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Full Length Research Paper

Antimicrobial activities of *plumeria acutifolia*

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Ethanolic extract of *Plumeria acutifolia* Poir. (Apocynaceae) stembark was tested for antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Klebsiella Pnumoniae, Psuedomonas aeruginosa, Salmonella typhimurium)* and fungi (*Aspergillus niger* and *candida albicans*) by disc diffusion method. Minimal inhibitory concentration (MIC) and acute toxicity were also assayed. The ethanol extract showed the strong *in vitro* antimicrobial activity against *E. faecalis, B. subtilis, S. aureus, P. aeruginosa, S. typhimurium, A. niger* and *C. albicans.* The extract did not show any toxic symptoms against the tested mice.

Key words: *Plumeria acutifolia* Poir, antibacterial activity, antifungal activity, disc diffusion, M.I.C, M.B.C/M.F.C, acute toxicity.

INTRODUCTION

The steadily increasing bacterial resistance to existing drugs is a serious problem in antibacterial therapy and necessitates continuing research into new classes of antibacterials (Essawi and Srour, 2000; Woodford, 2003). One way to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). Plants and plant derived agents have long history to clinical relevance as source of potential chemotherapeutic agents (Cushnie et al., 2005). Thousands of plant species have been screened for their antimicrobial activity, but relatively few were found to be sufficiently active (Poyart – Salmeron et al., 1990; Meng et al., 1998) and non toxic to humans (Izzo, 2004). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new antiinfective agents (Amani et al., 1998; Salvat et al., 2001; Ordonez et al., 2003; Arias et al., 2004) with possibly novel mechanisms of action (Hamil et al., 2003; Machado et al., 2003; Motsei et al., 2003; Barbour et al., 2004).

Plumeria acutifolia Poir. (Apocynaceae) is extensively cultivated in India and familiarly known as temple tree or tree of love. The stem bark of this plant has been employed against abscess, gonorrhoea and fevers. The Juice of stembark from this plant has been applied to carious teeth, ulcers and wounds and is rubifacient and anti rheumatic (Bastin, 1895). This work is the first attempt to study the antimicrobial activity and cytotoxicity assay of ethanol extract of *P. acutifolia* stembark.

MATERIALS AND METHODS

Plant material

The stembark of *P. acutifolia* Poir. was collected from a temple, Mahanandi, Nandyal, A.P, India. The Plant was identified and voucher specimen (NPGC/PV/009) was deposited at department of Botany, National Postgraduate College and Research Centre, Nandyal, A.P, India. Information on the use of these medicinal plants was gathered from a literature review and interviews had with traditional healers.

Preparation of plant extracts

The stembark of the plant was shade dried and ground into powder. About 1 kg of powdered material was soxhlet extracted with n–hexane. The n–hexane exhausted material was extracted with absolute ethanol. The ethanol extract was filtered using whattman filter paper (No.1) and then concentrated under vacuum at 40^{o}C using a Rotary Evaporator. The residue obtained was dissolved in 10% Dimethyl sulfoxide to a concentration of 100 mg/ml and stored

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at -20°C prior to analysis.

Antimicrobial activity

Microbial strains and growth media

The antimicrobial activity of ethanol extract of *P. acutifolia* stembark was evaluated using the following strains of bacteria and fungi, Gram-positive bacteria: *Bacillus subtilis* (ATCC 26633), *Enterococcus faecalis* (ATCC29212), *Staphylococcus aureus* (ATCC 25923), Gram – negative bacteria: *Escherichia coli* (ATCC 25922), *Klebsiella pnumoniae* (ATCC 13883), *Psuedomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 19430) and fungal strains : *Aspergillus niger (ATCC 16404) and* and *Candida albicans* (ATCC 10231). Stock cultures were maintained at 4°C on slants of tryptic soy broth amended with 5 g/L yeast extract and 15 g/l Agar agar (Himedia chemicals, India). Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to flasks of Mueller – Hinton broth (MHB) (Himedia, India) for bacteria and Sabouraud dextrose broth (SDB) (Himedia, India) for fungi that were incubated at 37 and 25°C for 24 h. The cultures were diluted with fresh Mueller–Hinton and Sabouraud dextrose broth to achieve optical densities corresponding to 2.0 x 10^6 CFU/ml for bacteria and 2.0 x 10^5 spores/ml for fungal strains.

