WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 8, 1518-1527. Research Article ISSN 2277– 7105

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF LEAF AND BARK ETHANOL EXTRACTS OF MARINE AND TERRESTRIAL PLANTS OF *XYLOCARPUS* SPECIES.

Shaik Babu Saheb[1], Naragani Krishna[1], Shaik Ibrahim Khalivulla [2] and Kokkanti Mallikarjuna[1*]

[1]Department of Botany and Microbiology, AcharyaNagarjuna University, Nagarjuna Nagar, Guntur - 522510, India.

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, No.1, Jalan Menara Gading, 56000 Cheras, Kuala Lumpur, Malaysia.

Article Received on
15 June 2016,

Revised on 06 July 2016,
Accepted on 27 July 2016

DOI: 10.20959/wjpr20168-6849

***Corresponding Author**
Kokkanti Mallikarjuna
Department of Botany and Microbiology, AcharyaNagarjuna University, Nagarjuna Nagar, Guntur - 522510, India.

ABSTRACT

True mangrove species of *Xylocarpus* belongs to meliaceae family and are distributed in the coastal ecosystems. Mangrove plants represent a unique set of plants with high adaptability to extreme environments. The unique adaptations of mangrove represent a rich source of bio active compounds. In the present work we report the anti-microbial activity of *Xylocarpus grantum* and *Xylocarpus moluccensis*. The antimicrobial activity of the leaf and bark of *X.granatum* and *X.moluccensis* were evaluated against antibiotic resistant bacteria and fungi like *Staphylococcus aureus, Bacillus subtilis*, *Bacillus cereus*, *Eschrichia coli* and *Candida albicans*. Ethanol extractions of leaf and bark were prepared using cold maturation and antimicrobial activity is evaluated by using agar well diffusion method. Marine and terrestrial plants of *Xylocarpus granatum* showed promising antibacterial activity against *S.aureus* (18mm), *B.subtilis* (15mm), *B.cereus* (18mm) and *E.coli* (21mm) and also antifungal activity against *C. albicans* (25mm) when compared to *Xylocarpus moluccensis*. The results indicated that *Xylocarpus granatum* is a potent source of bio active compounds when compared to *Xylocarpus moluccensis.*

KEY WORDS: *Xylocarpus granatum*, Antimicrobial activity, Agar well diffusion method.

INTRODUCTION

Mangrove plants are rich sources for bioactive compounds and widely distributed throughout the coastal areas of India, especially estuaries. Mangrove plants and their products have been used in traditional medicine. These plants are well known to have natural products with great pharmaceutical importance; they exhibit antimicrobial, anti-larval, antiviral and insecticidal activity (Kokpal et al., 1990; Premnathan et al., 1992, 1998). Mangrove plants are the best choice to isolate bioactive natural products active against bacterial and fungi (Han et al., 2007; Wu et al., 2008).

Xylocarpus granatum and *Xylocarpus moluccensis* belongs to meliaceae family. *Xylocarpus granatum* is rare and *Xylocarpus moluccensis* is distributed well (Raju, 2003). Availability and distribution of *Xylocarpus granatum* in Krishna mangroves were reported in Kothapalem (Pullaiah, 2000 and Nabi et al., 2012). The field survey indicated that there is a vast variation in distribution of *Xylocarpus granatum* and *Xylocarpus moluccensis* in the Krishna and Godavari mangroves (Raju, 2003).

In the present work, we prepared solvent extracts for antimicrobial activity from *Xylocarpus granatum* and *X.moluccensis* and assayed against range of microbes. We thought that evolutionary and ecological adaptations of mangrove species (*X.granatum* and *X.moluccensis*) might have resulted in accumulation of valuable biologically active molecules which need to be screened, isolated and identified. In the present work, we tried to understand the differential response of above plants against a range of microbes, which may be a reflection of their differential phytochemical composition.

MATERIALS AND METHODS

Collection of plant material

Bark and leaves of *Xylocarpus granatum* and *Xylocarpus moluccensis* were collected from mangrove forest located at Kothapalem, Guntur district, Andhra Pradesh in the month of December 2014.

Sample preparation

The obtained bark and leaves were washed with tap water to remove visible dust and then were shade dried at room temperature. Then crushed to fine powder using mortar and pestle and stored at refrigerator (4^{o}C) for further use.

