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ORIGINAL ARTICLE

Screening of Salt-stress, Antioxidant Enzyme, and Antimicrobial Activity of Leave extracts of Mangroves *Avicennia marina* L. from Hodaidah, Yemen

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Key words: Salinity, Antioxidant enzymes, Antimicrobial effect, Mangrove, Avicennia marina

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Key words: Salinity, Antioxidant enzymes, Antimicrobial effect, Mangrove, Avicennia marina

Mangroves are evergreen woody plants that grow at the interface between land and sea, and form the dominant intertidal vegetation in tropical and subtrobical regions (Blasco, 1984). These plants, and the associated microbes, fungi, plants, and animals, constitute the mangrove forest community or mangal (Tomlinson, 1994). Mangroves trees are characterized with broad leaves, aerial roots, like pneumatophores or stilt roots, and viviparous germinated seedlings (Naskarand Mandal, 1999).

Area of mangrove habitats in Yemen was estimated to be approximately 22.55 km^2 (Nagi *et al.*, 2012). Yemen's coastal and marine ecosystems which include extensive mangroves such as *Avicennia marina* and *Rhizophora mucronata* that is only growing in two locations (Nagi and Abubakr, 2013), coral reefs, and sea grass areas are of major economic importance for fisheries and tourism. *A. marina* is a mangrove tree species that is extraordinarily adaptable with a wide latitudinal range closely associated with its flexible growth

pattern (Vioux-Chagnoleau *et al.*, 2006).

Mangrove habitats along with their associated biota are of extreme importance, as a large coastal population depends on these resources for their livelihood. They have been used as traditional medicine in South Asian countries including Yemen (EPA, 2009). Recently, it has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values (Bandarnayake, 2002). *Avicennia marina* (Forssk.) Vierh. (Avicenniaceae) has received some attention in determining its important chemical constituents. Phenolic compounds are secondary plant metabolites and are involved in a wide range of specialized physiological functions. They are very important for the normal growth, development and defense mechanisms of plants (Miles *et al.*, 1998). These compounds are capable of inhibiting free radicals, and hence can retard the aging process (Maisuthisakul *et al.*, 2007).

Extracts and chemicals from mangroves are used mainly in folkloric medicine (*e.g.* bush medicine), as insecticides and pesticides and these practices continue to this day (Natarajan *et al.*, 2003). Methanol extract of *Excoecaria agallocha* leaves and shoots (Ravikumar *et al.*, 2010) and antifungal activity of methanol extract of *E. agallocha* and *Bruguiera gymnorrhiza* trunks are some other examples of pharmaceutical potential of mangrove plants.

There are no reports on salinity stress, antioxidant and antimicrobial activities of *A. marina* in Yemen, the present study was designed and carried out based on the reasons mentioned above. In this study, *A. marina* leaf extract was examined to evaluate the antioxidant potential and the same crude extracts were also used to indicate the antimicrobial activities of the samples.

MATERIALS AND METHODS

Preparation of plant extract

Mangrove leaves of *A. marina* were collected from Al Rage'ah Island (14° 54′ 53″ N and 42° 55′ 39″ E) which is located in front of Al Gabbanah village north of Al Hodaidah city. The collected leaf samples are air dried and then ground with sterilized distilled water. After grinding, filtration process conducted and the filtrate collected with Whatman No.1 filter paper and evaporated at 40°C for 48 hours. The residue was preserved for experimental analysis.

Plant materials and culture conditions

The preliminary experiments were carried out on A. *marina* at different concentrations of NaCl (50, 120, 190, 260, 330, 400, and 500 mM) for 0, 6, 12, 18, 23, 35, and 50 days in order to determine the viable range of salinities. Since, 500 mM NaCl was found lethal for this plant and at 330 mM NaCl, the plant can survive for more than 25 d, therefore, 330 mM NaCl was chosen as suitable concentration for investigating long-term effect of salinity in *A. marina*. The cultures were aerated continuously with an air bubbler. Leafs of plant from different days of treatment were collected to measure the experimental parameters and the experiments were repeated twice with three replicas in each treatment.

Extraction and estimation of total leaf protein

Total leaf protein was extracted by the acetone–TCA precipitation method as described earlier by (Damerval *et al.*, 1986) and estimated following the method of (Lowry *et al.*, 1951). Bovine serum albumin was used as standard protein.

