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Invitro Antibacterial Potentiality of *Luminitzera racemosa* Against Multiple Drug Resistant And Drug Sensitive Bacterial Strains

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Abstract: *Ethno-medicinal plants have been used throughout the world for centuries as drugs and remedies for various diseases. Since they have a great potential of producing diverse secondary metabolites that are benefit to mankind, scientists are attempting many approaches to identify the new biologically active principles in plants of different habitat. Green medicine is safer than synthetic drugs as these are associated with less cytotoxicity. This study was designed to evaluate the antibacterial activity of Luminitzera racemosa stem extract in different solvents against clinically important drug resistant Gram-positive strains viz., Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, drug resistant Gram-negative strains -Escherichia coli & Klebsiella pneumonia and drug sensitive Gram-positive strains- Bacillus subtilis and drug sensitive Gram-negative strains- Enterobacter aerogenes and Pseudomonas aeruginosa by agar well diffusion method. All the crude extracts were found to exhibit antibacterial activity against the drug resistant and drug sensitive test cultures. However, antibacterial potential varied from one extract to another in terms of zone of inhibition. The zone of inhibition was compared with standard broad spectrum antibiotics Gentamycin (30mcg/disc). Bioactive compounds infused in acetone exerted higher antibacterial potential against all tested strains, followed by ethanol, methanol and water. Drug resistant Klebsiella pneumonia strain was poorly inhibited by hexane extract. This study provides the necessary data for extraction and characterization of bioactive principles that possess the antibacterial action from acetone stem extracts of L.racemosa.*

Keywords: *Luminitzera racemosa; phyto chemical screening, Antibacterial activity and MIC.*

I. Introduction

Historically, the discovery of antibiotic penicillin was the conquest over the disease causing bacteria is one of the greatest success stories of modern medicine. Bacteria have adopted numerous defense mechanisms against antimicrobial agents and drug resistant pathogens are on the rise. In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented [1].These multidrug resistant bacteria have also created immense clinical problems around the world. Hence there is a need for the discovery of potent drugs to control multidrug resistant microorganisms. Plants remain the most common source of antimicrobial agents. Their use as traditional health care remedies is most popular in 80% of the world population in Asia, Latin America and Africa and reported to have minimal side effects [2, 3].

Medicinal value of plants lies in one or the other chemically active substances that produce a definite physiological action in living organisms. The most important among the bioactive constituents of plants are alkaloids, flavonoids tannins, steroids, etc [4, 5]. The natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [6]. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [7, 8]. Therefore, it is of great interest to carry out the screening of plants in order to validate their use in folk medicine, and to reveal the bioactive principle by isolation and characterization of their constituents. Systematic screening of plant extracts may result in the discovery of novel bioactive compounds [9, 10].

Mangroves are the plants found in the intertidal zones in tropical shorelines or estuaries. Because of their diverse habitat; these plants have been used in the treatment of human diseases in naturopathy and their products have pharmaceutical significance; they exhibit antimicrobial, antioxidant, antiviral, antihyper cholestermic and anti-insecticidal activity [11-14].

Luminitzera racemosa (*L.racemosa*) belongs to family combretaceae, order-myrtales. These are widely distributed in islands, East Africa to the West pacific including Fiji and Tonga, India and Northern Australia. L.racemosa is a black mangrove; white flowered, grey and fissured bark. Bark contains 15-19% tannins [15], while the leaves and wood contain smaller quantities. Studies have reported a long-chain rubber like polyisoprenoid alcohol from the leaves [16], flavonoids and long chain fatty acids and low molecular weight carbohydrates [17].Fluid obtained from incisions made in the stem was reported to be employed as an external application for the treatment of herpes and itches [18].Antihypertensive, antibacterial activity has been recently reported for the aqueous acetone extract of the plant [19, 20]. The main objective of this study is to screen the

antibacterial activity and phytochemicals of the nonpolar and polar solvents of *L.racemosa* (S) collected from Coringa Reserve Forest, Kakinada, India.

