ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(2):348-351

ORIGINAL RESEARCH ARTICLE

Phytochemical Characterization and Antimicrobial Efficiency of Mangrove Plants *Avicennia marina* and *Avicennia officinalis*

R. Shanmugapriya, T. Ramanathan* , G. Renugadevi

Centre of Advanced study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608502, Tamil Nadu, India

Received 28 Dec 2011; Revised 22 Mar 2012; Accepted 29 Mar 2012

ABSTRACT

Mangrove plants have been used in folklore medicines. In the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemical in *Avicennia officinalis* when compared to the *Avicennia marina*. In the DPPH scavenging assay, both the mangrove extracts showed high antioxidant activity. The *Avicennia marina* samples have more effective antioxidant activity when compared to the *Avicennia officinalis*. And the percentage of scavenging was found to be about 89.85% *for Avicennia marina* and 68.67% for *Avicennia officinalis* sample. The rapid TLC assay is considered as the rapid test to evaluate the antioxidant activity of natural compounds. The compounds showing the bands at hRf = >12, 25 and 93 of both the mangrove extracts and hRf = 53 in *Avicennia marina* alone were proved to be having antioxidant activity. The results of antimicrobial activity by the well diffusion assay also clearly expressed that *Avicennia marina* has high concentration of active principles when compared to the *Avicennia officinalis*.

Key words: *Avicennia marina, Avicennia officinalis,* Mangrove plants, alkaloids, anti-atherosclerotic activities, anti-inflammatory.

INTRODUCTION

Mangroves are woody trees and shrubs that grow in the intertidal zones of tropical and sub-tropical regions [1].Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven activity against human, animal and plant pathogens. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance [2]. [3] Had also reported the bioactive compounds from mangrove plants. Some mangrove plants had shown insecticidal activity [4, 5]. [6] Reported the cytotoxic and antiplatelet aggregation activity of methanol extract of *Aglaia elliptifolia*. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins [7]. Antimicrobial activity of plant constituents such as phenol, quinines, flavones, flavonoids, tannins, terpenoids, essential oils and alkaloids have been reported by several authors [8]. There is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases [9]. *A. officinalis* is a commonly available as white mangrove plant in almost all the coastal states of India. It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease, ulcer. A detection of the plant with sugar candy and cumin is used in dyspepsia with acid eructation's [10, 11]. *A. marina* have been shown to exhibit marked inhibitory effect on mouse skin tumor promotion [12], Phenolic compounds such as flavonoids, phenolic acid and tannins possess diverse biological activities such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These activities might be related to their antioxidant activity [13]. Hence in the present study is concerned screening of phytochemicals, antioxidants and antimicrobial properties of *Avicennia marina* and *Avicennia officinalis*.

MATERIALS AND METHODS

The leaves of *Avicennia marina* and *Avicennia officinalis* were collected from the southeast coast of Parangipettai, Tamilnadu, India. The mangrove leaves sample was dried in the shadow; it was

***Corresponding Author:** Dr .T. Ramanathan**, Email:** drtrcasmb@gmail.com, **Phone No:** +91- 9894175200

powdered and stored at room temperature. About 5g of the leaves were extracted with 100 ml of Methanol for 1 week at room temperature. These samples were filtered using Whatmann filter paper and the filtrate was evaporated to dryness under vacuum at 40^0C. Each concentrated extracts were made into different concentrations (200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml) using ethanol.

Preliminary Phytochemical Analysis

The preliminary phytochemicals from the mangrove sample extracts were determined. In the preliminary phytochemical analysis of crude extracts of *Avicennia marina* and *Avicennia officinalis* for screened the presence of glycosides, tannin, terpenoids, phenolics, saponins, amino acid, Sterol, alkaloid, phenolics and flavanoids were carried out by [14]method.

Antioxidant Assay

The antioxidant properties of the mangrove extracts were studied by their ability to scavenge free radicals using the 2, 2- Diphenyl-1-picryl hydrazyl (DPPH) reducing power.

Preparation of Test Extracts

5ml of hydroponics test sample was dissolved in 5ml of pure methanol for analysis.

