Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 5, 2011

Research Article

COMPARATIVE ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF THE METHANOLIC EXTRACT OF LEAVES OF *PLUMERIA ALBA* LINN. (APOCYNACEAE)

SANGITA KUMARI *[1], AVIJIT MAZUMDER [1], S. BHATTACHARYA[2], RUPA MAZUMDER [1] AND SHILPA PAHWA[1]

[1.] Dept. of Pharm. Technology, Noida Institute of Engineering and Technology, Greater Noida U.P. India, [2.] NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan India. Email: sangi_mph04@yahoo.co.in

Received: 12 Aug 2011, Revised and Accepted: 16 Nov 2011

ABSTRACT

The present study was designed to evaluate the antibacterial and antifungal efficacy of the methanolic extract of the leaves of *Plumeria alba* Linn. (Apocynaceae) against various diarrhea and dysentery causing drug resistant microorganisms isolated from patients admitted in hospitals. The methanolic extract of the leaves was found to be most effective against *Shigella flexneri* type 36 NK 381, *Sh. flexneri* type BCH 995, *Sh. boydii* 22461, *Sh. soneii* BCH 397, *Sh. soneii* E08869, *Sh. dysenteriae* 9, *E. coli* 18/9, *S. aureus* MTCC 96, *P. auriginosa* AP585 NLF, *Klebsiella pneumoniae* and *Proteus vulgaris* AP679 NLF in concentration range between 50-1000μg/ml. The methanolic extract of the leaves inhibited the growth maximum against *Candida albicans* 5, *Aspergillus niger* MTCC 281 and *Penicillium chrysogenum* MTCC 2725. This study has pointed to the potential application of *P.alba* as a bacteriocide and fungicide.

Keywords: *Plumeria alba*, Diarrhea, Dysentery, Bacteriocide and Fungicide

INTRODUCTION

An increase in microbial resistance to antimicrobial agents has been worldwide during past decades[1,2&3].The development of new antimicrobial compounds against different microorganisms is becoming critically important, as infectious diseases are still one of the leading causes of death in the world. The pharmaceutical industry is searching for new lead compounds with novel chemical structures to overcome the increasing resistance to known antibiotics. Green plants represent a useful source of reservoir of effective chemotherapeutics and can provide valuable source of natural antimicrobials [4&5]. Antimicrobials of plant origin are effective in the treatment of infectious diseases while fewer side effects often associated with natural products as compared to synthetic antimicrobials [6]. The plant *Plumeria alba* is commonly known as Frangipani flower or white champa belongs to the family Apocynaceae. Traditionally the latex is applied to ulcers, herpes and scabies. The seeds possess haemostatic properties. Moreover its bark is bruised and applied as plaster over hard tumours. The leaves and bark also finds its application as purgative, cardiotonic, diuretic, infective and hypotensive [7&8]. The preliminary phytochemical study revealed the presence of phytosteroids, alkaloids, flavonoides, terpenoides and glycosides in the methanolic extract of the leaves [9]. So, the present study focused on the antimicrobial properties of the methanolic extract of the leaves. The minimum inhibitory concentration of the extract was determined against various microorganisms by serial dilution checker technique. The agar dilution or cup plate method was used to determine the zone of inhibition against the sensitive strains at MIC so determined.

MATERIALS AND METHODS

Plant Material

The plant was collected from Greater Noida and authenticated by Dr. Anjula Pandey, Principal Scientist, NBPGR, Pusa Campus, New Delhi. The voucher number was NHCP/NBPGR/2010-18) and a voucher specimen is also retained in our laboratory for future references. The leaves were sun dried after washing and then grinded to a coarse powder in a mechanical grinder.

Method of extract preparation

The coarse powder of the leaves (50 gm) was extracted in a soxhlet apparatus with methanol and the solvent was removed by evaporation a heating mantle by taking care that the temperature did not rise above 60ºC. A semisolid dark viscous crude extract (yield 6.38% w/w) thus obtained was tested for its antibacterial and antifungal potentiality.