Analysis of protein profile of leaf by SDS-PAGE

Leaf samples were harvested from control and

NaCl treated plants after 7, 14, 30 and 45 days of treatment for analysis of soluble protein by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). Samples (0.5 g) were homogenized with 2 ml of a buffer containing 50 mm Tris(hydroxymethyl) aminomethane (Tris)-Glycine (pH 8.3), 0.5 m sucrose, 50 mm EDTA, 0.1 m KCl, 2 mm PMSF and 0.1% (v/v) 2-mercaptoethanolin a chilled pestle and mortar at 4 ^{0}C. The homogenate was centrifuged in a refrigerated centrifuge at 14,000r.p.m for 10 min. Protein concentration in the supernatant samples was estimated according to the method of (Laemmli, 1970). The supernatants were stored in small aliquots at 85 ^{0}C for SDS-PAGE. Supernatant samples (40 μg protein) were mixed with equal volumes of solubilizing buffer [62.5 mm Tris-HCl, pH 6.8, 20% (w/v) glycerol, 2%(w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.01% bromophenol blue] and heated for 4 min at 95 ^{0}C, cooled on ice before loading on 12.5% polyacrylamide slab gels. Gels were made according to (Laemmli, 1970).

A 10 % separating gel containing 375 mm Tris-HCl, pH 8.8, 0.1% (w/v) SDS, 0.05% (w/v) ammonium persulfate and 0.4 μl·TEMED was used for resolving the polypeptides whereas a 4% stacking gel containing 125 mm Tris-HCl, pH 6.8.

Extraction of antioxidative enzymes and their assays

Extraction of enzymes and assays:

Two grams of young leaf buds were macerated to powder with liquid nitrogen with a mortar-pestle; then 0.1 g PVP and 5 ml of extraction buffer (consisting of 1 M Sucrose, 0.2 M Tris-HCL and 0.056 M β-Marcaptoethanol; pH adjusted at 8.5) was added and homogenized. The extractants were centrifuged at 10,000 rpm for 20 min at 48^{0}C. The supernatant were collected and used for the assay of catalase (CAT), peroxidase (POX) and guaicol peroxidase (GPX).

Estimation of Catalase (CAT)

Catalase was measured according to (Sadasivam and Manickam, 1991) by change in absorbance at 240 nm. An assay mixture contained 3 ml H_2O_2 phosphate buffer (0.64 ml of H_2O_2 diluted to 100 ml with 0.1M phosphate buffer pH 7.0) and 0.2 ml enzyme extract. The amount of enzyme required to change the absorption (Δ OD) by 0.01 min^{-1} mg^{-1} protein was taken as unit enzyme activity.

Estimation of Peroxidase (POX)

Peroxidase activity was measured according to (Maehly and Chance, 1954) by following the change in absorbance at 470 nm due to guiacol oxidation in the presence of H_2O_2 and enzyme. The assay mixture 2 ml 0.1M phosphate buffer (pH 7.0), 1 ml 20 mM guaiacol, 0.05 ml H_2O_2 (20 mM) and 0.5 ml enzyme extract. The amount of enzyme required to change the absorption (Δ OD) by 0.01 min^{-1} mg^{-1} protein was taken as unit enzyme activity.

Estimation of guaiacol peroxidase (GPX)

GPX activity was measured spectrophotometrically at 25^{o}C by following the method of (Tatiana *et al.*, 1999). The reaction mixture (2ml) consisted of 50 mM potassium phosphate (pH 7.0), 2 mM H_2O_2, and 2.7 mM guaiacol. The reaction was started by the addition of an enzyme extract equivalent to 5 µg protein. The formation of tetra guaiacol was measured at 470nm (ε=26.6 $mM^{-1}cm^{-1}$).

Screening for antimicrobial activity of *Avicennia marina* Extracts

The inhibition activity on bacteria (*Escherichia coli* ATCC8739, *Staphylococus aureus* ATCC 6538, *and Bacillus subtilis* ATCC6633) and fungus

(*Candida albicans* ATCC 2091, and *Aspergillus niger* ATCC 16404) Were tested using Agar well diffusion method (He and Zhou, 2007). The 1 mL of bacterial and fungus cultured at equal turbidity of McFarland No.0.5 was swab and placed into the surface of Mueller-Hinton Agar. The agar media was punctured into 6 holes per each culture plates of 0.5 cm diameter. 5, 10, 15, 20, 25, and 30µl of *A. marina* extracts were poured into 5 holes of agar and another hole was used as control (without the *A. marina* extract). The culture plates were incubated Finally, and the diameters of inhibition zone (DIZ) were measured in millimeter (mm). The experiment was repeated three times and the mean values were presented. Data were expressed as means ± standard deviations (SD) of triplicate experiments.