Disc diffusion method

The ethanol extract was tested for antibacterial and antifungal activities by the disc diffusion method (Bauer et al., 1966; NCCLS, 2001, 2004; CLSI, 2006b) using 100 µl of suspension of the tested microorganisms, containing 2.0x 10^6 CFU/ml for bacteria and 2.0 x 10^5 CFU/ml spores for fungal strains. Mueller – Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were sterilized in flasks, cooled to 45 – 50°C and then distributed to sterilized Petri dishes (9 cm). Sterile filter paper discs of 6 mm diameter were impregnated with 25 µl of extract solution (equivalent to 2.5 mg of the dried extract) and then placed on to agar plates which had previously been inoculated with the tested microorganisms. The Petri dishes were kept at 4°C for 24 h. The plates were inoculated with bacteria incubated at 37°C for 24 h and at 30°C for 48 h for the fungal strains. The diameters of the inhibition zones were measured in millimeters. All the tests were performed in triplicate. Gentamycin (30 µg) and nystatin (30 µg) served as positive controls and 10% Dimethyl sulfoxide served as a negative control.

Broth microdilution method

A broth microdilution method was used to determine M.I.C and M.B.C or M.F.C (NCCLS, 2001, 2004; CLSI, 2006b). All tests were performed in Mueller Hinton broth (MHB) supplemented with Tween – 80 detergent to a final concentration of 0.5% (v/v), with the exception of the fungal strains (Sabouraud dextrose broth – SDB + Tween – 80). Geometric dilutions ranging from 0.03 to 500 µg/ml of the ethanol extract were prepared in a 96 – well microtitre plate. Overnight broth cultures of each strain were prepared and the final concentration in each well was adjusted to 2.0 x 10^6 CFU/ml for bacteria and 2.0 x 10^5 CFU/mL spores for fungal strains. Petri dishes were kept at 4°C for 2 h. Bacteria were incubated at 37°C for 24 h and fungal strains were incubated at 25°C for 48 h. The M.I.C was defined as the lowest concentration of test sample that resulted in a complete inhibition of visible growth. The microbial growth was indicated by the presence of a white “pellet” on the well bottom. To determine M.B.C/M.F.C, broth was taken from the well that showed no visible growth and inoculated in MHA for 24 h at 37°C for bacteria or in SDA for 48 h at 27°C for fungi. M.B.C or M.F.C were defined as the lowest concentration of test sample at which 99.9% of the microorganisms were killed. All determinations were performed in triplicate and two growth controls consisting of MHB and SDB media with 0.5% Tween-80 were included. Gentamycin and nystatin served as positive controls for bacteria and fungi respectively.

Determination of acute toxicity

The acute toxicity test was performed according to Lorke (1983) and Shilpi et al. (2006). Swiss Albino mice (Weighing 20 – 25 g) of either sex were obtained from animal house, Bangalore University, Bangalore, India. The animals were kept in the departmental animal house at 23.0 ± 2.0°C, relative humidity 44 – 56% and light dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. The animals were fed with a standard diet and water *ad libitum*. All experiments were performed in the morning according to current guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

Test animals were divided into groups (n = 6 per group) which were administered different doses of the crude extract (50, 100, 200, 400, 800, 1600 and 3200 mg/kg p.o). The control group received 1% Tween –80 in water, p.o. The general signs and symptoms of toxicity were observed for 24 h.

RESULTS AND DISCUSSION

The *in vitro* antimicrobial activity of *P. acutifolia* stembark ethanol extract against the microorganisms employed was assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters, M.I.C and M.B.C or M.F.C values. The ethanol extract of *P. acutifolia* stembark showed great *in vitro* antimicrobial activities against all 7 bacteria and 2 yeast species tested. In this study, the antimicrobial activities of ethanol extract were compared with standard antibiotics such as gentamycin and nystatin used as positive controls. Results from the antimicrobial disc diffusion assay were furnished in Table1. The data indicated that Gram – positive *e. faecalis* was the most sensitive strain tested to the ethanol extract of *P. acutifolia* stembark with the greatest inhibition zone of 21 mm. The *B. subtilis* and *S. aureus* were also found to be more sensitive with inhibition zones of 26 and 26 mm respectively. The ethanol extract of *P. acutifolia* stembark also showed excellent activity against tested Gram – negative bacteriae. *E. coli* was the most sensitive organism among Gram – negative bacteria with the inhibition zone of 33 mm more than that of standard gentamycin positive control. *K. pneumoniae, P. aeruginosa* and *S. typhimurium* also exhibited significant sensitivities to the tested ethanol crude extract with the inhibition zones of 18, 16 and 20 mm respectively. The ethanol extract of *P. acutifolia* stembark also exhibited the highest antifungal activity against *Aspergillus niger* and *C. albicans* with the inhibition zones of 19 and 20 mm respectively.

