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Antibacterial and Phytochemical Screening of *Xylocarpus moluccensis* Leaf and Stem on Selected Drug Resistant and Sensitive Bacteria

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Abstract: Many efforts have been made to discover new antimicrobial compounds from different sources such as microorganisms, animals and plants. One of such resources is folk medicines. Systematic screening may result in the identification of novel and effective compounds. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies. Mangroves are the plants found in the intertidal zones of sheltered shores and have been used in the treatment of human diseases for many centuries in naturopathy because of their diverse habitat. *Xylocarpus moluccensis* belongs to Meliaceae family. The present study mainly focused to determine the effect of *X. moluccensis* leaf and stem organic and aqueous solubles against medically important drug resistant Gram positive bacteria viz., *Bacillus cereus, Bacillus subtilis*, *Staphylococcus aureus* and Gram negative bacteria viz., *Escherichia coli*, *Klebsiella pneumoniae* and drug sensitive Gram positive *Bacillus subtilis*, Gram negative *Enterobacter aerogenes* and *Pseudomonas aeruginosa* by agar well diffusion method. All the extracts were found effective against the tested drug resistant and drug sensitive strains. Among the extracts, acetone, ethanol, methanol and water extracts exhibited maximum activity against drug resistant *E. coli, B. subtilis, B. cereus* and drug sensitive *P. aeruginosa* and *B. subtilis*. Phytochemical diversity might be responsible for the antibacterial activity of acetone, ethanol, methanol and water extracts of *X. moluccensis*.

Key words: Antibacterial Activity · Xylocarpus moluccensis · Agar Well Diffusion · Phytochemicals

INTRODUCTION

Plants and plant products are widely used in ethno medicine around the world [1]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, which have been found in vitro to have antimicrobial properties [2]. Mangrove forests are the rich source for Biodiversity 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, antilarval, antiviral and insecticidal activity [3-5].

Pathogenic microorganisms are continuously developing resistance to the existing antimicrobial compounds [6,7]. Hence there is a need to search and design new alternative drugs from natural products to combat microbial infections [8]. Mangrove plants are the best choice to isolate bioactive natural products active against bacteria and fungi [9, 10].

Xylocarpus moluccensis (X. moluccesis) belongs to the order Geraniales of the family Meliaceae. The genus *Xylocarpus* is distributed in the coastal regions of India, Ceylon, Burma and Malaya [11] and Indonesia. The fruit of *X. moluccensis* is a green color, lemon fruit sized, hard and heavy, leading to the common name 'cannon ball tree'. This mangrove provides a several phytochemical compounds. It can be used for health therapy. Several biological properties have been attributed to *X. moluccensis*: aphrodisiac, fever, malaria, hair preservatives, astringent, antidiarrhoea, anthelmentic

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and haemostatic properties [12, 13]. So the main objective of this study was to screen antibacterial activity and phytochemicals of the organic solvent and aqueous extracts of *X. moluccensis.*

MATERIALS AND METHODS

Plant Material: The healthy plant of *X. moluccesis* was collected from Coringa Reserve Forest, Kakinada andhra Pradesh, India. The leaves and stems were surface sterilized with 1% mercuric chloride and thoroughly washed with plenty of distilled water. Later, the leaves and stems were shade dried after chopped into small pieces.

Extraction: The chopped leaf and stem material of *X. moluccesis* (100g) was extracted separately in to different solvents in the increasing order of polarity viz, hexane, benzene, ethylacetate, chloroform, acetone, absolute alcohol, methanol and distilled water [14]. The chopped material was extracted sequentially into 500 ml of the respective solvent by initial soaking for 12 hours followed by refluxing for about 10 hours below the boiling point of the respective solvent. Resulting extracts in different solvents were evaporated and concentrated using the rotary evaporator. Concentrated extracts were dissolved in 1-2 ml of dimethyl sulfoxide (DMSO) and the concentration was adjusted to 100 mg/ml with water and stored at 4°C.