RESULTS

Changes in total leaf soluble protein content

Total soluble protein content in *A. marina* decreased upon exposure to 500mM NaCl for a short period of 7 days (Figure 1). Total soluble leaf protein content in *A. marina* decreased 3.4 fold after 7 days of salt treatment as compared to control.

Changes in leaf protein SDS-PAGE profiling

The leaf protein was extracted from control and salt treated plant leaves samples and analyzed by SDS-PAGE (Figure 2). SDS-PAGE analysis of total protein profiling indicated that no differences were found in number of protein band upon exposure to salt treatment. But the intensities of several protein bands having apparent molecular mass by reduced severely in salt treated samples compared to control in *A. marina.*

Effect of salinity on antioxidant enzymes

The effect of NaCl on the catalase, guaiacol peroxidase, and peroxidase activities in the leaves at 500 mM NaCl concentration in different days of treatment is presented in Table 1. There was a steady increase in all the enzyme activities in *A. marina.*

Antimicrobial activity assay

The result of antimicrobial activity of *A. marina* extracts was presented in Table 2 and Figure 3. Extract of leaves showed antimicrobial activity against *E. coli*, *S. aureus,* and *B. subtiliss,* and antifungal activity against the growth of *C. albicans* and *A. niger*.

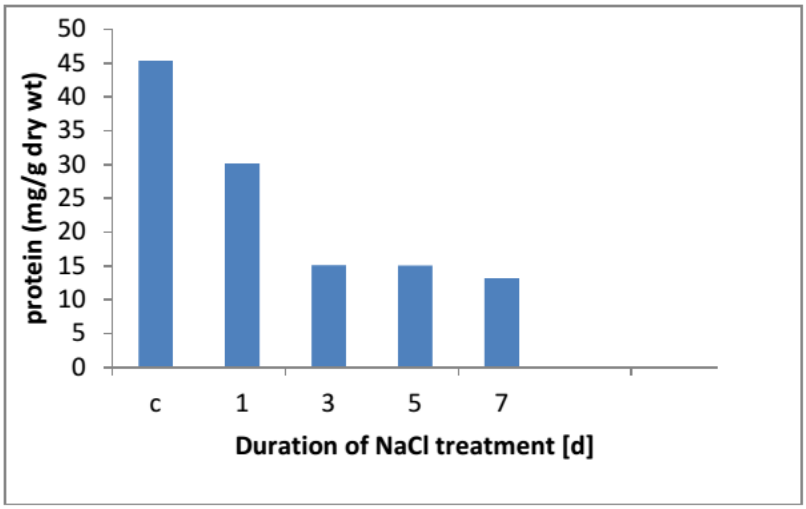

Figure 1: Effect of NaCl on total soluble proteins in leaves of *Avicennia marina* in different days for 500mM NaCl treatment.

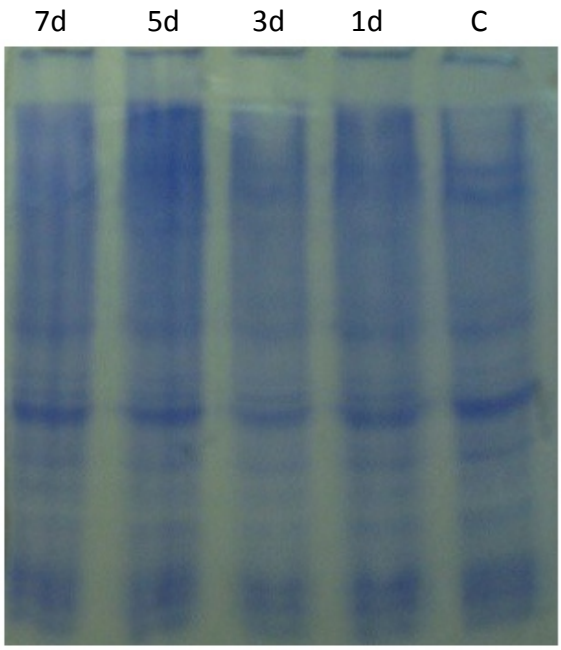

Figure 2: Effect of NaCl on total soluble proteins in leaves of *Avicennia marina* in different days for 500mM NaCl treatment.