Test micro-organisms

The test bacteria used were *Shigella flexneri* type 36 NK 381,*Sh.flexneri* type 6B 999, *Sh. flexneri* type BCH 995, *Sh. boydii* 22461, *Sh. boydii* 16552, *Sh. boydii* 8, *Sh. soneii* BCH 397, *Sh. soneii* E08869, *Sh. soneii* NK 840, *Sh. soneii* BCH 937, *Sh. soneii* I, *Sh. soneii* DN3, *Sh. soneii* F11001, *Sh. soneii* NK 29, *Sh. dysenteriae* 1, *Sh. dysenteriae* 9, *V. cholerae* 1023, *V. cholerae* BD 1/81, *V. cholerae* 1341, *V. cholerae* 452, *V. cholerae* 1033, *V. cholerae* 575, *V. cholerae* 765, *V. cholerae* 1311, *V. cholerae* 756*, V. cholerae* DN6, *V. cholerae* A 26, *E. coli* AP600, *E. coli* 383, *E. coli* RH 07/12, *E. coli* 18/9, *E. coli* 597, *E. coli* 798, *E. coli* 35B, *E. coli* 306, *E. coli* K88, *E. coli* 872, *Enterobacter spp* AP596 , *S. typhi* Type 2, *S. aureus* ML 267, *S. aureus* ATCC 6538, *S. aureus* MTCC 96, *S. aureus* 381, *B. subtilis* MTCC 441, *B. cereus* MTCC 1305, *B. pumilus* 8241, *Pseudomonas putida* MTCC 2252, *P. auriginosa* AP585 NLF, *Klebsiella pneumoniae* and *Proteus vulgaris* AP679 NLF.

The test fungi used were *Candida albicans* ATCC 10231, *Candida albicans* 5, *Aspergillus niger* MTCC 281, *Penicillium chrysogenum* MTCC 2725, *Phaenorochaete chrysporium* MTCC 787 and *Ralstonia entropha* MTCC1255. These microbial strains included various drug resistant hospital isolates collected and characterized in Department of Pharmaceutical Technology, Jadavpur University, India. All strains were maintained on Nutrient Agar (NA) for bacteria and Sabourauds's Dextrose Agar (SDA) slants for fungi at 4º C prior to use for antibacterial and antifungal tests respectively.

Determination of minimum inhibitory concentration by Serial Dilution Technique

The leaf extract (stock solution) was reconstituted with a minimum amount of dimethyl sulfoxide (DMSO). This solvent did not posses any antimicrobial activity of its own. Calculated volume of this stock solution were dispensed in a series of McCartney bottles previously containing calculated volume of sterile cooled molten nutrient agar media (40-45ºC) to prepare final volume of 30ml each with dilutions of 5, 10, 25, 50, 100, 200, 400, 800 and1000 μg /ml. The stock solution were dispensed into molten SDA to prepare varying dilutions of 100, 200, 400, 800, 1500 and 2000 μg /ml while determining the MIC against the fungi. Then these molten media containing varying concentration of extract were poured aseptically in presterilized petridishes (70 mm) to give sterile nutrient agar plates with varying dilution of extract. These plates were then kept in refrigerator at 4ºC for 24hrs to ensure uniform diffusion of extract. Then these plates were dried at 37ºC for bacteria and 25ºC for fungi for 2 hours before spot inoculations. One loopful (loop diameter: 3mm) of an overnight grown bacterial strains suspension (10^5 CFU/ml) were added in each quadrant as marked by checker

board technique [10&11].The spotted plates were incubated at 37ºC and 25ºC for 24 hours for bacteria and fungi respectively in an incubator and MIC values were obtained.