Preparation of plant extracts

100g of bark and leaves samples were taken and extracted individually with ethanol. Samples were kept for 72 hours at room temperature and it's were stirred with a glass rod after every 2 hours. After 3 days, the mixtures were filtered using the whatman's filter papers no.1. The extracts obtained were dried and concentrated in rotary vacuum evaporator and concentrated extracts were stored in refrigerator (4^{o}C) for analysis (Alade and Irobi's cold extraction method).

Antimicrobial Activity against Test Organisms

The antimicrobial activity of the crude extract of *Xylocarpus granatum* and *Xylocarpus moluccensis* was determined by agar well diffusion method (Naragani *et al.,* 2014). Nutrient agar (NA) and Czapek-Dox (CD) agar media were used for culturing the test bacteria and fungi respectively. NA medium (100 ml) was sterilizedat 15 lbs pressure (121ºC) for 15min, cooled and inoculated with 0.5 ml of test bacterial suspension. After solidification of agar medium, wells of about 5 mm diameter were punched into it with sterilized cork borer. In case of antifungal assay, spore suspension of test fungi was mixed with the cooled, molten CD agar medium and poured into petri dishes. The crude extract dissolved in ethanol at a concentration of 50,100,150 mg/ml was added to the wells made in the medium. Adding only ethanol to the wells served as control. The plates were incubated at 30°C for 24 h for bacteria and 24-72 h for fungi and the diameter of the inhibition zones was measured.

The antimicrobial activity of the crude extract produced by the *Xylocarpus granatum* and *Xylocarpus moluccensis* were tested against bacteria viz. *Staphylococcus aureus (*ATCC6538), *Bacillus subtilis (*ATCC6633), *Bacillus cereus*, *Escherichia coli* (ATCC15597*)* and fungi such as *Candida albicans (*ATCC10231) by agar-diffusion assay.

RESULTS AND DISCUSSION

Mangrove plants are adapted well to extremely challenging environmental abiotic conditions by alterations in their physiological processes resulting in the synthesis of novel chemical compounds that offers protection against the various biotic and abiotic conditions (Edreva et al., 2008). A number of these phyto compounds or secondary metabolites have significant medicinal properties and are being used traditionally for treatment of number of ailments (Bandaranayake, 2002). The mangrove plants possess strong antimicrobial compounds (viz. flavonoids, tannins, terpenoids, coumarins, alkaloids, lectins) that may act against a broad range of disease causing microorganisms (Das et al., 2014). Extracts from different mangrove

plants and mangrove associates are found to be active against human and plant pathogens (Chandrasekaran et al., 2009).

The mangrove plant that belongs to the genus *Xylocarpus* have shown to be having enormous ethno medicinal potential; however, few reports are available about their principles responsible for their biological activities. The presence of alkaloids, flavonoids, triterpinoids, phenolics, limonoids and steroids etc. has been reported in the leaves; stem bark and fruits of these plants (Wangensteen et al., 2009).

A B C D

Fig: 1 A, B) Leaf ethanol extracts of *Xylocarpus granatum* and *X. moluccensis*
C, D) Bark ethanol extracts of *Xylocarpus granatum* and *X.moluccensis*

Qualitative phytochemical analysis indicated that *Xylocarpus granatum* and *X. moluccensis* ethanol extracts contain number of secondary metabolites such as alkaloids, terpenoids, flavonoids, tannins and saponins (Table.1).

Table. 1 Phytochemical analyses of *Xylocarpus granatum* and *Xylocarpus moluccensis*

S.No	Secondary metabolite	Test	*Xylocarpus granatum*		*Xylocarpus moluccensis*	
			Leaf	Bark	Leaf	Bark
1	Alkaloids	Mayer's test Fraction+	+	++	+	+
2	Terpenoids	General test Fraction+$SnCl_3$	+	+ +	+	+ +
3	Flavonoids	General test Fraction+Ethyl acetate+Dil HNO_3	+	+ +	+	+ +
4	Tannins	Fraction+Water+ 5% $FeCl_3$	++	+ +	+	+ +
5	Saponins	Frothing test Fraction+ distilled water	-	++	-	-