II. Materials And Methods

2.1 Plant materials:

L.racemosa stems were collected from Corangi Reserved Forest, Kakinada, East Godavari, Andhra Pradesh, India. Geographic location - between 16^o 39' N longitude - 17^o N longitude and 82^o 14' E latitude - 82^o 23'E latitude. All the stems were surface sterilized with 1% mercuric chloride and thoroughly washed with plenty of distilled water. The plant material was dried under shade with occasional mixing. Later, the stems are chopped into small piece and stored in an airtight container.

2.2 Extraction:

The chopped stem material *L.racemosa* (100g) was extracted separately into different solvents in the decreasing order of lyophillicity viz hexane, benzene, ethyl acetate, chloroform, acetone, absolute alcohol, methanol and distilled water [21].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 minimal volumes of dimethylsulfoxide (DMSO) and the concentration was adjusted to 100 mg/ml with water and stored at 4°C.

2.3 Bacterial strains:

Pure cultures of *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 430), *Escherichia coli* (MTCC40), *Klebsiella pneumonia* (MTCC 39) (drug resistant strains) (the strains are resistant to the following drugs *S.aureus*-erythromycin, *B.subtilis*-cephalexin, *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. The active cultures were generated by inoculating a loopful of culture in separate 100mL nutrient broths and incubated at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 15min, washed with normal saline, spun at 4000 rpm for 15 min again and diluted in normal saline to obtain 1-2×10 CFU/ml [22].The amount of bacterial culture (OD-0.01) needed to undertake the study was determined usingUV/Vis spectrophotometer (ELICO, India) at 625 nm.

2.4 Invitro determination of antimicrobial activity:

The antibacterial activity of *L.racemosa* stem extracts were performed by agar well diffusion method [23]. About 20 ml of melted (at about 50°C) nutrient agar was mixed with 0.5 ml of bacterial suspension homogeneously and allowed to solidify in petri dishes (143 mm diameter). Wells (10mm diameter) were punched using a sterile cork borer. The wells were filled with 200µl of the crude extract containing 5 - 20mg/ml and incubated at 37°C under macro aerophillic conditions. All the tests were performed in triplicates. Gentamycin (30mcg/disc) used as standard reference antibiotics; and the diameters of the inhibition zones were measured and their means were calculated. DMSO in water was taken as solvent control.

2.5 Determination of minimum inhibitory concentrations MIC:

The MIC was determined using the nutrient agar broth dilution method [24-26]. Sterile broth was dispensed into each test tubes containing crude extract between 5-20mg/ml to evaluate MIC. 0.1ml of standard culture was added to each tube and was incubated at 37°C for 24h. Each experiment was performed in triplicates and the mean standard deviation values were calculated. The lowest concentration of the extract that produced no visible sign of bacterial growth when compared with the control tubes was considered as MIC.

2.6 Phytochemical evaluation:

A qualitative phytochemical test to detect the presence of alkaloids, tannins, terpenoids, steroids, saponins, flavonoids, glycosides, anthraquinone and phenols were carried out using standard procedures [27-29] and the results were shown in Table 2.

2.7 Statistical analysis:

The experimental data were analyzed by applying mean and standard Deviation using excel software, windows 8.1 version.

III. Results And Discussion

The antibacterial and phytochemical assessment of studied *L.racemosa* stem extracts was found to be