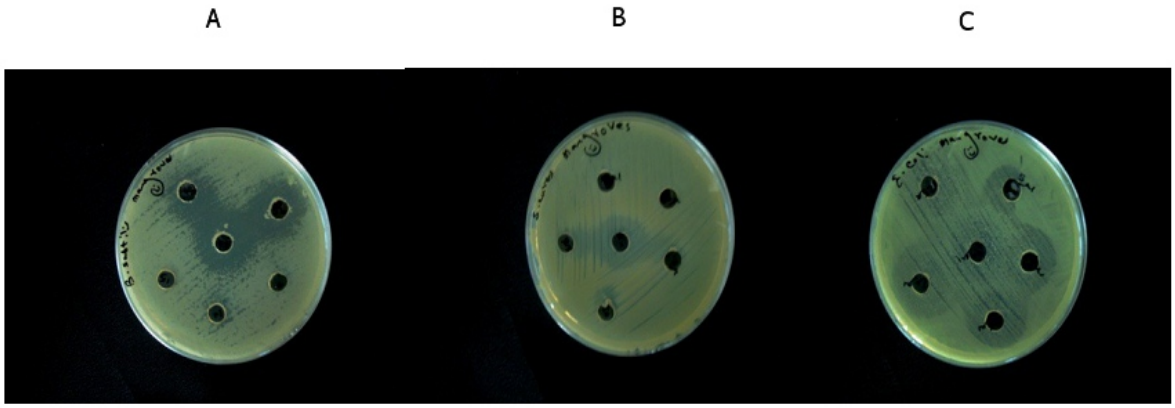

Figure 3: Anti-microbial activity of different levels from leave extract of *Avicennia marina* (**A**) with *Escherichia coli.* (**B**) with *Staphylococus aureus*. (**C**) with *Bacillus subtilis.* (Antifungal activity not shown)

Table 1. Effects of 500 mMNaCl treatment on enzyme GPX, POX, CAT and Protein content in leaves of *Avicennia marina* in different days of treatment

	Control	500mM NaCl			
		1d	3d	5d	7d
Protein (mg)	45.41	30.20	15.13	15.13	13.22
CAT U/mg of protein	0.173±0.113	0.186±0.013	0.224±0.012	0.276±0.011	0.332±0. 113
POX U/mg of protein	1.034±0.023	1.265±0.043	1.454±0.054	1.856±0.013	2.234±0.003
GPX U/mg of protein	2.122±0.003	2.321±0.003	2.445±0.002	3.765±0.001	4.683±0.002

Table 2: Anti-microbial activity of different levels from leave extract of *Avicennia marina*.

Microorganisms	levels of Mangroves extract					
	30 µl	25 µl	20 µl	15 µl	10 µl	5 µl
	Inhibition zone, mm					
Escherichia coli ATCC8739	12	11	10	8	7	4
Staphylococus aureus ATCC 6538	6	0	0	0	0	0
Bacillus subtilis ATCC6633	7	6	5	0	0	0
Candida albicans ATCC 2091	9	8	6	0	0	0
Aspergillus niger ATCC 16404	10	9	8	0	0	0

DISCUSSION

The total protein content of leaf gradually decreased with increasing concentration of NaCl. This decrease in protein content might be due to stimulation of protein hydrolysis (Uprety and Sari, 1976), or the increasing activity of acid and alkaline proteases. As reported earlier, levels of free amino acid increase as a result of salt stress in *B. parviflora* (Parida *et al.*, 2002).

In this study, three antioxidant enzymes were estimated, *i.e.* CAT, POX, and GPX. A quantitative study of these enzymes from saline and fresh water grown plants revealed that enzymes activity were higher in salt-stressed plant.

