barks collected from 3 saline zones of the Sundarbans. In the present study, TAC of the barks of HS, MS and LS zones were 6.35±0.32 >5.78±0.23 >5.23±0.36 g/100g extract, respectively.

Total saponin contents

In **Graph 2,** it was indicated that the total saponin contents of *S. caseolaris* barks of of HS, MH and LS zones of the Sundarbans were 10.02±0.4, 9.18±0.4 and 8.07±0.5 mg/g extract, respectively. Highest saponin contents were observed in the barks of HS zones and lowest saponin contents in LS zones.

Total tannin contents

Total tannin contents of *S. caseolaris* barks collected from HS, MH and LS zones of the Sundarbans are shown in **Graph 3.** In the present study, total tannin contents of the barks of HS, MH and LS zones were 5.15±0.29 > 4.81±0.21 > 4.39±0.24 g TAE/100g extract, respectively.

Total phenolics

Graph 4 showed that the amounts of phenolics of *S. caseolaris* barks of HS, MS and LS zones were 4.61 ± 0.25, 5.75 ± 0.3 and 4.87 ± 0.19 g GAE/100g extract, respectively. From **Graph 4,** it was observed that *S. caseolaris* barks of MS zones contained highest total phenolics (5.75 ± 0.3 g GAE/100g extract) and lowest phenolics was in HS zones (4.61 ± 0.25 g GAE/100g extract).

Total flavonoid contents

Total flavonoid contents of *S. caseolaris* barks collected from HS, MH and LS zones of the Sundarbans are shown in **Graph 5.** In the present study, the values of TFC of the plant barks of HS, MS and LS zones were 8.05±0.39, 8.97±0.28 and 8.21±0.47 g GE/100g extract, respectively. The *S. caseolaris* barks collected from MS zones of Sundarbans showed the highest TFC (8.97±0.28 g QE/100g extract) and from HS zones showed lowest TFC (8.05±0.39 g QE/100g extract).

Discussion

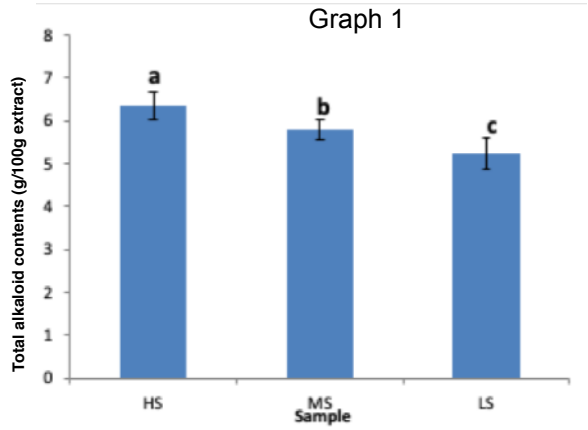
The previous studies indicated that polyphenol contents and flavonoid contents of leaflets of *Simarouba glauca* seedlings were increased with increasing the salinity treatments. It was observed that increased levels of polyphenols under salinity stress in *Aegiceros corniculatum* [16,17]. In *Bruguiera parviflora*, Parida and Das noticed an increase in polyphenols with the increasing the amounts of salinity [18]. Salt stress found to cause rise in total phenolic contents in the halophytic species such as *B. parviflora*, *Agiceras corniculatum, Cakile maritima, Salvadora persica* [17,18,19]. Total polyphenol contents of *Moringa oleifera* was increased when salinity was increased from 2 dS/m to 12 dS/m [20]. In the majority of the halophytes higher polyphenol accumulation can indicate higher antioxidant activity [19,21].

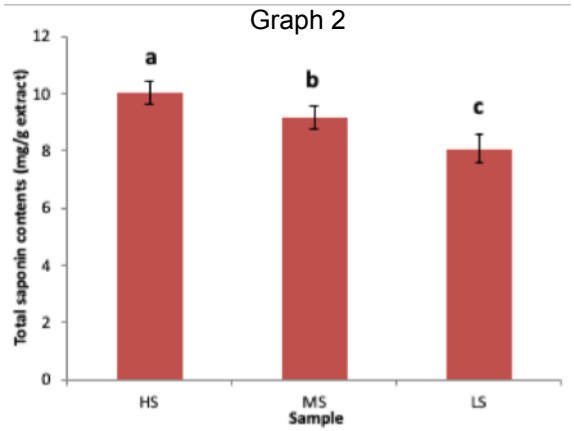
The impact of salt pressure (50 and 100 mM NaCl) was observed on flavonoid content in shoots and roots of barley. Flavonoid content both in shoots and roots of barley was significantly increased in response to salt stress [22]. In leaves of the *Paulownia* clones (TF 01 and EF 02) total flavonoid content was increased with increasing salt pressure (50, 100 and 200 mg/l NaCl treatment) [23]. A steady increase in flavonoid level in the leaf tissue along with increase in salinity was noticed in *S. glauca* which may contribute to the antioxidant metabolism in salt stressed leaf

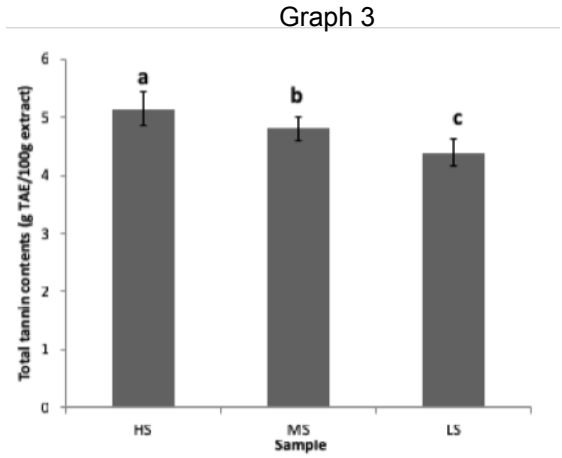
Table 1: Determination of Electrical Conductivity (EC) and salt percentage of soil sample

