Bangladesh Pharmaceutical Journal ***19****(2): 147-151, 2016*

Antioxidant and Membrane Stabilizing Activities of Bark of *Sonneratia apetala*

Md. Emdadul Hasan Mukul[1,2], Mohammad Salim Hossain[1], Sayed Koushik Ahamed[1,3], Pankaj Debnath[1] and Mariyam Akter[1]

[1]Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh
[2]Department of Pharmacy, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh
[3]Department of Pharmacy, Comilla University, Comilla, Bangladesh

Received: March 06, 2016; Accepted: April 10, 2016; Published (Web): July 31, 2016

Abstract

This study was carried out to investigate the antioxidant activity in terms of free radical scavenging capacity and membrane stabilizing ability of methanol extract of bark of *Sonneratia apetala*. The total phenol content of the extract was measured by Folin-Ciocalteu reagent. The antioxidant potential was investigated using 2,2 diphenylpicrylhydrazyl (DPPH), reducing power assay and chelating power determination. The membrane stabilizing capacity was assessed by monitoring the hypotonic solution - and heat-induced haemolysis of human erythrocytes. The total phenolic content was found 50.75 mg/gm of gallic acid equivalent. The extract exhibited significant antioxidant activity in DPPH free radical scavenging assay with IC_{50} value of 81.42 µg/ml as compared to the standard, BHT (IC_{50}=42.56 µg/ml), Fe^{2+} ion reducing power assay and chelating power ability. In case of assay for chelating ability, EDTA represented 98.76% chelation while the plant extract showed 82.83% at concentration of 100 µg/ml. In addition, the methanol extract of bark of this plant was found to moderately inhibit the haemolysis of human erythrocyte. In conclusion, the methanol extract of *S. apetala* could be valuable candidate for future development for antioxidant activity.

Key words: *Sonneratia apetala*, Phenolic content, Free radical scavenging activity, Chelation, Reducing power.

Introduction

During the oxidative stress, various reactive oxygen species (ROS) like superoxide, hydroxyl and peroxyl radicals are generated. These ROS play important role for the pathogenesis of several health problems like cancer, Alzhimer's disease (Ames, 1983; Gey, 1990; Smith *et al*., 1996), cellular aging (Finkel, 2000), diabetes (Stadler, 2012), and inflammation (Aruoma, 1998). Thus minimizing the effect of these free radicals is important for the maintaining a healthy life (Davies, 2000). Human beings are using plants to treat various illnesses for thousand of years and discovery of new medicines from plant sources has been always a major area of research (Samuelsson, 2004). Antioxidants from natural sources have gained benefits of having better efficacy with less side effects.

Sonneratia is a plant genus of plants having 20 identified species in the family of Lythraceae. *S. apetala* Buch.-Ham. is found in Bangladesh, Sri Lanka, Myanmar, and India, including the Andaman and Nicobar islands. It has been introduced into Fujian and Guangdong provinces of China. *S. apetala* is a highly adaptable, fast growing plant that acts as a pioneer species in ecological succession in many degenerated mangrove forests (Chen *et al.*, 2003). The fruits of *S. apetala* are extensively consumed by the coastal people of Bangladesh for antidiabetic, antibacterial and antioxidant capacities (Hossain *et al*, 2013). Thus, we aimed to study the total phenolic content and free radical scavenging activity related to antioxidant capacity and membrane stabilizing ability of the bark of

Correspondence to: Mohammad Salim Hossain; Tel: + 88-01711-200-410; Email: pharmasalim@yahoo.com

S. apetala and we, here in, report the results of our preliminary investigations.

Materials and Methods

Drugs and chemicals: 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, quercetin and gallic acid were obtained from Sigma Chemical Co (MO, USA). Folin- ciocalteu reagent (FCR) was purchased from Merck, Germany. All other chemicals and reagents were of analytical grade.

Extraction: The plant *S. apetala* is locally known as kewra. The bark of *S. apetala* was collected from Sundarban, in February, 2013 with the help of local people. A specimen has been kept in our laboratory for future reference. After drying and crushing, the powdered material (500 gm) was fully soaked into methanol (2.5L) and allowed to stand for 15 days with occasional shaking and stirring. The extract was filtered and solvents were evaporated to dryness.

Determination of total phenol content: Total phenol content of *S. apetala* extract was measured as per published method by employing Folin-Ciocalteu reagent (Foiln and Ciacalteu, 1927). Total phenolic content in the plant extract was expressed in terms of gallic acid equivalent (mg/gm of dry mass), which is a common reference compound.