Increase in CAT activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of H_2O_2 produced during cell metabolism and protection against oxidative (Bor *et al.*, 2003). (Takemura *et al.*, 2000) reported an inductive response in CAT activity in the mangrove *B. gymnorrhiza* under salt stress. Similarly in the present study, the salt induced enhancement of CAT activity in *A. marina* may suggest its effective scavenging mechanism to remove H_2O_2 and imparting tolerance against salinity induced oxidative stress.

POX activity of *A. marina* was increased with increasing salt concentrations. This result agrees with *Aegiceras corniculatum* (Manikandan and Venkatesan, 2004). The enzyme POX involves in the decomposition of cosubstrates such as phenolic compounds and/or antioxidants. GPX activity also increases when exposed to salt stress. Exposure to high NaCl imposes oxidative stress due to changes in the osmotic and ionic environment in plant (Allakhverdiev *et al.*, 2000).

It is important to study scientifically plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds (Hammer *et al.*, 2001). Plants are employed as important source for traditional medications (Neves *et al.*, 2009).

Mangrove plant *A. marina* extracts showed antimicrobial activity against *E. coli*, *S. aureus*, and *B. subtilis*. The *A. marina* specie studied, was effective against *E. coli, S. aureus* and *B. subtilis*at (5, 10, 15, 20, 25, 30µl), (30 µl) and (20, 25, 30 µl), respectively. The inhibition zone was found to be better in *E. coli*. The result for antibacterial activity agrees with many leaves extract of mangrove, (Imdadul *et al.*, 2011a; Natarajan *et al.*, 2011; Imdadul *et al.*, 2011b).

Antifungal metabolites mangrove plant leaves include alkaloids, flavonoids and related

compounds, fatty acids, oxygen heterocyclics, proanthocyanidins, quinones, stilbenes, terpenoids and triterpenoid, saponins. These compounds have toxicological characteristics, such as anti-fungal activity (Bandaranayake, 2002). The antifungal activity was effective against fungus at (20, 25, 30µl) for both *C. albicans* and *A. niger.* Different levels of mangrove extract have been used to consider its antimicrobial effect. Fatty acids are widely occurring compounds in natural fats and dietary oils, and they are known to have antibacterial and antifungal properties (Agoramoorthy *et al.*, 2007; Shelar *et al.*, 2012).

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REFERENCES

Agoramoorthy, G., Chandrasekaran, M., Venkatesalu, V., and Hsu, M. (2007) Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Brazilian J. Microbio.,* **38(4)**, 739-742.

Allakhverdiev, S.I., Sakamoto, A., Nisihiyama, Y., Inaba, M. and Murata, N. (2000) Ionic and osmotic effects of NaCl induced inactivation of photosystems I and II in *synechococcus sp. Plant Physiol.*, **123**, 1047-1056.

Arivuselvan, N., Silambarasan, D., Govindan, T. and Kathiresan, K. (2011) Antibacterial Activity of Mangrove Leaf and Bark Extracts Against Human Pathogens. *Advan. Biol. Res.,* **5(5)**, 251-254.

Bandaranayake, W.M. (2002) Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wet. Ecol. Manage.,* **10(6)**, 421-452.

Blasco, F. (1984) Climatic factors and the biology of mangrove plants. In Snedakar, S. C. and Snedakar, J. G. (Eds.), *The mangrove ecosystem: research methods*. UNESCO, Bungay, UK, pp. 18-35.

Bor, M., Ozdemir, F. and Turkan, I. (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. *Plant Sci.*, **164**,77-84.

Damerval, C., Vienne, P., Zivy, M., and Thiellement, H. (1986) Technical improvement in two-dimensional electrophoresis increase the level of genetic variation detected in wheat seedling proteins. *Electrophoresis*, **7**, 52-54.

EPA (2009) Assessing Progress towards the 2010 Target - The 4th National CBD Report. Ministry of Water and Environment, the Republic of Yemen.

Hammer, K.A., Carson, C., and Riley, T. (2001) Antimicrobial activity of essential oils and other plant extracts. *J. Appli. Microbio.*, **86(6)**, 985-990.

He, F. and Zhou, J. (2007) A new antimicrobial susceptibility testing method of *Escherichia coli* against ampicillin. *J. Microbio. Meth.*, **68(3)**, 563-567.

Imdadul, H., Wirakarnain, S., Koshy, P., Arash, R., Shariff, H.A.B.M. and Mat, T.R. (2011) Valuable antioxidant and antimicrobial extracts from *Rhizophora Mucronata* of Asiatic mangrove

forests. *Res. J. Biotech.*, **6(1)**, 10-14.