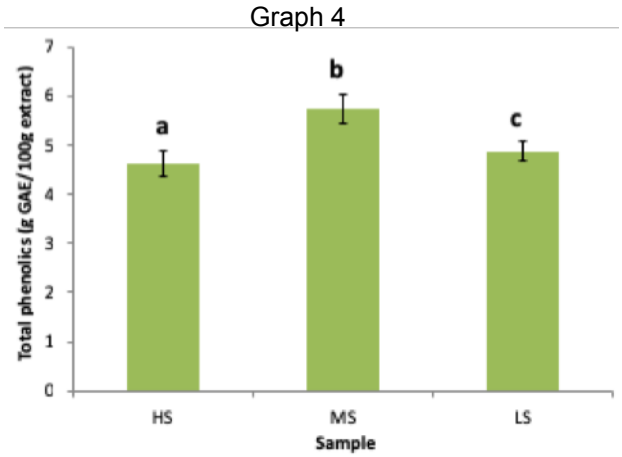
Soil sample	EC (mS/cm) ± SD	Salt % ± SD
HS	12.03 ± 1.27	0.77 ± 0.08
MS	6.30 ± 0.89	0.40 ± 0.06
LS	3.11 ± 0.52	0.20 ± 0.03

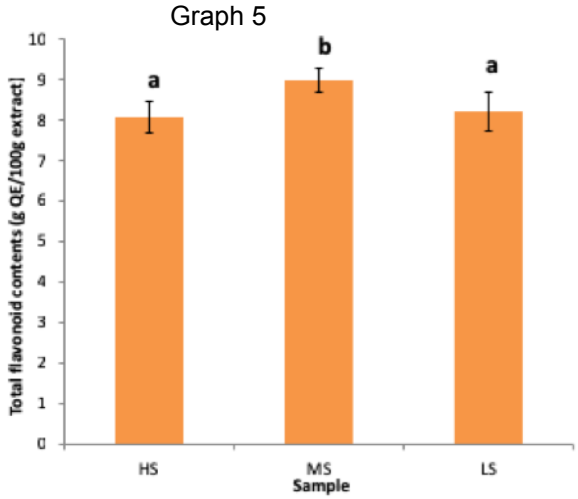
HS=High Saline, MS=Moderately Saline, LS=Less Saline, SD= Standard Deviation











Graph 1: Total alkaloid contents (g/100g extract) of *S. caseolaris* barks collected from HS (High Saline), MS (Moderately Saline) and LS (Less Saline) zones of Sundarbans. Values are presented as mean ± SD (bars), n=3. Different letters reveal the significant difference at P<0.05 level.

Graph 2: Total saponin contents (mg/g extract) of *S. caseolaris* barks collected from HS (High Saline), MS (Moderately Saline) and LS (Less Saline) zones of Sundarbans. Values are presented as mean ± SD (bars), n=3. Different letters reveal the significant difference at

Graph 3: Total tannin contents (g TAE/100g extract) of *S. caseolaris* barks collected from HS (High Saline), MS (Moderately Saline) and LS (Less Saline) zones of Sundarbans. (TAE=Tannic Acid Equivalent). Values are presented as mean ± SD (bars), n=3. Different letters reveal the significant difference at P<0.05 level.

Graph 4: Total phenolics (g GAE/100g extract) of *S. caseolaris* barks collected from HS (High Saline), MS (Moderately Saline) and LS (Less Saline) zones of Sundarbans. (GAE=Gallic Acid Equivalent). Values are presented as mean ± SD (bars), n=3. Different letters reveal the significant difference at P<0.05 level.

Graph 5: Total flavonoid contents (g QE/100g extract) of *S. caseolaris* barks collected from HS (High Saline), MS (Moderately Saline) and LS (Less Saline) zones of Sundarbans. (QE= Quercetin Equivalent). Values are presented as mean ± SD (bars), n=3. Different letters reveal the significant difference at P<0.05 level while same letters reveal the non-significant difference at P<0.05 level.

tissue. According to Rezazadeh *et al*, total polyphenol contents (TPC) and total flavonoid (TFC) contents were the highest in *Cynara scolymus* leaves of moderate saline region (6.45-6.9 dS/m) which are consistent with the findings of our present study [24]. Total saponins of *Plantago ovata* seed were significantly increased with the enlargement of NaCl in the medium [25]. Moreover, in the seeds of *Cichorium intybus*, the mean of saponin increased progressively by increasing NaCl concentration. The maximum saponin was greater by 25% than the control under the highest NaCl concentration (150mM) [26]. Results of the quantitative analysis showed that J*atropha curcas* leaves produced higher amount of saponin and alkaloid, flavonoid, steroid and tannin production were decreased under drought and salinity stresses [27].

Conclusion

The current investigation indicates that the barks of S*onneratia caseolaris* could serve as a source of natural antioxidants. When grown in moderate saline zones of Sundarbans, an effective antioxidant system was found with high phenolic contents of its bark. Salinity in *S. caseolaris* is linked with increased production of phenolic antioxidants which presents a potential to utilize saline resources by growing halophytes of high economic values. There might have genetic causes or other environmental factors except for salinity which may also contribute to these varieties of antioxidant contents. Further studies should be conducted to explore the actual causes which are responsible for these changes of antioxidant activity in *S. caseolaris* barks of HS, MS and LS zones of Sundarbans.

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