most active against all tested bacteria with inhibition zones in the range of 1-14mm as shown in Table 1a&Table1b. The minimal inhibitory concentration (MIC) values of eight solvent extracts against the tested bacteria were found to range from ≤ 5 to ≥ 20 mg/ml. The most active solvent extracts are acetone, methanol and aqueous extracts (MIC: ≤ 11-14mm). Of these extracts, acetone exerted highest antibacterial activity on drug resistant Gram -ve *K.pneumonia* (14mm) than the methanol & aqueous extracts 13mm, 12mm respectively, moderate activity was observed with acetone extract on drug resistant Gram +ve *B.cereus* (11mm) while a least activity was associated with hexane extract (MIC:≤1-3mm) on drug-resistant *K.pneumonia* (1mm), *E.coli* (2mm) and drug sensitive *B.subtilis* (3mm). Phytochemical analysis data shown in Table-2 clearly supports the activity of different crude extracts in different solvents on the tested bacterial cultures. The development of drug resistance limits the usefulness of all known antimicrobials. In addition to, there are so many reasons for development of resistance. Sensitive bacteria can acquire antibiotic resistance. Acquisition of antimicrobial resistance can also occur through spontaneous mutation or through DNA transfer [30]. This study also supports, that the phytochemicals of this plant controls the proliferation of both drug resistant and drug sensitive strains. In response to the increased resistance, the pharmaceutical industry is searching to develop new antimicrobial drugs. Recently, the acceptances of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics have led researches to investigate and isolate the antimicrobial activity of medicinal plants. In Previous references, a variety of biological activities have been published including anti inflammatory, antioxidant and others [31, 32]. Further study is necessary for the separation, purification and characterization of biologically active compounds. However this family of compounds has diverse range of activities in mammalian cells and in vivo confirmation of their side effects would be necessary for complete evaluation of their practical usefulness in modern medicine.

Table (2): Phytochemical analysis of *L.racemosa* stem extracts in different solvents

S.N	Phytochemical	H	B	EA	C	A	E	M	W
1	Alkaloids	-	-	+	-	-	+	-	+
2	Flavonoids	+	+	+	-	+	+	+	+
3	Glycosides	+	+	+	+	+	+	+	+
4	Phenols	-	-	-	-	+	+	+	+
5	Saponins	-	+	-	-	+	+	+	+
6	Steroids	+	+	+	-	+	+	+	+
7	Terpenoids	-	+	-	-	+	+	-	+
8	Tannins	-	-	-	+	+	+	-	+
9	Anthraquinones	-	-	-	-	-	+	+	+

Table (1a): Antibacterial activity of *L.racemosa* stem extracts on drug resistant microorganisms

S.N	Microorganism	Con	H	B	EA	C	A	E	M	W	Genta-mycin (30mcg)
1	Escherichia coli(MTCC 40)	5	-	-	1 ± 0.381	1±0.381	5.2±0.28	5.1±0.23	5±1.723	5.1±0.23	14
		10	-	-	4.1±0.88	1±0.412	5.1±0.23	5.1±0.23	6±1.512	7 ±0.319	
		15	1 ± 0.000	-	5 ± 1.723	2±0.319	7.2±0.91	6.2±0.16	7±2.770	8.5±1.20	
		20	2 ± 0.615	3.2±0.00	6.9±1.73	3±0.385	8 ± 0.35	7 ± 1.538	7±0.319	9 ± 3.505	
2	Klebsiella pneumonia(MTCC 39)	5	-	-	-	-	9±0.529	7.1±0.18	9±0.557	7 ± 1.044	17
		10	-	-	-	-	10±0.51	8 ± 0.458	9±0.623	8.2±0.37	
		15	-	2 ± 0.321	2.3±0.85	-	13±0.50	8±0.458	10±0.14	9±3.518	
		20	1 ± 0.381	3.3±0.38	3.5±1.35	2±0.281	14±0.50	9 ± 0.298	13±0.29	12±3.939	
3	Bacillus cereus(MTCC 430),	5	1 ± 0.312	-	4.1±0.18	1.5±0.2	9±0.634	7±0.294	8±0.312	4.1±0.18	13
		10	3 ± 0.554	-	5 ± 0.538	3±0.528	10±0.52	7.2±0.28	9±0.557	6.1±0.18	
		15	4 ± 0.110	2 ± 1.490	5 ± 0.557	5±0.319	10±0.40	8.1±0.15	10±0.14	6.6±0.09	
		20	6 ± 0.591	4.1±0.18	6 ± 0.529	6±0.472	11±0.42	8.1±0.15	10±0.51	7.2±0.28	
4	Bacillus subtilis(MTCC 441)	5	1 ± 0.000	-	1.9±0.57	1±0.000	8±0.883	7 ± 1.538	7±1.700	6 ± 0.332	18
		10	2.03±0.6	-	4 ± 1.499	3±3.000	9±0.529	8 ± 0.651	8±0.883	6.9±1.72	
		15	5.2±0.28	2 ± 0.281	5.2±0.63	4±1.499	10±0.51	8 ± 0.312	9±0.529	7 ± 0.410	
		20	6 ± 0.329	5 ± 0.000	6 ± 0.188	6.9±1.7	10±0.14	9 ± 0.557	9±0.557	8 ± 0.312	
5	Staphylococcus aureus (MTCC 87)	5	-	-	1 ± 0.412	2.5±0.9	5±1.001	6 ± 2.414	6±2.325	4.1±0.88	17
		10	-	-	2 ± 0.847	4.2±1.5	5±1.001	6 ± 2.325	7±1.538	5.1±1.20	
		15	2 ± 0.281	3.1±0.23	4.5±1.74	4±1.051	7±0.319	7 ± 2.755	7±1.700	5.5±1.84	
		20	4 ± 1.093	3.1±0.23	5.2±0.06	6±2.414	8±0.883	8 ± 0.651	8.1±2.6	6 ± 2.325	