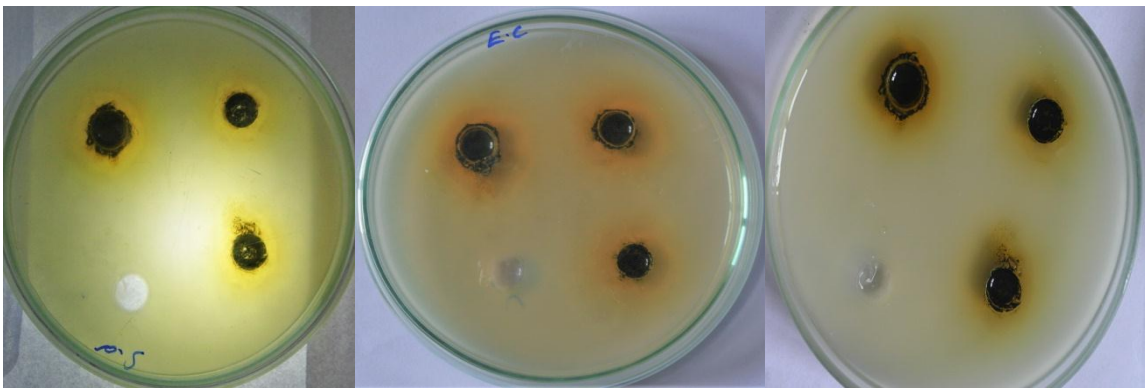
In our preliminary study, it is found that aqueous and ethanol extracts of some mangrove species have antimicrobial activities (Abeysinghe et al., 2000). In almost all tests, crude ethanol extracts showed better inhibition against all tested bacterial strains, indicating that active ingredients in plant materials could be extracted into ethanol (Babuselvam et al., 2012). However, the leaf extract has been tested for it antimicrobial effect against on *Staphylococcus aureus* (ATCC6538), *Bacillus subtilis* (ATCC6633), *Bacillus cereus, Escherichia coli* (ATCC15597) and fungi such as *Candida albicans* (ATCC10231) in the levels of 50, 100 and 150 mg/ml growth was inhibited by the effect of extract.The MIC ranged from 150 to 1000 µg/ml. For the interpretation of antibacterial assay results, we scale of measurement adopted was as zone of inhibition of >15 mm as strongly inhibitory, 10 - 15 mm as moderately inhibitory, and <10 mm as not inhibitory, according to Carvois-Stanko et al.(2010). *Xylocarpus granatum* leaf extract shows at the level of 150 mg/ml maximum and minimum inhibition zone was observed against in *E.coli* (21mm) and *B.subtilis* (10mm) respectively (fig.2). Maximum inhibition zone was observed in fungi such as *Candida albicans* (25mm) (Table.2).as well as *E.coli* (8mm), *Candida albicans* (12mm) inhibition zone was observed at terrestrial environment(Table.3) that could be due to lack of stressful marine environment. Whereas *Xylocarpus moluccensis* leaf extract shows at the level of 150 mg/ml maximum and minimum inhibition zone was observed against in *B.subtilis*, *B.cereus (*20mm) and *S.aureus* (15mm) respectively (fig. 3). Maximum inhibition zone was observed in fungi such as *Candida albicans* (18mm) (Table.2 & fig.3). But no promising inhibition zone was observed at terrestrial environment that could be due to lack of stressful marine environment (Table.3).The results indicated that at level of 150 mg/ml leaf extract of *Xylocarpus granatum* shows more activity against bacterial (21mm) and fungi (25mm) cultures when compared to the *Xylocarpus moluccensis* (Table.2&3).

Table: 2 Antimicrobial activity of leaf ethanol extract of *Xylocarpus granatum* and *Xylocarpus moluccensis* at marine environment.

S. No	Microorganism	Zone of inhibition (mm)					
		X.granatum (µg/ml)			*X.moluccensis* (µg/ml)		
		50	100	150	50	100	150
1	***Staphylococcus aureus***	7	12	15	10	12	18
2	***Bacillus subtilis***	13	15	20	5	10	15
3	***Bacillus cereus***	15	17	20	10	15	18
4	***Escherichia coli***	10	15	21	10	16	18
5	***Candida albicans***	13	15	25	10	18	18