Determination of Zones of inhibition by Disc Diffusion Method [12, 13]

The stock solution (each of 10µg/ml) of both extract and ciprofloxacin were prepared. From these stock solutions two sets of four dilutions (200, 400, 800, 1000 µg/ml) each of leaf extract (solvent: DMSO) and ciprofloxacin (solvent: sterile distilled water) were prepared in sterilized McCartney bottles. However we have compare the activity of griseofulvin with the extract at 1000, 1200, 1500, 2000 µg/ml. Sterile agar plates were prepared and incubated at 37ºC for bacteria and 25ºCfor fungi for 24 hours to check for the presence of any sort of contamination. Then each sterilized agar plates were flooded with liquid culture of the strains, dried for 30 minute at 37 ºC for bacteria and 25ºC for fungi. The sterile whatman filter paper disc (4mm diameter) were soaked in four different dilution of the crude extract and placed in appropriate position of the plates marked as quadrant at the back of petridishes. All the flooded plates with corresponding paper discs soaked with appropriates dilution of extract were incubated for 24 hours and diameter of zone of inhibition were measured in mm. Similar procedure was adopted for reference standard drug and corresponding zone diameters were measured and compared accordingly.

Determination of mode of action of the extract[14&15]

To determine whether the extract was bacteristatic or fungistatic and bactericidal or fungicidal in nature, plugs from the zone of inhibition were taken out and reincubated into fresh media which were then examined for their growth after 96 hours incubation at 37ºC and 25ºC in an incubator respectively.

RESULTS

Antibacterial Activity

The result in Table 1 depicted the MIC values of the methanolic extract of the leaf of *Plumeria alba* Linn. against various tested bacterial pathogens. It is evident that the extract is highly active against *Shigella flexneri*, *Sh. soneii, Sh. dysenteriae* and *S. aureus*.

The result of determination of zone of inhibition of the crude extract of the leaf of the plant and comparison with standard antibacterial agent ciprofloxacin against the bacterial strains is recorded in Table 2.

The sensitivity pattern of the bacterial organisms to the extract was found to decrease in following order: *Sh .flexneri* type BCH 995, *Sh. soneii* E08869, *E.coli* 18/9, *Shigella flexneri* type 36 NK 381, *P.auriginosa* AP585 NLF, *S. aureus* ATCC 96, *Proteus vulgaris* AP679 NLF, *Klebsiella pneumoniae*, *Sh. boydii* 22461 and *Sh .dysenteriae* 9 as evident from the study of Table 1 and 2.

Antifungal Activity

The observation suggests that antifungal principles in the extract have a broad spectrum of activity which is quite comparable with that of griseofulvin. The sensitivity pattern of the fungal organisms to the extract was found to decrease in following order: *Candida albicans* 5, *Aspergillus niger* MTCC 281, *Penicillium chrysogenum* MTCC 2725, *Candida albicans* ATCC 10231 and *Phaenorochaete chrysporium* MTCC 787 as evident from Table 3 and Table 4.

Table 2: Determination of diameter of zone of inhibition (in mm) produced by the methanolic extract of the leaves of *Plumeria alba* and its comparison with Ciprofloxacin against selected sensitive bacterial strains*.

S. No	Name of Bacteria	Extract (µg/ml)				Ciprofloxacin (µg/ml)			
		200	400	800	1000	200	400	800	1000
1.	*Shigella flexneri* type 36 NK 381	8.0	9.5	11.0	11.5	9.0	11.0	12.0	14.0
2.	*Sh. flexneri type* BCH 995	10.0	10.5	12.0	12.5	11.0	11.5	12.5	14.0
3.	*Sh. boydii* 22461	7.0	7.5	9.0	10.0	8.0	9.5	11.0	13.0
4.	*Sh. soneii* E08869	9.0	9.5	10.5	12.0	10.5	11.5	13.0	14.5
5.	*Sh. dysenteriae* 9	6.0	7.5	8.0	10.0	8.0	9.5	11.0	13.0
6.	*S. aureus* MTCC 96	8.5	9.0	10.0	11.5	11.5	12.5	13.0	13.5
7.	*P. auriginosa* AP585 NLF	8.5	9.0	10.5	11.5	10.5	11.0	12.5	13.5
8.	*Klebsiella pneumonia*	7.0	7.5	9.0	11.0	9.0	11.0	11.5	12.0
9.	*Proteus vulgaris* AP679 NLF	7.5	8.0	8.5	10.5	11.0	12.0	13.0	14.5
10.	*E. coli* 18/9	9.0	9.0	10.0	12.0	11.0	11.5	12.5	14.5