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

The data presented as mean value ± standard deviation; n=triplicates. Standard antibiotic Gentamycin used for antibacterial activity against tested bacterial strains.The zone of inhibition was measured in mm.

Table (1b): Antibacterial activity of *L.racemosa* stem extracts on drug sensitive microorganisms

S.N	Microorganism	Con	H	B	EA	C	A	E	M	W	Gentamycin(30mcg)
1	Bacillus subtilis (MTCC 121)	5	-	-	0.1±0.06	2±0.847	5±1.895	5 ± 0.627	3±0.000	4.2±1.557	15
		10	-	2.5±0.942	4 ± 1.051	2±0.886	5±0.312	6.3±0.22	4.1±1.5	5 ± 0.554	
		15	0.1±0.05	2± 0.847	5 ± 1.895	3.1±0.88	6±0.543	7 ± 0.500	5±0.600	5 ± 0.07	
		20	3 ± 1.136	4.5±1.807	6 ± 2.325	4.5±1.71	7±0.555	7 ± 0.256	6.1±0.2	6 ± 0.338	
2	Enterobacter aerogenes (MTCC 111)	5	3 ± 0.472	3 ± 0.472	6 ± 0.538	4.7±0.83	8±0.642	9 ± 0.395	9±0.557	6 ± 0.538	19
		10	3 ± 0.472	5.1±0.319	6 ± 0.529	5±0.627	8 ±0.53	9 ± 0.623	9.7±0.5	7 ± 0.256	
		15	5.1±0.31	6 ± 0.472	7.6±0.30	6±0.529	9±0.623	10±0.360	10±0.42	7.6±0.309	
		20	6.2 0.884	7 ± 0.152	9.7±0.52	7±0.544	9±0.529	10±0.548	10±0.36	8 ± 0.651	
3	Pseudomonas aeruginosa(MTCC 424	5	2 ± 0.092	2± 0.651	2.5±0.94	-	7±0.663	5 ± 0.538	5±0.538	6 ± 0.529	15
		10	3.1 ± 0.230	3 ± 1.814	4 ± 1.528	-	8±0.557	7 ± 0.544	5±0.557	7 ± 0.529	
		15	5.2±0.28	5 ± 0.538	5.4±1.26	2±0.360	9±0.557	7 ± 0.529	6±0.529	7 ± 0.529	
		20	5.1±0.23	6 ± 0.529	6 ± 0.538	4.7±1.83	9±1.435	8 ± 0.642	7±0.544	8 ± 0.642	

IV. Conclusion

The crude stem extracts of *L.racemosa* screened for antibacterial activity against drug resistant and drug sensitive strains. Their potency is quantitatively assessed in terms of zone diameter and compared with Gentamycin. The acetone extracts showed the highest antibacterial activity compared to methanol and aqueous extracts. The result of the study shows that *L.racemosa* stems possess a broad spectrum of activity against a panel of microorganisms responsible for most common microbial diseases. Further research is necessary to identify and isolate the pure active compounds from *L.racemosa*.