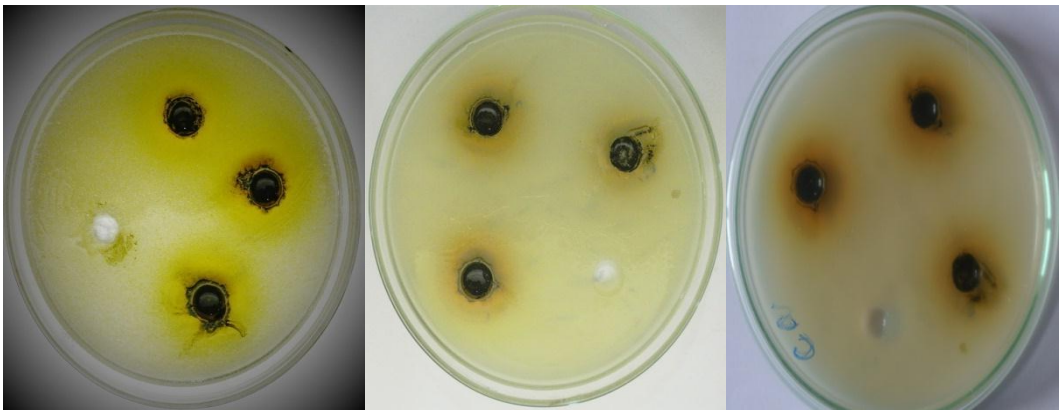
Table: 3 Antimicrobial activity of leaf ethanol extracts of *Xylocarpus granatum* and *Xylocarpus moluccensis* at terrestrial environment.

S.No	Microorganism	Zone of inhibition (mm)					
		X.granatum (µg/ml)			*X.moluccensis* (µg/ml)		
		50	100	150	50	100	150
1	***Staphylococcus aureus***	-	-	5	-	-	-
2	***Bacillus subtilis***	-	-	7	-	-	5
3	***Bacillus cereus***	-	-	5	-	-	-
4	***Escherichia coli***	-	-	8	-	-	5
5	***Candida albicans***	-	5	12	-	-	-



A) *S. aureus* **B) *E. coli*** **C) *C. albicans***

Fig.2. Antimicrobial activity (A, B and C) of leaf ethanol extract of *Xylocarpus granatum*



A) *S.aureus* **B) *E. coli*** **C) *C.albicans***

Fig.3 Antimicrobial activity (A, B and C) of leaf ethanol extracts of *Xylocarpus moluccensis*

Xylocarpus granatum bark extract shows at the level of 150 mg/ml maximum and minimum inhibition zone was observed against in *S.aureus, B.subtilis, B.cereus* (25mm) and *E.coli* (2.5mm) respectively. Maximum inhibition zone was observed in fungi such as *Candida albicans* (25mm) (Table.4 and fig.4). As well as *E.coli* (8mm) and *Candida albicans*(13mm)

at terrestrial environment (Table.5 and fig.5). Whereas *Xylocarpus moluccensis* bark extract shows at the level of 150 mg/ml maximum and minimum inhibition zone was observed against in *S.aureus, E.coli* (25mm) and *B.subtilis* (23mm) respectively. Maximum inhibition zone was observed in fungi such as *Candida albicans* (19mm) (Table.5). But very low (<5mm) activity was observed at terrestrial environment. The results indicated that at level of 150 mg/ml, the leaf extract of *Xylocarpus granatum* shows more activity against bacteria (25mm) and fungi (25mm) cultures when compared to the *Xylocarpus moluccensis* (Table.4&5).

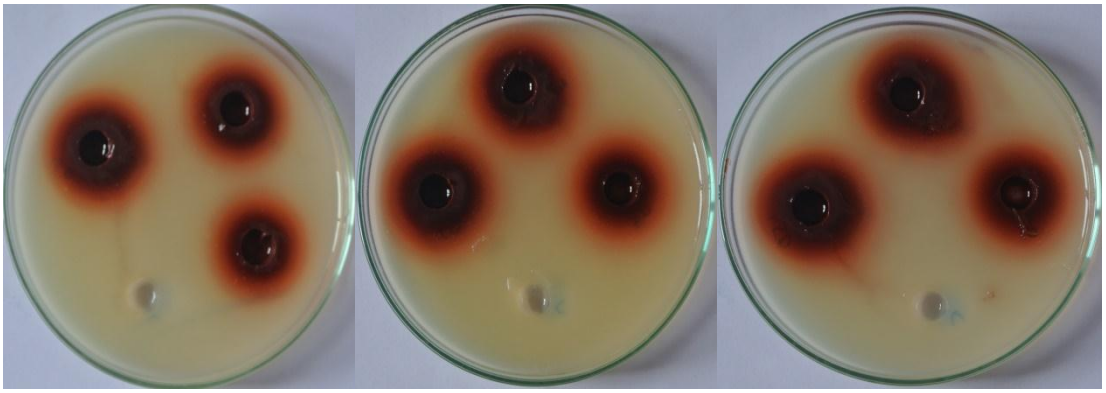
Our results also indicate clear difference in antimicrobial activity of leaf and bark of *Xylocarpus granatum* and *X.moluccensis*. Whereas, *Xylocarpus granatum* shows high activity, Which is a reflection of the rich sources of bio active compounds that a plants contains.