(* - Average of two plates)

Table 3: Determination of MIC of the methanolic extract of leaves of *P.alba* Linn. against different fungal strains

S. No.	Name of Fungi	Dilution of methanolic leaf extract (µg/ml) in Sabouraud's Dextrose Agar(SDA) media							
		0*	100	200	400	800	1000	1500	2000
1.	*Candida albicans* 5	+	+	±	±	-	-	-	-
2.	*Candida albicans* ATCC 10231	+	+	+	±	±	-	-	-
3.	*Aspergillus niger* MTCC 281	+	+	+	±	-	-	-	-
4.	*Penicillium chrysogenum* MTCC 2725	+	+	+	+	±	-	-	-
5.	*Phaenorochaete chrysporium* MTCC 787	+	+	+	+	±	±	-	-
6.	*Ralstonia entropha* MTCC1255	+	+	+	+	±	±	±	-

* = Control (without extract), ± = Inhibited Growth, + = Growth, – = No Growth.

Table 4: Determination of diameter of zone of inhibition (in mm) produced by the methanolic extract of the leaves of *Plumeria alba* and its comparison with Griseofulvin against different sensitive fungal strains*.

S. No	Name of Fungi	Extract (µg/ml)				Griseofulvin (µg/ml)			
		1000	1200	1500	2000	1000	1200	1500	2000
1.	*Candida albicans* 5	10.0	11.0	12.0	13.0	9.0	11.0	12.0	14.0
2.	*Aspergillus niger* MTCC 281	8.0	8.5	10.0	12.0	8.5	10.0	12.0	14.0
3.	*Penicillium chrysogenum* MTCC 2725	7.0	7.5	9.0	10.0	7.0	8.0	10.0	12.0
4.	*Phaenorochaete chrysporium* MTCC 787	4.0	4.0	5.0	7.0	7.5	8.5	10.0	11.5

(* - Average of two plates)

Table 1: Determination of MIC of the methanolic extract of leaves of *Plumeria alba* Linn. against different bacterial strains