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References

[1]. Jones,M.E.,Draghi,D.C.,Thornsberry,C.,Karlowsky,J.A.,Sahm,D.F.,Wenzel,R.P,Emerging resistance among bacterial pathogens in the intensive care unit-a European and North American Survivallence study(2000-2002) Ann.Clin.Microbiol.Antimicrob, 3 ,2004;14.
[2]. Kloucek P, Polesny Z, Svobodova B, Vlkova E, Kokoska L. Antibacterial screening of some Peruvian medicinal plants used in Calleria District,J. Ethnopharmacol, 99, 2005 ;309–312.
[3]. DuraipandiyanV, AuyanaM, IgnacimuthuS.Antimicrobial activity of some Ethno medicinal plants used by Paliyar tribe from TamilNadu, India.BMC Complementary and Alternative Medicine, 20, 2006; 6–35.
[4]. Edeoga HO, Okwu DE, M bebie BO, Phytochemical constituents of some Nigerian Medicinal plants, African Journal of Biotechnology, 4, 2005; 685-688.
[5]. Gutal Valyfathulla Shaik and M. Pavani, Antibacterial Activity of Leaf Extracts of Indian Medicinal Plant *Argyreia involucrate*, International Journal of Research in Pharmaceutical and Biomedical Sciences , 2(4),2011; 1701-1705.
[6]. Barbour EK, Al-Sharif M, Sagherian VK, Habre AN, Talhouk RS and Talhouk SN, Screening of selected indigeneous plants of Lebanon for antimicrobial activity, J. Ethnopharmacol, 93, 2004; 1 –7.
[7]. Murray M, The healing power of herbs (Rocklin, C.A: primapublishing, 1995) pp.163-172.
[8]. Eloff JN, Famakin JO, Katerere DRP.Combretum woodii (combretaceae) leaf extracts has high activity against gram-negative and gram-positive bacteria, Afr.J.Biotechnol, 4, 2005; 1161-1166.
[9]. PushpaD Damayanthi Abeysinghe,Antibacterial activity of aqueous and ethanol extracts of mangrove species collected from Southern Sri Lanka,Asian J Pharm Biol, 2 (1)2012; 79-83.
[10]. Janovská D, Kubíková K, Kokoška L, Screening for antibacterial activity of some medicinal plant species of traditional Chinese medicine, Czech J. Food Sci, 21, 2003; 107-110.
[11]. Bandaranayke, W.M.Traditional and medicinal uses of mangroves, Mangroves and Salt Marshes, 2, 1998; 133-148.
[12]. Premnathan, M., K.Chandra, S.K.Bajpai and K.Kathiresan, A survey of some Indian marine plants for antiviral activity, Botanical Marina, 35, 1992; 321-324.
[13]. Premnathan, M., H.Nakashima, K.Kathiresan, N.Rajendran and N. Yamamoto, In Vitro anti human immunodeficiency virus activity of mangrove plants, Indian Journal of Medicinal Research, 103,1996; 278-281.
[14]. Kokpal,V.,D.H.Miles,A.M.PayneandV.Chittarwong,Chemical constituents And Bioactive Compounds from mangrove plants, Studies in Natural products Chemistry, 7,1990;175-199.
[15]. Ta-chen L, Feng-lin H, Juei-Tang C, 56, (J. Nat. Prod , 1993) 629.
[16]. SkoczylasE, SwiezewskaE, Chojnacki T, Tanaka Y, 32 (Plant Physiol.Bioche, 1994) 825.
[17]. Ammanamanchi S.R.Anjaneyulu, Yellajosyula L.N.Murthy, Vadali Lakshmana Rao, Karanam Sreedhar.A new aromatic ester from the mangrove plant Lumnitzera racemosa willd, ARKIVOC. (3), 2003; 25-30.
[18]. V. P. Upadhyay, P. K. Mishra and J. R. Sahu. Distribution of Mangrove Species within, Bhitarkanika National Park in Orissa, India. Vol 3(Trees for Life Journal2008) 1-5.
[19]. Lin Ta-chen, Hsu Feng-lin, Cheng Juei-Tang,Antihypertensive Activity of Corilagin and Chebulinic Acid, Tannins from *Lumnitzera racemosa* ,J. Nat. Prod ,56(4)1993; 629-632.
[20]. Lisette D'Souza, Solimabi Wahidulla and Prabha Devi, Antibacterial phenolics from mangrove *Luminitzera racemosa*, Indian