Bacterial Strains: Pure cultures of *Staphylococcus aureus* (MTCC 87)*, Bacillus subtilis* (MTCC 441)*, Bacillus cereus* (MTCC 430), *Escherichia coli* (MTCC 40)*, Klebsiella pneumonia* (MTCC 39) (drug resistant strains) (these strains are resistant to the following drugs: *S. aureus*-erythromycin, B. subtilis-cephalexin and penicillin, *B. cereus*-penicillin *E. coli*-cephalexin, *K. pneumonia* - ciprofloxacin) *Bacillus subtilis* (MTCC 121), *Enterobacter aerogenes* (MTCC 111) and *Pseudomonas aeruginosa* (MTCC 424) (drug sensitive strains) were procured from Microbial Type Culture Collection (MTCC) Chandigarh to determine the antibacterial activity.

Determination Of Antibacterial Activity: The antibacterial activity of *X. moluccesis* extracts were performed by agar well diffusion method [15]. Bacterial suspensions of the test cultures were prepared by using 24 hour old bacterial culture. The amount of bacteria needed to undertake the study was determined using UV/Vis spectrophotometer (ELICO, India) at 625 nm so that the absorbance of the suspension was held at 0.01 which was assumed to contain 1-2×10^8 CFU/ml [16]. About 20 ml of melted (at about 50°C) Mueller Hinton agar was mixed with 1 ml of bacterial suspension homogeneously and allowed to solidify in petri dishes (143 mm diameter). Wells (10mm diameter.) were made using a sterile cork borer on the solidified medium. Wells were filled with 200 μl of the crude extract containing 5 -20 mg. All the tests were performed in triplicates. Tetracycline was used as a standard reference antibiotic. The diameters of the inhibition zones were measured and their means were calculated. DMSO in water was taken as control.

Phytochemical Analysis: The crude extracts were subjected to preliminary phytochemical analysis by standard protocols from Pharmacopia for the identification of different phytochemical constituents [17, 18].

Statistical Analysis: The mean value and standard deviation of triplicates were calculated.

RESULTS AND DISCUSSION

The antimicrobial activities varied considerably from leaf to stem, all the sixteen extracts prepared from both stem and leaf sequentially in the order of increased polarity were tested for their antimicrobial activity against five drug resistant and three drug sensitive bacterial strains by agar well diffusion method. They were able to inhibit the growth of one or more of the tested bacterial strains. The most susceptible organisms with both stem and leaf are *E. coli, B. subtilis* (both drug resistant and sensitive strains) and *B. cereus. K. pneumonia* found to be resistant among the tested microorganisms with stem extracts. However, the growth of *K. pneumonia* was significantly (using standard deviation) inhibited by most of the leaf extracts and ethyl acetate extract of the stem. *S. aureus, P. aeruginosa and E. aerogenes* were moderately inhibited by both the stem and leaf extracts. Experimental data also revealed that, stem and leaf extracts prepared in non polar aprotic solvents such as hexane, benzene and chloroform showed less antibacterial activity than polar aprotic ethyl acetate and acetone and protic solvents such as methanol, ethanol and water. Acetone solubles were found to exhibit more antibacterial activity than other solvents as results showed in Tables 1and 2.

Table 1 a.: Antibacterial activity of *Xylocarpus X. moluccensis* leaf extracts on drug resistant microorganisms