DPPH free radical scavenging activity: The DPPH oxidative assay is used worldwide in the quantification of radical scavenging capacity. The antioxidant activities of plant extracts and the standard were assessed on the basis of the free radical scavenging effect of the stable DPPH free radical activity described by Monzarro *et al* (1998). Here, BHT was used as standard. The following equation was used to calculate the scavenging activity.

DPPH scavenged (%) = $\{(A_{control} - A_{test})/ A_{control}\}$ x 100

where, $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the test sample.

Reducing power assay: The reducing power of the extract was determined by the method described by Benzie and Strain (1999). Briefly, various concentrations of the plant extract in 1.0 ml deionized water were mixed with potassium ferricyanide (2.5 ml) in phosphate buffer and incubated for 20 min at 50^{O}C. Trichloroacetic acid (2.5 ml) was added to this mixture and allowed to centrifuge at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared ferric chloride solution (0.5 ml) and the absorbance was measured at 700 nm using ascorbic acid as standard. Reducing power was calculated by using the following equation:

% Increase in the reducing power =

$\{(A_{test}/A_{blank})-1\}$ x 100

Chelating power ability: The chelation of ferrous ions by extracts was estimated by method of Dinis *et al.* (1986). Briefly, 50 µl of 2 mM $FeCl_2$ was added to 1 ml of various concentrations of extract. The reaction was initiated by addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigoriously shaken and allowed to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. Here EDTA was used as standard. The chelating power was determined by using the following equation:

% Inhibition of ferrozine–Fe^{2+} complex =

$[(A_0 - A_s)/A_s]$ x 100

where, A_0 was the absorbance of the control, and A_s was the absorbance of the extract.

Membrane stabilizing activity: The membrane stabilizing activity was assessed using hypotonic and heat-induced hemolysis of human erythrocyte by the method developed by Shindhe *et al.* (1999) with slight modification by Sikder *et al.* (2011). The result was compared with standard, acetyl salicylic acid.

Result and Discussion

Antioxidants play important roles in the defense mechanism of human body and help to minimize the oxidative stress caused by the reactive oxygen species (Davies, 2000). There is growing interest in the antioxidant activity of plant materials. Estimation of total phenolic contents is the first step to determine the antioxidant capacity of plant extract. Here we assessed

the total phenolic content of the extractive, which was found to be 50.75 mg of GAE/gm.

Several studies have indicated that the antioxidant activities of some plants are highly correlated with their total phenolic contents (Palave *et al.,* 2006; Gupta *et al.,* 2010). Taking this into consideration, the antioxidant activity of this extract was assessed. The results are presented in figure 1.

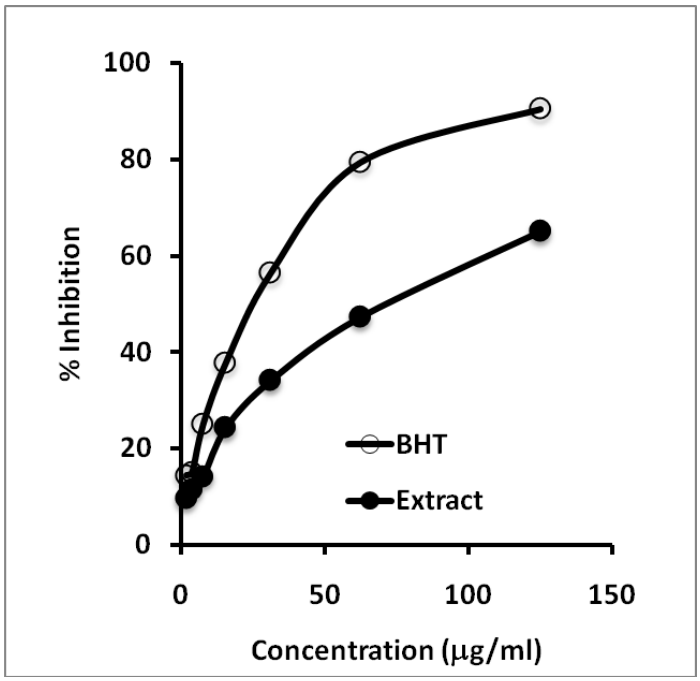

Figure 1. DPPH scavenging activity of bark of *S. apetala.*

In case of DPPH scavenging activity, IC_{50} value of the extractive was 81.42 µg/ml, whereas the standard BHT showed IC_{50} value of 42.56 µg/ml. The linearity range of calibration curve was 2 µg/ml to 125 µg/ml (R^2 =0.939 for extract and R^2 = 0.851 for BHT). Although, the extent of inhibition was less than that of BHT, the test extractive was shown to have a good DPPH scavenging activity.