Preparation of DPPH (2, 2-Diphenyl-1-picryl hydrazyl)

0.0025g of 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) was dissolved in 25 ml of methanol (0.25mM concentration). The content should be made and kept in dark condition, because DPPH is light sensitive.

DPPH free Radical Scavenging Assay

The free radical scavenging activity of mangrove extracts was measured by the DPPH method proposed [15]. Percentage inhibition or DPPH scavenging activity was calculated by following expressions.

Percentage of scavenging = [(Ao-A1)/Ao] x 100

Where Ao is the absorbance of control and A1 is the absorbance of test sample.

Rapid screening with TLC method

Thin Layer Chromatography was used to detect antioxidant activity of two mangrove samples based on spraying the plates with oxidizing reagents. The separated compounds on TLC plates were spraying with 0.004% DPPH stable radical in methanol [16] to located and detect antioxidant active compounds. The protecting against the scavenging DPPH radical gave pale yellow coloured spots were taken as positive results.

Antimicrobial Activity

Five pathogens were chosen for the present investigation and obtained from the Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu, India. Thus, the antimicrobial activity of the crude extract of *Avicennia marina* and *Avicennia officinalis* were determined by measuring the zone of inhibition in the Agar well diffusion method. The results were compared with a standard antibiotic, tetracycline (20µg/ml).

RESULTS

Phytochemical analysis

In the preliminary phytochemical analysis of crude extracts of *Avicennia marina* and *Avicennia officinalis* contains Alkaloid, Flavanoid, terpenoids and phenolics. In *Avicennia marina* extract obtained Saponins and Amino acid but absence in *Avicennia officinalis*. Tannins, Sterols and Glycosides were absent in *Avicennia marina* but present in *Avicennia officinalis* (**Table 1**).

Table1: Preliminary phytochemical screening of crude extracts of *Avicennia marina* and *Avicennia officinalis*.

S. No	Name of the test	*Avicennia marina*	*Avicennia officinalis*
1	Alkaloid	Present	Present
2	Flavanoid	Present	Present
3	Terpenoids	Present	Present
4	phenolics	Present	Present
5	Tannins	Absent	Present
6	Saponins	Present	Absent
7	Sterols	Absent	Present
8	Glycosides	Absent	Present
9	Amino acid	Present	Absent

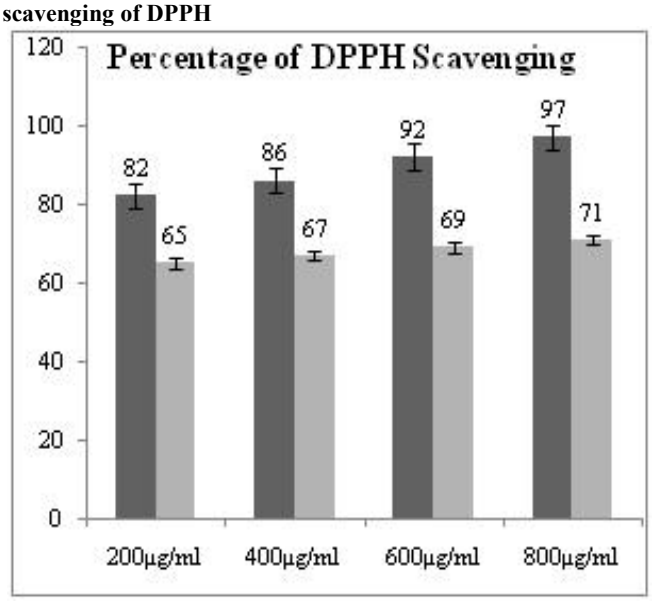
Antioxidant activity

DPPH scavenging assay

In the present study the mangrove extracts has high DPPH scavenging capacity, which increased with increasing concentration (**Fig 1**). The DPPH assay was carried out at different concentrations of mangrove samples, namely 200µg/ml, 400µg/ml, 600µg/ml and 800µg/ml. DPPH assay did not show any significant difference at 200µg/ml and 400µg/ml concentrations in *Avicennia marina* sample; however, it was significant for 600µg/ml and 800µg/ml for the extracts.

DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off, and the solution losses colour stoichiometrically depending on the number of electrons taken up. Hence this assay provided information on reactivity of test samples with a stable free radical. The decrease in the absorbance of the DPPH radical caused by test samples was due to the scavenging of radical by electron donation.

Fig 1: Effect of crude extract of mangrove samples on scavenging of DPPH



Rapid screening with TLC method

Rapid TLC- screening assay based on decolorization of ethanolic DPPH· radical that sprayed into TLC plates, as a rapid test to evaluate the antioxidant activity of natural compounds [17]. The development of pale yellow spots on the separated TLC plate confirms the antioxidant activity of the two samples. Among all separated bands, bands at hRf = >10, 25, 53 and 93 of *Avicennia marina* and *Avicennia officinalis* extracts showed an excellent antioxidant activity (**Table 2**).

Table 2: TLC profile of *Avicennia marina* and *Avicennia officinalis*

No. of spots obtained	*Avicennia marina*		*Avicennia officinalis*	
	R_f value	hR_f value	R_f value	hR_f value
1	0.22	22	0.22	22
2	0.53	53	0.76	76
3	0.74	74	0.93	93
4	0.93	93	1	1

Antimicrobial activity

The antimicrobial activity of crude extracts of *Avicennia marina* and *Avicennia officinalis* against nine human pathogenic bacterial strains were done and their zone of inhibition compared with standard antibiotic, tetracycline. The mangrove extracts were shown more active antimicrobial proficiency against *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeroginosa, Bacillus subtilis* and *Escherichia coli* when compared to the standard antibiotic, but little antibacterial activity against *Enterobacter aeruginosa, Proteus sp. Salmonella paratyphi, Citrobacter sp.* is a highly resistant against both test samples as well as standard antibiotics. (**Table 3**).

Table 3: Antimicrobial activity of the crude extract of *Avicennia marina* and *Avicennia officinalis* against human pathogenic bacteria

S.No	Pathogenic bacteria	Zone of inhibition (mm)		
		Avicennia marina	*Avicennia officinalis*	Standaard antibiotic Tetracycline
1	*Staphylococcus aureus*	18	21	32
2	*Klebsiella pneumonia*	24	25	27
3	*Pseudomonas aeroginosa*	26	23	26
4	*Bacillus subtilis*	16	8	18
5	*Escherichia coli*	27	25	31
6	*Enterobacter aeruginosa*	8	7	19
7	*Proteus sp*	12	11	20
8	*Salmonella paratyphi*	5	5	7
9	*Citrobacter sp*	1	1	12

From the present study, preliminary phytochemical analysis concluded that due to the presence of extra phytochemical in test extracts, they are beneficial one for its biological activity. In the DPPH scavenging assay, the mangrove extracts showed high antioxidant activity. The test extract samples have more effective antioxidant activity, the percentage of scavenging was found to be about 88.93% *for Avicennia marina* and 67.67 % in *Avicennia officinalis*. The rapid TLC assay is considered as the rapid test to evaluate the antioxidant activity of natural compounds. The compounds showing the bands at hRf = >10, 25 and 93 of both the mangrove extracts and hRf = 53 in *Avicennia marina* alone were proved to be having antioxidant activity.

DISCUSSION

The mangroves are a promising source of natural products. Mangroves have been a source on several bioactive compounds. Those bands that have developed into yellow spots were suspected as carotenoids and other phenolic compounds with respect to references (Hanaa *et al*., 2008). Phenolic compounds from plants are known to be good natural anti-oxidants. However, the activity of synthetic anti-oxidants was often observed to be higher than that of natural anti-oxidants [18]. The results of antimicrobial activity by the well diffusion assay also clearly expressed that test extracts have high concentration of active principles.

CONCLUSION

This work is a successful attempt of phytochemical characterization and antimicrobial efficiency of mangrove plants *Avicennia marina* and *Avicennia officinalis*. In recent years,

screening of mangrove plants for a variety of biological activities, further attention should be paid to develop the novel drugs from natural product.