S.No.	Microorganism	Conc.	H	B	EA	C	A	E	M	W	Tetracycline
1	Escherichia coli(MTCC 40)	5	0.5 0.1	3 0.616	4.5 1.056	1 0.523	5 1.140	3 1.141	2 0.734	2.5 0.890	32
		10	0.5 0.066	4.1 0.883	5.1 1.203	2.5 1.212	8.1 2.667	6 2.325	5.1 2.141	5.5 1.844	
		15	1.5 0.273	5.2 1.159	8.5 2.200	4.5 1.807	12.1 4.354	9 3.868	8.2 2.945	11 4.330	
		20	2 0.614	6.5 1.705	10 1.829	6 2.0652	12.1 4.354	10 4.220	10.8 3.876	11 4.330	
2	Klebsiella pneumonia(MTCC 39)	5	0.1 0.055	0.1 0.062	2 1.050	1 0.229	3 1.136	2.5 0.966	11 0.650	1.5 0.520	26
		10	0.1 0.055	0.1 0.062	4 1.051	2 0.886	7 2.555	5 1.895	2.5 0.942	4.2 1.557	
		15	0.5 0.146	0.4 0.084	7 2.079	3 1.097	9 3.054	10 3.868	5 0.639	6.5 2.564	
		20	1 0.058	1 0.227	9 1.974	5 1.885	11 2.774	12 4.564	10 3.883	11 4.421	
3	Staphylococcus aureus(MTCC 87),	5	0.1 0.045	0.2 0.086	0.3 0.146	0.1 0.043	0.9 0.386	1.1 0.347	0.1 0.040	0.2 0.081	22
		10	0.1 0.042	0.5 0.222	1.5 0.581	0.5 0.243	2 0.629	2.4 0.921	1.5 0.587	1.4 0.515	
		15	0.5 0.230	1 0.486	3 1.206	1.5 0.635	4.5 1.815	3.5 1.503	3 1.194	3.4 1.508	
		20	1 0.412	2 0.847	6 2.414	3 1.222	9 1.435	7 2.915	6 2.311	7 3.022	
4	Bacillus subtilis(MTCC 441),	5	0.1 0.044	1 0.419	1.1 0.387	1.3 0.531	5 2.065	5.5 2.222	1.5 0.597	1 0.381	30
		10	0.1 0.042	2 0.880	2.2 0.667	2.3 0.768	7 3.206	7.5 2.802	3.5 1.369	2.5 0.940	
		15	0.4 0.172	3 1.227	4.7 1.830	4 1.528	10 3.851	10 3.044	5 1.964	5.5 2.071	
		20	1 0.381	5 1.922	7 2.755	6 2.325	10 3.851	10 3.044	9 3.505	10 3.481	
5	Bacillus cereus(MTCC 430),	5	-	-	1.5 0.597	0.1 0.038	4 1.830	1.2 0.477	0.9 0.343	1.5 0.577	24
		10	-	-	3.1 1.380	0.2 0.096	6 2.532	2.1 0.812	2.3 0.852	3.5 1.358	
		15	-	-	4.5 1.747	1.5 0.608	11 3.939	4.5 1.718	4.1 1.585	5 1.964	
		20	-	-	6 2.165	3 1.237	11 3.939	9 3.518	7 2.770	9 3.518	

H- Hexane; B- Benzene; C- Chloroform; EA- Ethyl Acetate; M- Methanol; A- Acetone; E- Ethanol (Absolute alcohol); W- Water.

b) Antibacterial activity of *X. moluccensis* leaf extracts on drug sensitive microorganisms

S. No.	Microorganism	Conc.	H	B	EA	C	A	E	M	W	Tetracycline
1	Bacillus subtilis (MTCC 121)	5	-	0.1 0.075	0.9 0.379	0.8 0.289	5 0.627	7 0.256	5 0.600	6.3 0.220	22
		10	0.1 0.038	0.2 0.095	1.5 0.620	1.2 0.477	6 0.671	8 0.189	8 0.591	7.1 0.835	
		15	0.3 0.136	0.3 0.142	3.5 1.382	1.5 0.601	10 1.096	10 0.552	9.5 1.169	8 0.969	
		20	1 0.464	1 0.492	6 2.339	2 0.823	10 1.096	10 0.552	10 0.953	10 0.885	
2	Enterobacter aerogenes (MTCC 111)	5	-	-	0.9 0.076	0.1 0.073	4.3 0.225	3.2 0.638	2.5 0.663	1 0.175	32
		10	-	-	1.5 0.350	0.2 0.075	5.9 0.742	4.3 0.693	3.2 0.708	2 0.614	
		15	-	-	1.8 0.381	0.8 0.175	6.4 0.554	5.1 0.312	4.9 0.200	3 0.621	
		20	-	-	2 0.728	1 0.162	7 0.500	6 0.653	6 1.109	5 0.671	
3	Pseudomonas aeruginosa(MTCC 424)	5	-	0.1 0.010	1 0.150	1 0.422	2 0.319	2 0.319	4 0.969	5 0.614	30
		10	-	0.5 0.157	3 0.633	2 0.669	4 1.124	3 0.319	5 0.602	8 0.458	
		15	-	1 0.312	4 0.110	3 0.554	5 0.661	4 0.178	6 0.591	9 0.634	
		20	-	2 0.312	7 0.599	5 1.001	7 0.319	7 0.319	7 0.663	10 0.312	