Furthermore, as the Fe^{+2} reducing power is also considered to be a parameter to address the antioxidant activity, the reducing power capability of the extractive was estimated. The results presented in figure 2 shows that the reducing power of the extractive was seen to increase as a function of concentration. The reducing power of the extract was compared with ascorbic acid. Although, at lower concentration, the extract showed the fairly similar absorbance with that of the standard, it was evident that the extract was less potent that ascorbic acid to reduce the Fe^{+2} at higher concentration.

Moreover, another mechanism of antioxidative action is chelation of transition metals, thus preventing catalysis of hydroperoxide decomposition and Fenton type reactions (Gordon, 1990). In the presence of chelating agents, the complex formation is disrupted with the reduction of the red color of the complex. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals (Aboul-Enein *et al.,* 2003). The result of chelation assay is presented in figure 3. At concentration of 5µg/ml, EDTA showed 36.97% chelating ability, where as the plant extract displaced 24.08%. On the other hand, at 100 µg/ml concentration, EDTA represented 98.76% chelation, as compared to 82.83% by the plant extract. All these data agree with the strong chelating ability of bark of *S. apetala*.

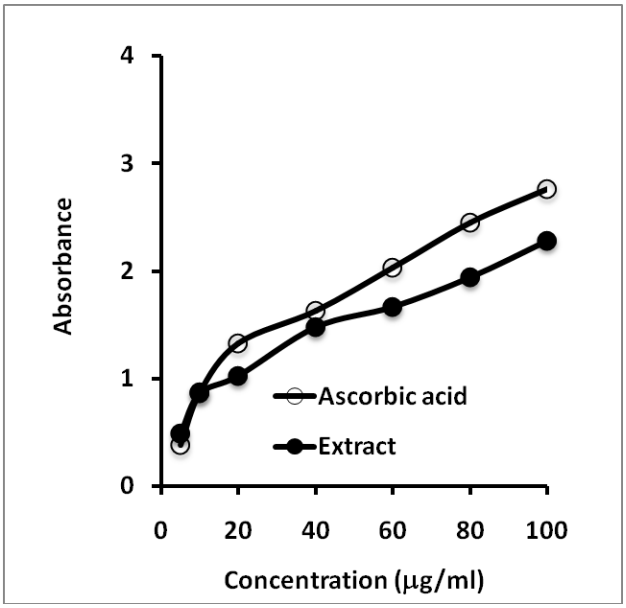

Figure 2. Reducing power assay of *S. apetala.*

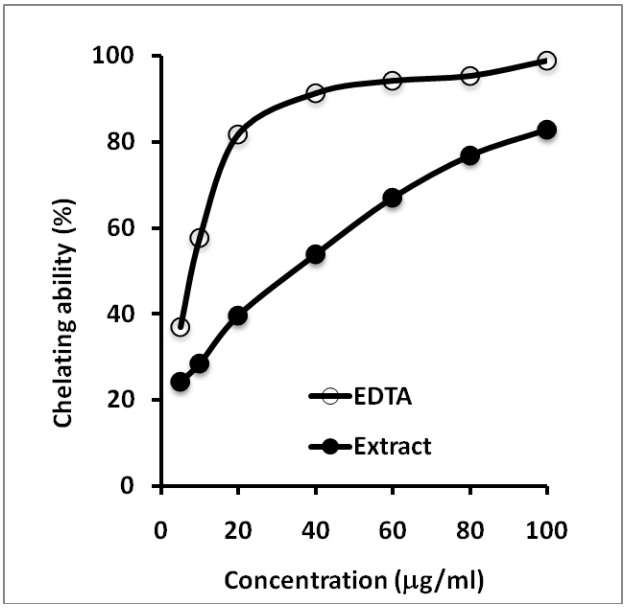

Figure 3. Chelating ability of bark of *S. apetala.*

The anti-inflammatory potentials of the crude extract was assessed through the membrane stabilizing activity by using acetyl salicylic acid as positive control. Membrane stabilization is well studied as a mechanism of anti-inflammatory response (Shinde *et al.*, 1999). Our data (Table 1) revealed that the methanolic extract of *S. apetala* has moderate inhibitory

Table 1. Membrane stabilizing effect of bark of *S. apetala.*

Treatment	% Inhibition of haemolysis	
	Hypnotic solution induced	Heat induced
Acetyl salicylic acid (Standard)	77.3 ± 1.45	61.5 ± 0.46
Extract of *Sonneratia apetala*	56.0 ± 1.76	42.5 ± 1.45

capacity of haemolysis of erythrocyte in hypotonic solution- and heat-induced conditions. As erythrocyte membrane is similar to lysosomal membrane components, it is extrapolated that the drugs which stabilizes erythrocyte membrane can also stabilizes lysosomal membrane (Omale *et al.*, 2008) and thus interfere with the release and or action of mediators responsible for inflammation like histamine, serotonin, prostaglandins, etc. (Shinde *et al*., 1999). Thus, the test extract may be considered to be moderately effective to inhibit the release of inflammatory mediators.