The results of the M.I.C and M.B.C or M.F.C were shown in Table 2. The data indicated that the ethanol extract of *P. acutifolia* stem bark exhibited significant levels

Table 1. Antimicrobial activity of the ethanol extract of *P. acutifolia* stembark (11.8% yield) by disc diffusion method.

Microorganisms	Ethanol extract*	Antimicrobial agents
	Inhibition zone (mm)	Inhibition zone (mm)
Gram – positive		Gentamycin [a]
Bacillus subtilis	26	26
Enterococcus faecalis	21	18
Staphylococcus aureus	26	27
Gram – negative		
Escherichia coli	33	31
Klebsiella pneumoniae	18	19
Pseudomonas aeruginosa	16	19
Salmonella typhimurium	20	28
Fungi		Nystatin [b]
Aspergillus niger	19	20
Candida albicans	20	23

Values are mean of triplicates.
*Ethanol extract concentration (2.5 mg/ disc)
[a]Gentamycin (30 µg/disc)
[b]Nystatin (30 µg/disc)

Table 2. Antimicrobial activity expressed as minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC) of ethanol extract of *P. acutifolia* stembark by broth microdilution method.

Microorganisms	Ethanol Extract*		Antimicrobial agents	
	MIC[a]	MBC / MFC[b]	MIC[a]	MBC / MFC[b]
Gram-positive			Gentamycin	
Bacillus subtilis	250	250	0.5	2.0
Enterococcus faecalis	125	250	1.5	12.0
Staphylococcus aureus	125	250	0.25	10.0
Gram-negative				
Escherichia coli	125	250	0.02	2.0
Klebsiella pnumoniae	250	500	0.08	6.0
Psuedomonas aeruginosa	125	250	0.05	10.0
Salmonella typhimurium	250	250	1.0	2.0
Fungi			Nystatin	
Aspergillus niger	125	250	0.50	2.0
Candida albicans	125	250	1.5	2.0

Values are mean of triplicates.
*Ethanol extract yield (11.8 %).
[a]Minimal Inhibitory Concentration in µg / mL.
[b] Minimal Bactericidal or Fungicidal Concentration in µg / mL.

of antimicrobial activity against the investigated bacteriae and fungi. The inhibitory properties of the crude ethanol extract were observed with in a range of concentrations from 125 to 250 µg/ml. Maximum activity was observed against the Gram – positive strains such as *E. faecalis* and *S. aureus*, the Gram – negative strains such as *E. coli* and *P. aeruginosa* and the yeasts such as *C. albicans* and *A. niger* with M.I.C. value of 125 and M.B.C/M.F.C value of 250 µg/mL to the tested ethanol extract. Most antibacterial medicinal plants attack Gram-positive strains while few are active against Gram–negative bacteria (Herrera et al., 1996; Meng et al., 2000; Srinivasan et al., 2001). *P. aeruginosa* is a serious problem to the world with its multidrug resistant properties (Tacconelli et al., 2002). However, our results showed that Gram – negative bacteria such as *P. aeruginosa* showed the significant MIC of 125 and inhibition zone of 16 mm to the tested ethanol extract.

Lethal effects were not observed within 24 h after the administration of the ethanol extract of *P. acutifolia* stembark at any of the doses used, even at the highest dose tested (3200 mg/kg). There fore, the lethal dose (LD 50)

of the ethanol extract in mice could not be deter-mined. There were no previous published reports on the antimicrobial activity of *P. acutifolia* and therefore our results can be considered as the first report. Further investigation to obtain information on chemical composition, to purify and determine the structures of active principles has been in progress in our laboratory.

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REFERENCES

Amani S, Isla MI, Vattuone M, Poch M, Cudmani N, Sampietro A (1998). Antimicrobial activities in some Argentine Medicinal Plants. Acta Horticulture. 501:115-122.

Arias ME, Gomez JD, Cudmani N, Vattuone MA, Isla MI (2004). Antibacterial activity of ethanolic and aqueous extract of *Acacia aroma* Gill ex Hook et. Life Science. 75:191-202.

Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN (2004). Screening of selected indigenous plants of Lebanon for antimicrobial activity. J. Ethnopharmacol. 93:1-7.