Table: 4 Antimicrobial activity of bark ethanol extracts of *Xylocarpus granatum* and *Xylocarpus moluccensis* of marine areas.

S.No	Microorganism	Zone of inhibition(mm)					
		X.granatum(µg/ml)			*X.moluccensis* (µg/ml)		
		50	100	150	50	100	150
1	*Staphylococcus aureus*	20	22	25	15	20	25
2	*Bacillus subtilis*	18	20	24	15	20	23
3	*Bacillus cereus*	20	25	25	18	20	23
4	*Escherichia coli*	20	25	25	15	20	20
5	*Candida albicans*	15	20	25	14	16	19

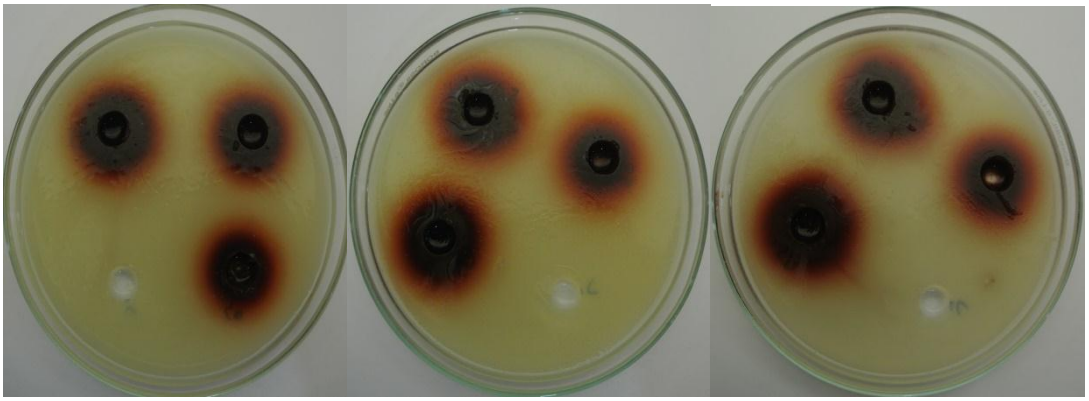
Table: 5 Antimicrobial activity of bark ethanol extracts of *Xylocarpus granatum* and *Xylocarpus moluccensis* of terrestrial areas.

S.No	Microorganism	Zone of inhibition(mm)					
		X.granatum(µg/ml)			*X.moluccensis* (µg/ml)		
		50	100	150	50	100	150
1	*Staphylococcus aureus*	5	5	5	0.0	0.0	5
2	*Bacillus subtilis*	5	7	8	0.0	0.0	4
3	*Bacillus cereus*	0	5	5	0.0	0.0	5
4	*Escherichia coli*	0	3	5	0.0	0.0	5
5	*Candida albicans*	5.0	9.0	13	0.0	3.0	3.0



A) *S.aureus* B) *B.subtilis* C) *C.albicans*

Fig.4. Antimicrobial activity (A, B and C) of bark ethanol extract of Xylocarpus *granatum*



A) *S.aureus* B) *B.subtilis* C) *C.albicans*

Fig.5.Antimicrobial activity (A, B and C) of bark ethanol extract of *Xylocarpus moluccensis*

CONCLUSIONS

In the present work, we provide data to show the potential of mangrove extracts for development of anti-microbial agents with changing environments. *Xylocarpus granatum* showed more activity than *Xylocarpus moluccensis* and indicates that *Xylocarpus grantum* more potent than *Xylocarpus moluccensis.*

ACKNOWLEDGEMENTS

The first author (Shaik Babu Saheb) is thankful to UGC for providing BSR – SAP Fellowship to carry out this work. The corresponding author (Kokkanti Mallikarjuna) is grateful to UGC for sanctioning Major Research Project (No: 42-949/2013 (SR)) to support to this work.