Conclusion

The present study suggests that, the methanolic crude extract of bark of *S. apetala* possesses potential free radical scavenging, reducing power, chelating abilities and moderate inhibitory effect on membrane stabilization. Further studies are necessary to isolate the specific active compounds from the bark of *S. apetala* and elucidate the mechanism of action.

Acknowledgement

The research was partly supported by a grant (BS 142/2012) obtained from Ministry of Science and Technology, Government of The Peoples' Republic of Bangladesh.

References

Aboul-Enein, A.M., El Baz, F.K., El-Baroty, G.S., Youssef, A.M., and Abd El-Baky, H. H. 2003. Antioxidant activity of algal extracts on lipid peroxidation. *J. Med. Sci*. **3**, 87-98.

Ames, B.N. 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science*. **221**, 1256-1264.

Aruoma, O.I. 1998. Free radicals, oxidative stress and antioxidants in human health and disease. *J. Am. Oil. Chem. Soc.* **75**, 99-212.

Benzie, I.F.F. and Strain, J.J. 1999. Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.* **299**, 15–27.

Chen, Y., Liao, B., Peng, Y., Xu, S., Zheng, S., and Chen, D. 2003. Researches on the northern introduction of mangrove species *Sonneratla apetala* Buch.-Ham. *Guangdong Forest*. **19**, 9-12.

Dinis, T.C.P., Madeira, V.M.C., and Almeida, M.L.M. 1986. Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.* **315**, 161-169.

Davies, K.J.A. 2000. Oxidative stress, antioxidant defenses and damage removal, repair and replacement systems. *IUBMB Life.* **50**, 279-289

Finkel, T. 2000. Oxidants, oxidative stress and the biology of aging. *Nature* **408**, 239-248.

Folin, O. and Ciocalteu, V. 1927. On tyrosine and tryptophane determination in proteins. *J. Biol. Chem.* **27**, 627-650.

Gey, K.F. 1990. The antioxidant hypothesis of cardiovascular disease: epidermiology and mechanisms. *Biochem. Soc. Trans.* **18**, 1041-1045.

Gordon, M.H. 1990. The mechanism of the antioxidant action *in-vitro*. In: B.J.F. Hudson (Ed.), Food Antioxidants. Elsevier London/New York, pp. 1-18.

Gupta, P.K., Langridge, P., and Mir, R.R. 2010. Marker-assisted wheat breeding: present status and future possibilities. *Mol. Breed.* **26**, 145-161.

Hossain , S.J., Basar, M.H., Rokeya, B., Arif, K.M.T., Sultana, M.S., and Rahman, M.H. 2013. Evaluation of antioxidant, antidiabetic and antibacterial activities of the fruit of *Sonneratia apetala* (Buch.-Ham.) *Oriental Pharm. Exp. Med.* **3**, 95-102.

Manzorro, L., Anese, M., and Nicoli, M.C. 1998. Antioxidant properties of tea extracts as affected by processing. *Lebens- mittel-Wissenschaft Und-Technol.* **31**, 694-698.

Omale, J., and Okafor, P.N. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata. Afr. J. Biotechnol.* **7**, 3129-33.

Palave, Y.K., and D'mello, P.M. 2006. Standardization of selected Indian medicinal herbal raw materials containing polyphenols as major phytoconstituents. *Ind. J. Pharm. Sci.* **68**, 506-509.

Samuelsson, G. 2004. Drugs of Natural Origin: A Text of Pharmacognosy. 4th revised ed., Swedish Pharmaceutical Press, Stockholm, Sweden, pp. 417-429

Shindhe, U.A., Phadke, A.S., Nair, A.M., Mungantiwar, A.A., Dikshit, V.J. and Saraf, M.N. 1999. Membrane stabilizing activity - a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia.* **70**, 251-257.

Sikder, M.A., Rahman, M.A., Kaisar, M.A., Rahman, M.S., Hasan, C.M. and Rashid, M.A. 2011. *In vitro* Antioxidant, reducing power, free radical scavenging and membrane stabilizing activities of seeds of *Syzygium cumini* L. *Lat. Am. J. Pharm*. **30**, 781-785.

Smith, M.A., Perry, G., Richey, P.L., Sayre, L.M., Anderson, V.E., Beal, M F. and Kowal, N. 1996. Oxidative damage in Alzheimer's disease. *Nature* **382**, 120-121.

Stadler, K. 2012. Oxidative stress in diabetes. *Adv. Exp. Med. Biol.* **771**, 272-287.