Table 2 a. Antibacterial activity of X. moluccensis stem extracts on drug resistant microorganisms

S.No.	Microorganism	Conc.	H	B	EA	C	A	E	M	W	Tetracycline
1	Escherichia coli(MTCC 40)	5	0.1 0.01	0.5 0.132	5.6 0.340	-	3 0.336	6 1.491	8 0.603	4 0.546	30
		10	0.5 0.202	1.5 0.132	8.5 0.268	0.1 0.01	5 0.355	8 0.860	9.6 0.553	6 0.482	
		15	1 0.104	2.5 0.305	10 0.332	0.5 0.175	13 0.503	10 0.529	10 0.405	8 0.602	
		20	2 0.319	5 0.312	10 0.332	1 0.305	13 0.503	10 0.529	10 0.405	11 0.425	
2	Klebsiella pneumonia(MTCC 39)	5	-	-	1.5 0.275	-	5 0.319	6 0.472	3 0.528	2 0.900	25
		10	-	-	3.5 0.268	-	6 0.360	8 0.603	4 0.463	4 0.548	
		15	-	-	5.5 0.431	-	7.5 0.284	10 0.417	6 0.900	7 1.036	
		20	-	-	7 0.557	-	9 0.332	10 0.417	8 0.557	9 0.557	
3	Staphylococcus aureus(MTCC 87),	5	0.5 0.2	0.2 0.06	2 0.360	-	6 0.653	2 0.354	1 0.332	4 0.480	21
		10	1 0.240	1 0.404	3 0.534	0.2 0.081	8 0.520	3 0.368	3 0.900	5 0.557	
		15	2 0.321	2 0.464	4 0.340	0.5 0.208	10 0.340	5 0.312	5 0.332	7 0.595	
		20	3 0.385	5 0.554	6 0.338	1 0.748	10 0.340	7 0.319	8 0.312	8 0.883	
4	Bacillus subtilis(MTCC 441),	5	01 0.01	-	2 0.324	0.1 0.030	7 0.814	5 0.305	6.5 0.266	2.5 0.532	29
		10	0.8 0.208	-	4 0.300	0.2 0.030	9 0.378	8 0.360	8.1 0.416	5.1 0.468	
		15	1.5 0.268	-	6 .543	1 0.312	11 0.360	10 0.360	10 0.548	7 0.901	
		20	2 0.360	-	8 0.358	2 0.234	11 0.360	10 0.360	10 0.548	9 0.395	
5	Bacillus cereus(MTCC 430),	5	0.2 0.031	0.3 0.087	5 0.332	0.1 0.074	3 0.472	2 0.284	2 0.423	3 0.609	22
		10	0.6 0.098	0.7 0.152	7.6 0.309	0.3 0.129	5 0.529	4 0.332	5 0.537	6 0.472	
		15	1 0.284	1.1 0.100	8.1 0.565	0.5 0.177	6 0.332	7 0.410	7 0.416	8 0.244	
		20	2 0.281	2 0.229	9 0.557	1 0.142	8 0.651	9 0.529	9 0.298	10 0.510	

b) Antibacterial activity of X. moluccensis stem extracts on drug sensitive microorganisms

S.No.	Microorganism	Conc.	H	B	EA	C	A	E	M	W	Tetracycline
1	Bacillus subtilis (MTCC 121)	5	-	-	1.1 0.150	-	4.1 0.307	3.1 0.529	2.1 0.160	4.1 0.305	21
		10	-	-	2.5 0.500	-	8 0.243	5.2 0.571	3.6 0.732	5.6 0.750	
		15	-	-	5 0.312	-	10 0.340	7.5 0.321	4 0.615	7.7 0.608	
		20	-	-	7 0.362	-	10 0.340	9 0.413	6 0.543	9 0.529	
2	Enterobacter aerogenes (MTCC 111)	5	-	01 0.045	2.2 0.307	-	2 0.388	1.7 0.332	1.1 0.297	1.5 0.422	31
		10	-	0.5 0.157	3.7 0.398	-	3 0.557	2.5 0.475	2.5 0.289	3.2 0.559	
		15	-	1.1 0.286	4.9 0.500	-	4 0.221	3.1 0.526	3.5 0.683	4.5 0.427	
		20	-	2 0.651	6 0.538	-	6 0.529	5 0.538	5 0.557	6 0.529	
3	Pseudomonas aeruginosa(MTCC 424)	5	-	-	2 0.137	-	4.3 0.407	2 0.251	3.9 0.088	1 0.301	29
		10	-	-	3 0.472	-	6.2 0.884	4 0.265	5.1 0.319	3 0.500	
		15	-	-	5 0.319	-	7 0.557	6 0.525	6.3 1.200	5 0.428	
		20	-	-	7 0.544	-	9 0.623	8 0.642	8 0.539	7 0.529	