S. No.	Name of microorganism	Dilution of methanolic leaf extract (µg/ml) in nutrient agar media									
		0*	5	10	25	50	100	200	400	800	1000
1.	*Shigella flexneri* type 36 NK 381	+	+	+	+	-	-	-	-	-	-
2.	*Sh. flexneri* type 6B 999	+	+	+	+	+	+	+	-	-	-
3.	*Sh. flexneri type* BCH 995	+	+	+	+	-	-	-	-	-	-
4.	*Sh. boydii* 22461	+	+	+	+	±	±	-	-	-	-
5.	*Sh. boydii* 16552	+	+	+	+	+	+	+	+	+	+
6.	*Sh. boydii* 8	+	+	+	+	+	+	-	-	-	-
7.	*Sh. soneii* BCH 397	+	+	+	+	+	+	-	-	-	-
8.	*Sh. soneii* E08869	+	+	+	+	-	-	-	-	-	-
9.	*Sh. soneii* NK 840	+	+	+	+	+	±	-	-	-	-
10.	*Sh. soneii* BCH 937	+	+	+	+	±	±	±	±	-	-
11.	*Sh. soneii* I	+	+	+	+	+	+	+	+	+	+
12.	*Sh. soneii* DN3	+	+	+	+	+	+	+	+	+	+
13.	*Sh. soneii* F11001	+	+	+	+	+	+	+	+	+	+
14.	*Sh. soneii* NK 29	+	+	+	+	+	+	+	+	+	+
15.	*Sh. dysenteriae* 1	+	+	+	+	+	+	+	+	-	-
16.	*Sh. dysenteriae* 9	+	+	+	+	+	+	+	-	-	-
17.	*V .cholerae* 1023	+	+	+	+	+	+	±	±	±	-
18.	*V. cholerae* BD 1/81	+	+	+	+	+	+	+	+	+	+
19.	*V. cholerae* 1341	+	+	+	+	+	+	+	+	+	+
20.	*V. cholerae* 452	+	+	+	+	+	+	+	+	+	+
21.	*V. cholerae* 1033	+	+	+	+	+	+	+	+	+	+
22.	*V. cholerae* 575	+	+	+	+	+	+	+	+	+	+
23.	*V. cholerae* 765	+	+	+	+	+	+	+	+	+	+
24.	*V. cholerae* 1311	+	+	+	+	+	+	+	+	+	+
25.	*V. cholerae* 756	+	+	+	+	+	+	+	+	+	+
26.	*V.cholerae* DN6	+	+	+	+	+	+	+	+	+	+
27.	*V.cholerae* A 26	+	+	+	+	+	+	+	+	+	+
28.	*E. coli* AP600	+	+	+	+	+	+	±	±	±	±
29.	*E. coli* 383	+	+	+	+	+	+	+	+	+	±
30.	*E. coli* RH 07/12	+	+	+	+	+	+	+	+	+	+
31.	*E. coli* 18/9	+	+	+	+	-	-	-	-	-	-
32.	*E.coli* 597	+	+	+	+	+	+	+	+	+	+
33.	*E.coli* 798	+	+	+	+	+	+	+	+	+	+
34.	*E.coli* 35B	+	+	+	+	+	+	+	+	+	+
35.	*E.coli* 306	+	+	+	+	+	+	+	+	+	+
36.	*E. coli* K88	+	+	+	+	+	+	+	+	+	+
37.	*E. coli* 872	+	+	+	+	+	+	+	+	+	+
38.	*Enterobacter* spp AP596	+	+	+	+	+	+	+	±	±	±
39.	*S. typhii* Type 2	+	+	+	+	+	+	+	+	+	+
40.	*S. aureus* ML 267	+	+	+	+	+	+	±	-	-	-
41.	*S. aureus* ATCC 6538	+	+	+	+	+	+	-	-	-	-
42.	*S. aureus* MTCC 96	+	+	+	+	+	-	-	-	-	-
43.	*S. aureus* 381	+	+	+	+	+	+	+	+	+	+
44.	*B. subtilis* MTCC 441	+	+	+	+	+	+	+	+	+	+
45.	*B. cereus* MTCC 1305	+	+	+	+	+	+	+	+	+	+
46.	*B. pumilus* 8241	+	+	+	+	+	+	+	-	-	-
47.	*Pseudomonas putida* MTCC 2252	+	+	+	+	+	+	+	+	+	+
48.	*P. auriginosa* AP585 NLF	+	+	+	+	-	-	-	-	-	-
49.	*Klebsiella pneumonia*	+	+	+	+	±	±	-	-	-	-
50.	*Proteus vulgaris* AP679 NLF	+	+	+	+	+	+	-	-	-	-

* = Control (without extract), ± = Inhibited Growth, + = Growth, – = No Growth.

Reincubation of inoculum from zone of inhibition resulted into normal growth, which suggests that the extract is basically fungistatic in mode of action.

DISCUSSION

Plants contain many biologically active compounds which have potential for development as medicinal agents. Herbal medicines already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too [16]. The phytochemical constituents of *P.alba* have been established in previous studies, which show the presence of phytosteroids, alkaloids, flavonoides, terpenoides and glycosides in it. Several studies have linked presence of these bioactive compounds in plant materials to antimicrobial activity. The leaf extracts also possess antimicrobial potential against maximum pathogens which may be due to the presence of steroids [17].