REFERENCES

1. Abeysinghe PD, Vithanawasam M, Pathirana RN, Abeysinghe S. Preliminary in *vitro* screening of some mangrove plant extracts for antimicrobial compounds against clinical isolates from different sourecs.1st science symposium proceedings 2000; 1: 22-5.
2. Babuselvam M., Farook K. A. M., Abideen S., Mohamed M. P., Uthiraselvam M., 2012 Screening of antibacterial activity of mangrove plant extracts against fish and shrimp pathogens. International Journal of Applied Microbiology Science, 2012; 1: 20-25.
3. Bandaranayake WM. Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management. 2002; 10: 421– 52.
4. Chandrasekaran M, Kannathasan K, Venkatesalu V and Prabhakar K. Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant Staphylococcus aureus. World J. Microbiol. Biotechnol. 2009; 2: 155–60.
5. Carovic-Stanko, K., Orlic, S. and Politeo, O. Composition and Antibacterial Activities of Essential Oils of Seven Ocimum Taxa. Food Chemistry, 2010; 119: 196-201.
6. Das, S.K., Samantaray, D. & Thatoi, H., 2014. Ethnomedicinal, antimicrobial and antidiarrhoeal studies on the mangrove plants of the genus *Xylocarpus*: A mini-review. Journal of Bioanalysis and Biomedicine. 2014; 12: 04, 7.
7. Edreva A., Velikova V., Tsonev T., Dagnon S., Gurel A. et al. Stress-protective role of secondary metabolites: diversity of functions and mechanisms. Gen Appl Plant Physiol, 2008; 34: 67-78.
8. Han, X., T. Shen and Lou, H. Dietary polyphenols and their biological 558 significance. Int J Mol Sci. 2007; 8: 950-988.
9. Kokpal V, Miles DH, Payne AM and Chittawong V. Chemical constituents and bioactive compounds from mangrove plants. Studies in Natural Products Chemistry, 1990; 7: 175-199.
10. Nabi A, Rao BP, Prasad AVR, Analysis of mangrove vegetation of diviseema region, Krishna District, Andhra Pradesh. International Journal of Plant, Animal and Environmental Sciences. 2012; 2(4): 99-108.
11. Naragani Krishna, Rajesh Kumar Munaganti, Usha Kiranmayi Mangamuri and Vijayalakshmi Muvva Optimization of Culture Conditions for Enhanced Antimicrobial Activity of RhodococcuserythropolisVLK-12 Isolated from South Coast of Andhra Pradesh, India. British Microbiology Research Journal 2014; 4(1): 59-75.
12. Premanathan M, Kathiresan K, Yamamoto N, Nakashima H. In vitro anti-human Rhizophora mangle L. Rev Cubana Med Trop. 1999; 30: 181-187.

13. Pullaiah T, RamakrishnaiahV, Sandhya Rani S, P.N Rao, Flora of Guntur District, Andhra Pradesh, India. Regency publishers, New Delhi. pp32. 2000.
14. Raju, ISSN, Xylocarpus (Meliaceae), A less known mangrove taxon of the mangrove in Godavari estuary, India. Current Science, 2003; 84(7): 879-881.
15. Wangensteen H, Alamgir M, Duong GM, Gronhaug TE, Samuelsen AB, Malterud KE. Chemical and biological studies of medicinal plants from the Sundarbans mangrove forest. In: Eddouks M, editor. Advances in Phyto therapy Research. India: Research Signpost; 2009; 59–78.
16. Wu, J., X. Qiang, X Jing, M. Li, J. Pan and M. Yang,. "Natural Products from True Mangrove Flora: Source, Chemistry and Bioactivities", Natural Products Report 2008; 25: 955-981.
17. Wu, J., X. Qiang, X. Jing, M. Li, J. Pan, and M. Yang,. "Natural Products from True Mangrove Flora: Source, Chemistry and Bioactivities", Natural Products Report 2008; 25: 955-981.