Table 3 a: Phytochemical analysis of *X. moluccensis* leaf extracts in different solvents.

S. No	PHYTOCHEMICAL	H	B	C	EA	M	A	E	W
1	Flavonoids	+	+	+	+++	+ ++	++ +	+++	+++
2	Alkaloids	++	++	++	++	++	++	++	++
3	Saponins	+	-	-	-	+	+	+	+
4	Terpenoids	-	+	-	-	+	+	-	-
5	Steroids	-	+	+	+	+	+	-	-
6	Tannins	-	-	+	+	+	+	+	+
7	Glycosides	++	++	++	++	++	++	++	++

b) Phytochemical analysis of *X. moluccensis* stem extracts in different solvents.

S.No	PHYTOCHEMICAL	H	B	C	EA	M	A	E	W
1	Flavonoids	-	-	-	+	-	+	-	-
2	Alkaloids	+	+	+	+	+	+	+	+
3	Saponins	_	+	+	-	-	-	-	-
4	Terpenoids	+	+	+	+	-	+	-	-
5	Steroids	-	-	+	-	-	-	-	-
6	Tannins	-	-	+	-	+	+	-	+
7	Glycosides	+	+	+	+	+	+	+	-

H- Hexane; B- Benzene; C- Chloroform; EA- Ethyl Acetate; M- Methanol; A- Acetone; E- Ethanol (Absolute alcohol); W- Water.

The beneficial effects of plant based medicines in therapy are mainly due to the action of a combination of different secondary metabolites present in different parts of the plant. Flavonoids and phenolic compounds of the plants share most of the pharmacological properties. Phytochemical diversity of *X. moluccesis* stem and leaf were shown in the Table 3. This data reveals that, flavonoids, alkaloids and glycosides were found to be at higher levels than other secondary metabolites. Flavonoids and phenolic compounds from natural sources like plants are known to associate with diverse biological activities such as antioxidant properties, anti-inflammatory actions and anticancer activities.

Plant extract are extensively studied to isolate different active principles exhibiting antibacterial activity against Gram positive and Gram negative drug resistant and sensitive bacteria to over come the toxic effects of the presently prescribing synthetic pharmacological preparations [19, 20].

X. moluccesis stem and leaf extracts were screened for antibacterial activity against drug resistant Gram positive bacteria *B. cereus, B. subtilis*, *S. aureus* and Gram negative *E. coli*, *K. pneumoniae* and drug sensitive Gram positive *B. subtilis*, Gram negative *E. aerogenes* and *P. aeruginosa*. The results in the Table 1 and 2 clearly demonstrate that the extracts were found to be effective against Gram positive and Gram negative drug resistant and drug sensitive strains.

The reference antibiotic (tetracyclin) showed the highest antimicrobial activity against tested strains (Tables 1 and 2) with MIC ranging from 5-20mg/ml. The results of the present study support the traditional use of *X. moluccensis* as an ethno medicine. The present work on *X. moluccensis* leaf and stem needs further extension to isolate and characterize the active anti-bacterial principles.

In conclusion, our findings showed antimicrobial activity of *X. moluccesis* stem and leaf extracts. It is also concluded that the active principles isolated with protic polar solvents to associate with more antibacterial activity than non polar solubles against both Gram positive and Gram negative drug resistant and sensitive

selected bacterial strains. Further studies were aimed to isolate the active compounds from the positive extracts.