ACKNOWLEDGEMENTS

The authors would like to thank Prof.Dr.T.Balasubramanian, Dean& Director, Faculty of Marine Science, Annamalai University, Tamil Nadu for providing all facilities during the study period.

REFERENCES

1. Duke NC. Mangrove floristics and biogeography. *In:* A.I. Robertson and D.M.Alongi (eds) Tropical Mangrove Ecosystems. American Geophysical Union, Washington DC. (1992), 63-100.
2. Bandaranayake WM. Bioactives, bioactive compounds and chemical constituents of mangrove plants. Wetland Ecol. Manage (2002), 10: 421-452.
3. Kokpal V, Miles DH, Payne AM, Chittawong V. Chemical constituents and bioactive compounds from mangrove plants. Stud Nat Prod Chem (1990), 7: 175-199.
4. Miki T, Sakaki T, Shibata M, Inukai Y, Hirosue H, Ikema Y, Yaga S. Soxhlet extraction of mangrove and biological activities of extracts. Kyushu Kogyo Gijutsu Kenkyusho Hokoku, (1994), 53: 3347-3352.
5. Ishibashi F, Satasook C, Isman MB, Neil Towers GH. Insecticidal 1HCyclopentatetrahydro [b] Benzofurans from *Aglaia odorata*. Phytochemistry (1993), 32: 307-310.
6. Wu TS, Liou M.J, Kuon CS, Teng CM, Nagao T, Lee KW. Cytotoxic and antiplatelet aggregation principles from *Aglaia elliptifolia.* J Nat Prod (1997), 60: 606-608.
7. Bandaranayake WM. Survey of mangrove plants from Northern Australia for phytochemical constituents and uv-absorbing compounds. Curr Topic Phytochem (1995), 14: 69-78.
8. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol(2005), 4: 685-688.
9. Rojas R, Bustamante B, Bauer Fernandez I, Alban J, Lock O. Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol (2003), 88: 199-204.
10. Kathiresan, K., and T.Ramanathan, 1997. Medicinal plants of Parangipettai Coast. Monograph, Annamalai University, Parangipettai, India, 1: 79
11. Ramanathan, T., 2000. Studies of Medicinal Plants of Parangipettai Coast (South East Coast of India). Ph.D. thesis, Annamalai University, Parangipettai, India, pp: 181
12. M. Itigowa C. Ito HT.Tan. Okuda H. Tokuda H. Nishino H .Furukawa (2001). Cancer chemopreventive activity of naphthoquinones and their analogs from Avicennia plants. Cancer Lett. 174: 135-139.
13. Chung, K. T., Wong, T. Y., Huang, and Y. Lin, (Tannins and human health: A review. Critical Reviews in Food Science and Nutrition (1998).38: 421–464.
14. Hanaa, H Abd El- Baky., Farouk, K. El Baz and Gamal, S. El. Baroty. 2008. Evaluation of marine algae *Ulva lactuca*. L as a source of Natural Preservative Ingridient. American- Eurasian J. Agric & Environ. Sci., 3 (3): 434- 444.
15. Hatano, T., Kagawa, H., Yasuhara, T. 1988. Two new flavonoids and other constituents in licorice root; their relative stringency and radical scavenging effects. Chemical and Pharmaceutical Bulletin 36: 2090-2097.
16. Jaime, L., J.A, Mendiola., M, Herrero., C, Soler Rivas., S, Santoyo, F.J. Senorans, a.cifuentes and E.Ibanez. 2005. Separation and characterization of antioxidants from *Spirulina platensis* microalga combining pressurized liquid extraction, TLC and HPLCDAD. Sep. *Sci*., 28: 2111-2119.
17. Molyneux, P. 2004. The use of the stable free radical Diphenyl picryl hydrazyl (DPPH) for estimating antioxidant activity, *Songklanakarin J. Sci. Technol*., 26 (**2**): 211 – 219.Che. 2004. Marine products as a source of antiviral drug leads. *Drug Dev. Res*. 23 (3) 201-218.
18. M.B. Ningappa, R. Dinesha, and L. Srinivas, Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf extract (Murraya koenigii L.). Food Chemistry. (2008).106: 720–728.