Journal of Marine Sciences, 39(2), 2010; 294-298.
[21]. Choudhury, S., A. Sree, S.C. Mukherjee, P. Patnaikand M. Bapuji, In Vitro Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens, Asian Fisheries Science, 18, 2005; 285-294.
[22]. Varaprasad Bobbarala, Varahala Rao Vadlapudi and K.Chandrasekhar Naidu, Antimicrobial potentialities of Mangrove, *Avicennia marina*.Journal of Pharmacy Research, 2 (6), 2009; 1019-1021.
[23]. Sharief, N. and V. Uma Maheswara Rao, Antibacterial activity of stem and root extracts of *Avicennia officinalis* L. International Journal of Pharmaceutical Applications, 2, 2011; 231-236.
[24]. Bonjar Shahidi GH, Evaluation of Antibacterial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumonia and Bordetella bronchoseptica, Asian. j.sci 3 (1), 2004; 82-86.
[25]. Okoli S, Iroegbu CU,*In vitro* antibacterial activity of Synclisa scabrida whole root Extracts,African journal of biotechnology, 4, 2005; 946-952.
[26]. Damintoti K, Mamoudou HD, Jacques S, Alfred ST,Antioxidant and antibacterial activities of polyphenols from ethno medicinal plants of Burkina faso,African Journal of Biotechnology 4(8) 2005;823-828.
[27]. Nagababu P and V. Uma MaheswaraRao, Antibacterial activity and phytochemical screening of leaves and stem extracts of *Avicennia alba* blume, International Journal of Applied BiologyandPharmaceutical Technology, 3, 2012; 399-405.
[28]. Zaid O Ibraheem, Muvavvar A. Sattar, Nor A Abdullah, Rathoore Hassan, and Edward J Johns,Toxicity ,Phytochemical content and antioxidant assessment studies for a standardized ethanolic fraction of palm oil leaf extract ,Pharmacognosy Communication , 2(1) 2012,21-30.
[29]. Rahmi Nurdiani, Muhamad Firduas, and Asep Awaludin Prihanto, Phytochemical screening and antibacterial activity of methanol extract of mangrove plant *Rhizophora mucronata* from Porong River Estuary, Journal of Basic Science and Technology, 1(2) ,2012; 27-29.
[30]. Masuda, T., K.Iritani, S. Yonemori, Y. Oyama andY. Takeda, Isolation and antioxidant activity of Galloyl Flavonal, Glycosides from the seashore plants *Pemphis acidula*.Bioscience, Biotechnology and Biochemistry, 65(6), 2001; 1302-1309.
[31]. Lin Y M, AndersonH, flavinMT& paiYHS,Invitro Anti-HIV activity of bioflavonoids isolated from Rhus succedanea and Garcinia multiflora ,J.Nat.prod, 60, 1997;884&references within.
[32]. VlietinckAJ, De BruyneT, Apers S& PietersLA,Plant-derived leading Compounds For chemotherapy of humanimmuno deficiency virus (HIV) infection,PlantaMed , 64,1998;97-109.