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REFERENCES

1. Ncube, N.S., A.J. Afolayan and A.I. Okoh, 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotechnol, 7: 1797-1806.
2. Arshad, M. and A. Rao, 2000. Medicinal plants of Farooqi, 1998, P: 1. Dahanukar S.A., R.A. Kulkarni, N.N. Rege, Pharmacology of Medicinal Plants and Natural Products. Indian J. Pharmacol., 32: S81-S118.
3. Premnathan, M., K. Chandra, S.K. Bajpai and K. Kathiresan, 1992. A survey of some Indian marine plants for antiviral activity. Botanica Marina., 35: 321-324.
4. Premnathan, M., H. Nakashima, K. Kathiresan, N. Rajendran and N. Yamamoto, 1996. *In Vitro* anti human immunodeficiency virus activity of mangrove plants. Indian Journal of Medicinal Research, 103: 278-281.
5. Kokpal, V., D.H. Miles, A.M. Payne and V. Chittarwong, 1990. Chemical constituents and bioactive compounds from mangrove plants. Studies in Natural products Chemistry, 7: 175-199.
6. Nascimento, G.G.F., J. Locatelli, P.C. Freitas, G.L. Silva, 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Braz. J. Microbiol., 31: 247-256.
7. Mijovie, G., B. Andric, D. Terzic, M. Lopicic, B. Dupanovic, 2012. Antibiotic susceptibility of Salmonella Spp.: A Comparison of two surveys with A 5 years interval. Journal of IMAB- Annual Proceeding (Scientific Papers), 18(1): 216-219.
8. Sangeeta, R., R. Smita and G. Alekha, 2012. Phytoceutical evaluation and antimicrobial properties of Eclipta alba and *Tylophora indica*.International Journal of Microbiology Research, 4: 227-230.
9. Han, L., X. Huang, H.M. Dahse, U. Moellmann, H. Fu, S. Grabley, I. Sattler and W. Lin, 2007. Unusual naphthoquinone derivatives from the Twigs of Avicennia marina. Journal of Natural Products, 70: 923-927.
10. Wu, J., Q. Xiao, J. Xu, M.Y. Li, J.Y. Pan and M.H. Yang, 2008. Natural products from true mangrove flora: source, chemistry and bioactivities. Natural Product Reports, 25: 955-981.
11. Lakshmi, V. and P. Gupta, 2008. An Overview of the genus Xylocarpus. Natural Product Research, 22: 1197-1224.
12. Bandaranayake, W.M., 1998. Traditional and medicinal uses of mangroves. Mangroves and Salt Marshes, 2: 133-148.
13. Kokpol, U., W. Chavasiri, V. Chittawong and D.H. Miles, 1990. Taraxeryl *cis*-*p*-hydroxycinnamate, a novel taraxeryl from Rhizophora apiculata. Journal of Natural Products, 53: 953-955.
14. Choudhury, S., A. Sree, S.C. Mukherjee, P. Patnaik and M. Bapuji, 2005. *In Vitro* Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens. Asian Fisheries Science, 18: 285-94.
15. Sharief, N. and V. Uma Maheswara Rao, 2011. Antibacterial activity of stem and root extracts of Avicennia officinalis L. International Journal of Pharmaceutical Applications, 2: 231-236.
16. Debnath, S., S. Palchoudhuri, N. Chatterjee, D. Sinharoy, S. Bhowmick, T.K. Pal, S. Das and S.G. Dastidar, 2013. Experimential evaluation of synergistic action between antibiotics and the antipsychotic antimicrobial triflupromazine. International Journal of Microbiology Research, 5: 430-434.
17. Nagababu, P. and V. Uma Maheswara Rao., 2012. Antibacterial activity and phytochemical screening of leaves and stem extracts of Avicennia alba blume. International Journal of Applied Biology and Pharmaceutical Technology, 3: 399-405.
18. Mukesh Chandra Sharma and Smita Sharma, 2010. Phytochemical and pharmacological screening of combined Mimosa pudica Linn and Tridax procumbens for *In vitro* antimicrobial activity. International Journal of Microbiological Research, 3: 171-174.
19. Sumathi, P. and A. Parvathi, 2010. Antimicrobial activity of some traditional medicinal plants. Journal of Medicinal Plants Research, 4: 316-321.
20. Varahalarao, V. and N.K. Chandrashekar, 2010. In vitro bioactivity of Indian medicinal plant Calotropis procera (Ait.). Journal of Global Pharma Technology 2(2): 43-45.