Imdadul, H., Wirakarnain, S., Shariff, H.A.B.M., Mat, T.R. and Monneruzzaman, K.M. (2011b) Total phenolic contents, antioxidant and antimicrobial activities of *Bruguiera gymnorrhiza*. *J. Med. Plant. Res.,* **5(17)**, 4112-4118.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.*, **193**, 265-275.

Maehly, A.C. and Chance, B. (1954) Methods of biochemical analysis, Vol. I. (Glick, D. ed.). Interscience Publishers Inc., New York, pp. 357-424.

Maisuthisakul, P., Suttajit, M. and Pongsawatmanit, R. (2007) Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants, *Food Chem.,* **100**, 1409-1418.

Manikandan, T. and Venkatesan, A. (2004) Influence of NaCl on growth, organic constituents and certain antioxidant enzymes of *Aegiceras corniculatum* Blanco. *Geobios.*, **31**, 30-33.

Miles, D.H., Kokpol, U., Chittawong, V., Tip-Pyang, S., Tunsuwan, K. and Nguyen, C. (1998) Mangrove forests-The importance of conservation as a bioresource for ecosystem diversity and utilization as a source of chemical constituents with potential medicinal and agricultural value. IUPAC, **70(11)**, 1-9.

Nagi, H.M. and Abubakr, M.M. (2013) Threats status to mangrove ecosystem along the coastal zone of Yemen. *J. King AbdulAziz Uni. – Mar. Sci.*, **24(1)**, 101-117.

Nagi, H.M.; Khanbari, K.M. and Al-Sameh, A. (2012) Estimating total area of mangrove habitats in the republic of Yemen using remote sensing and GIS. *Fac. Sci. Bull.*, **24**, 75-84.

Naskar, K. and Mandal, R. (1999) *Ecology and biodiversity of Indian mangroves; Part I: Global status*. Daya Publishing House, Delhi.

Natarajan, V., Venugopal, P., Menon, T. (2003) Effect of *Azadirachtaindica* (neem) on the growth pattern of dermatophytes. *Indian J. Med. Microbio.*, **21(2)**, 98-101.

Neves, J.M., Matos, C., Moutinho, C., Queiroz, G. and Gomes, L.R. (2009) Ethnopharmacological notes about ancient uses of medicinal plants in Tras-os-Montes (northern of Portugal). *J. Ethnopharmacol.*, **124(2)**, 270-283.

Parida, A., Das, A.B., and Das, P. (2002) NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J. Plant Biol.*, **45**, 28–36.

Ravikumar, S., Gnanadesigan, M., Suganthi, P. and Ramalakshmi, A. (2010) Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. *Inter J. Med. Sci.*, **2(3)**, 94-99.

Sadasivam, S. and Manickam, A. (1991). *Biochemical methods for agricultural sciences*. Wiley Eastern Limited, New Delhi.

Shelar, P.S., Reddy, S.V.K., Shelar, G.S. and Reddy, V.S. (2012) Medicinal value of mangroves and its antimicrobial properties – a review Continental. *J. Fish Aquatic Sci.,* **6(1)**, 26-37.

Takemura, T., Hanagata, N., Sugihara, K., Baba, S.,

Karube, I. and Dubinsky, Z. (2000) Physiological and biochemical responses to salt stress in the mangrove, *Bruguiera gymnorrhiza*. *Aquat. Bot.*, **68**, 15–28.

Tatiana, Z., Yamashita, K., Matsumoto, H. (1999) Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber roots. *Plant Cell Physiol*., **40**, 273-280.

Tomlinson, P.B. (1994) *Thebotany of mangroves*. Cambridge University Press, New York, USA.

Uprety, D.C. and Sarin, M.N. (1976) Physiological studies on salt tolerance in *Pisumsativum* L. IV, Ionic composition and nitrogen metabolism. *Acta Agron. Hung.*, **25**,455–460.

Vioux-Chagnoleau, C., Lejeune, F., Sok, J., Pierrard, C., Marionnet, C. and Bernerd, F. (2006) Reconstructed human skin: from photodamage to sunscreen photoprotection and anti-aging molecules, *J. Dermatol. Sci*. (Supplement), **2(1)**, S1-S12.