The test organisms used in this study are associated with different human infections. From a clinical point of view, *Shigella* causes dysentery that result in the destruction of the epithelial cells of the intestinal mucosa in the cecum and rectum. The main symptoms are profuse watery diarrhea and vomiting. *E. coli* strains can cause serious food poisoning in humans, and are occasionally responsible for product recalls. Hence, the results of the above study supports the use of the crude methanolic leaf extract of *P. alba* Linn. to be effective in infections especially those of diarrhea and dysentery.

The ability of the extract to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum

antimicrobial potential of *P. alba*, which makes the plant a candidate for bioprospecting for antibiotic and antifungal drugs.

Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view of formulating novel chemotherapeutic agents should be the future path of investigation.

REFERENCES:

1. Livermore DM. ß- Lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995; 8:557-584.
2. Jones RN, Baquero F, Privitera G, Inoue M, Wiedemann B. Inducible ß- Lactamase mediated resistance to third-generation cephalosporins. Clin Microbiol Infect 1997; 3: 7-20.
3. Kolar M, Urbanek K, Latal T. Antibiotic selective pressure and development of bacterial resistance. Intern J Antimicrob Agents 2001; 17: 353-363.
4. Balandrin MF, Klocke JA, Wutule ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985; 228: 1154-1160.
5. Satish S, Raveesha KA, Janardhana GR. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. Lett Appl Microbiol 1999; 28: 145-147.
6. Kokosha L, Polesny Z, Rada V, Nepovim A, Vanek T. Screening of some Siberian medicinal plants for antimicrobial activity. J Ethnopharmacol 2002; 82:51-53.
7. Anonymous. The Wealth of India-A Dictionary of Indian Raw Materials and Industrial Products. New Delhi, India: Council of Scientific and Industrial Research; 2005.p.164-166.
8. Prajapati ND, Purohit SS, Sharma AK, Kumar T. Handbook of Medicinal Plants. 2nd ed. India: Agrobios ; 2004:336-338.
9. Radha R, Shivakumar T, Arokiyaraj S. Pharmacognostical evaluation of *Plumeria alba* Linn. Res J of Phar and Tech 2008; 1(4): 496-501.
10. Mazumder R, Mendiratta T, Mandal SC, Mazumder A. Antimicrobial potency of the leaf stalk extract of *Curcuma longa* Roxb. Anc. Sc. of Life 2000; XX: 92-96.
11. Mazumder A, Singh SK, Mazumder R, Basu SP, Saha BP. Antimicrobial action of the leaf extract of *Lagerstroemia parviflora* Roxb. Anc Sc of Life 2001; XXI (3):198-201.
12. Mishra US, Behera SR, Murthy PN, Kumar M, Kumar D. Antibacterial and Analgesic effects of the leaves of *dichrostachys cinerea* . Int J of Pharm and Pharm sci 2009; 2(1):141-45.
13. Jayaveera KN, Yoganandham RK, Yadav G, Kumanan R. Phytochemical Screenings, Antibacterial activity and Physico chemical constants of ethanolic extract of *euphorbia thymifolia* Linn. Int J of Pharm and Pharm sci 2010; 2(3):81-82.
14. Karuba RM, Siboe GM, Dossaji SF. Antifungal activity of *Schizozygia coffaeoids* Bail. (Apocynaceae) extract. J Ethnopharmacol 2001; 74: 41-44.
15. Perucci S, Mancianti F, Cioni PL, Flamini G, Morelli I, Machioni G. In vitro antifungal activity of essential oils against some isolates of *Microsporum canis* and *Microsporum gypsum*. Planta Medica 1993; 60: 184-187.
16. De N, Ifeoma E. Antimicrobial effects of components of the bark extracts of neem (*Azadirachta indica* A. juss). J Technol Dev 2002; 8: 23-28.
17. Cowan MM. Plants products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4): 564-582.