Bastin ES (1895). Botanical medicine monographs and sundry. *Am. J. Pharmacy* 67:18-19.

Bauer A W, Kirby W M M, Sheriss J C, Turck M (1966). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clinical pathol 45:493-496.

CLSI - Clinical and Laboratory standards Institute (2006b). Performance standards for anti-microbial susceptibility testing. 16th informational supplement. M100-S16. Wayne, PA, USA : CLSI.

Cushnie TP, Lamb AJ (2005). Anti microbial activities of flavonoids. Int. J. Antimicrob. Agents 26:343.

Essawi T, Srour M (2000). Screening of some Palestinian medicinal plants for antibacterial activity. J. Ethnopharmacol. 70:343–349.

Hamil FA, Apio S, Mubiru NK, Bukenya-Ziraba R, Mosango M, Maganyi OW, Soejarto DD (2003). Traditional herbal drugs of southern uganda, II : Literature anályis and antimicrobial assays. J. Ethnopharmacol. 84:57-78.

Herrera RM, Perez M, Martin-Herrera DA, Lopez-Garnia R, Rabanal RM (1996). Antimicrobial activity of extracts from plants endemics to the canary Islands. Phytotheraphy Research 10: 364-366.

Izzo AA (2004). Drug interactions with St. John's wort (*Hypericum perforation*): a review of the clinical evidence. Int. J. Clin. Pharmacol. Thera 42:139-148.

Lorke D (1983). A new apporach to acute toxicity testing. Archives of Toxicol. 54:275-287.

Machado T B, Pinto A V, Pinto M C F R, Leal I C R, Silva M G, Amaral A C F, Kuster R M, Netto – dosSantos K R (2003). Invitro activity of Brazilian medicinal plants, naturally occurring naphthoqinones and their analogues, against methicillin–resistant staphylococcus aureus. Int. J. of Antimicrobial Agents 21:279-284.

Meng JC, Zhu QX, Tan RX (2000). New antimicrobial mono- and sesquiterpenes from *soroseris hookeriana Subsp.erysimoides*. Planta Medica 66: 541-544.

Motsei ML, Lindsey KL, Van Staden J, Jaeger AK (2003). Screening of traditionally used South African plants for anti fungal activity against *candida albicans*. J. Ethnopharmacol. 86: 235-241.

NCCLS - National Committee for Clinical Laboratory Standards(2001) . Performance standards for anti-microbial susceptibility testing : eleventh informational supplement. Document M100 – S11. National Committee for Clinical Laboratory Standars, Wayne, PA, USA.

NCCLS – National Committee for Clinical Laboratory Standards (2004). Performance standards for Antimicrobial susceptibility testing. Fourteenth informational supplemented. M100-S14, Wayne, PA, USA.

Ordonez AA, Cudmani NM, Gomez D, Vattuone MA, Isla MI (2003). Antimicrobial activity of nine extracts of sechium edule (Jacq) Swartz. Microbiology Ecology in Health and Disease 15:33-39.

Poyart-Salmeron C, Carlier C, Trieu-cuot P, Courtieu A L, Courvalin P (1990). Transferable plasmid – mediated antibiotic resistance in Listeria monocytogenes. *The Lancet* 335:1422-1426.

Salvat A, Antonnacci L, Fartunato RH, Suarez EY, Godoy HM (2001). Screening of some plants from Northern Argentina for their antimicrobial activity. Letters in Applied Microbiology 32: 293-297

Shah PM (2005). The need for new therapeutic agents: What is in the pipeline? Clinical Microbiology and infection. 11: 36-42.

Shilpi JA, Taufiq-ur-Rahman MD, Jamal Uddin S, Shahanur Alam Md, Samir KS, Seidel V (2006). Preliminary pharmacological screening of *Bixa orellana* L. leaves. J. Ethnopharamcol. 108: 264-271.

Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P (2001). Antimicrobial activity of certain Indian Medicinal Plants used in folk loric medicine. J. Ethnopharmacol. 74: 217-220.

Tacconelli E, Tumbarello M, Bertagnolio S, Citton R, Sparu T, Fadda Q, Cauda R (2002). Multidrug – resistant pseudomonas aeruginosa blood stream infections: Analysis of trends in prevalence and epidemiology. Emerg. Infect. Dis 8: 220-221.

Woodford N, (2003).Novel agents for the treatment of resistant Gram-positive infections. Expert Opin. Investig. Drugs 12:117.

Zimmerman M (1983). Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16: 109-110.