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Antibacterial and antifungal activities of methanolic extract and the isolated fraction of *Plumeria alba Linn*.

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ABSTRACT

To examine the anti-bacterial and anti-fungal activities of methanolic extract and the isolated fraction of the plant *Plumeria alba* Linn. Antibacterial activity was assessed by standard dilution test using Mueller Hinton agar (MH) medium. The zone of inhibition was compared with that of Standard antibiotic ciprofloxacin (5µg/disc) by disc diffusion method. The Anti fungal activity was assessed by standard dilution technique using Sabouraud (SDA) dextrose agar medium. The results are compared with standard Clotrimazole (125mcg/ml). An attempt was made to isolate the fraction responsible for the antimicrobial property of the extract. The methanol extract showed potential anti-bacterial and anti-fungal properties comparable with standard Ciprofloxacin and Clotrimazole respectively against the organism examined. The minimum inhibitory concentration (MIC) of the extract for antibacterial activity was 200mcg/ml. The isolated fraction was also found to possess antimicrobial properties similar to that of the crude extract. The MIC of the fraction was 133.33mcg/ml and the thin layer chromatographic study of the fraction showed it as triterpenes. The study suggests that the plant is promising for development of phytomedicine for antimicrobial properties.

KEYWORDS

Plumeria alba Linn;
Extracts;
Fractions;
Antimicrobial activity;
Ciprofloxacin.

1. INTRODUCTION

The plant *Plumeria alba* Linn belongs to the family (Apocynaceae), is usually small laticiferous tree or shrub, native of tropical America and widely distributed in India. Commonly they are called white champa (Seemaiarali in Tamil)[1]. Earlier reports indicate that the plant *Plumeria acutifolia*, other species in the genus plumeria has been reported to possess antimicrobial property against, *Saccharomyces cerevisiae, candida lipolytica*[2].

The activity was attributed to the polar fractions of the alcoholic extract and the non-polar fraction was deficient in the antibacterial activity. The antimicrobial properties of the certain plants have been attributed to the presence of terpenoids. The presence of triterpenes in plant *Plumeria alba* Linn. has also been reported[3]. Considering this, in the present study an attempt was made to prepare methanolic extract of the plant and to investigate the extract for its antimicrobial activity[4]. Further the fraction contributing to the antimicrobial activity was also isolated and identified.

2. MATERIALS

The plant *Plumeria alba linn* was collected from Puducherry, Tamilnadu, India during the month of August 2007. The botanical identity of the plant was con-

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TABLE 1: Anti-bacterial activity of methanolic extract and isolated fraction of *Plumeria alba l*inn.

Description	Micro-organisms (MIC in µg/ml)								
	E.coli	*K.pneu-monia*	*P.aerug-inosa*	*P.mirab-ilis*	*S.aureus*	*CONS*	*S.typhii*	*S.paraA*	*S.paraB*
Control	+	+	+	+	+	+	+	+	+
Ciprofloxacin	5	5	5	5	5	5	5	5	5
Methanolic extract	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>133.33 <200	>33.33 <66.66	>66.66 <133.33	>66.66 <133.33	>33.33 <66.66
Isolated fraction	>66.66 <133.33	>66.66 <133.33	>66.66 <133.33	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66

(+) indicates the growth of the organism: values are an average of triplicate; Ciprofloxacin (5µg/ml) SD060 from Hi media laboratory limited, Mumbai-400086, India

firmed at the botanical survey of India (BSI), Coimbatore, Tamil Nadu. A voucher specimen has been deposited at the Museum of the Department of Pharmacognosy, Madras Medical College, Chennai-3.

The following microorganisons were selected for the study- Gram +ve and gram -ve Bactrial organisms and 4 fungi namely: (1) *Staphylococcus aureus,* (2) *Coagulase Negative Staphylococca,* (3) *Escherichia coli,* (4) *Klebsiella pneumoniae,* (5) *Pseudomonas aeuroginosa,* (6) *Salmonella typhi,* (7) *Salmonella typhi, Aspergillus niger, Pencillium Chrysogenum, Microsporum gypseum, Epidermatophyton flocossum.* These organisms were obtained from standard laboratory maintained in the Institute of Microbiology, Madras Medical College, Chennai.

Mueller Hinton agar (MH) medium was obtained from Himedia Laboratory Ltd., Mumbai 400 086, India.Ciprofloxacin disc (5µg/disc) and Sabouraud's dextrose agar medium were obtained from Himedia Laboratory Ltd., Mumbai.

3. METHODS

3.1 Preparation of plant extract

The freshly collected plant material (leaves) was dried in shade, then coarsely powdered and 500g of the powder was extracted successively with hexane, Chloroform, Ethyl acetate and Methanol (1.5 litres) in an aspirator bottle at room temperature. The powder was soaked in the solvent for 72 hours and nearly 80% of the solvent was removed by distillation on a water bath at atmospheric pressure and last traces were removed under reduced pressure using rotary evaporator. The residues were suitably diluted with dimethyl formamide (DMF) so as to get the final concentration of each extract 1000µg/ml and used for the study.

Thin layer chromatography (TLC) of extracts. The TLC plates using Silica gel G were prepared by standard procedure; the extract was spotted on the plates using a capillary tube 2cm above the bottom end of the plate. The chromatogram was developed using different solvent systems. The spots identified by different spraying reagents. The R_f for the different spots were recorded.

3.2 Fractionation of the plant

The extract was fractionated in the column packed with silica gel GF (chromatographic grade) and eluted with varying solvents by gradient elution technique. The fraction with similar R_f values were identified by thin layer chromatography using Hexane:Benzene(1:1) as mobile phase and 50% sulphuric acid as detecting agent for the presence of triterpenes and it was confirmed at Libermen-burchard reation(acetic anhydride with conc.sulphuric acid), it gave green blue colour.

3.3 Anti-bacterial activity[5,6]

The plates were prepared by using MH agar and different extracts of various dilution (TABLE 1), allowed to solidify and dry. Then a loopful of the bacterial cultures was inoculated at the labelled spot and the plates were incubated at 37^0C for 242 hrs. The results were read by the presence or absence of growth of organism (TABLE 1) and the Minimum inhibitory concentration (MIC) was determined. The procedure was repeated for the investigation of the isolated fraction of the extract.

3.4 Anti-fungal activity[7,8]

The slants were prepared by using Sabourauds dextrose agar medium as per stsndard procedures and allowed to set. The different fungi were inoculated into the slants and then incubated at 37^0C in an incubator for 1 week to 4 weeks. The results were read by noting, the presence or absence of growth of the organisms and compared with standard Clotimazole (125mcg/ml) (TABLE 2).

TABLE 2: Anti-fungal activity of Methanolic extract and isolated fraction of *Plumeria alba* Linn

Fungi	MIC in µg/ml		
	Clotrimazole	Methanolic extract	Isolated fraction
A.niger	125	>50<125	>50<125
P.chrysogenum	125	>50<125	>50<125
E.floccosum	125	>50<125	>50<125
M.gypseum	125	>50<125	>50<125

4. RESULTS

Both the methanol extract and the isolated fraction of the extract demonstrated anti-bacterial and anti-fungal activity (TABLES 1 and 2). The MIC of the extract and the purified fraction against the bacteria tested are as shown in Table1. The methanol extract and its fraction showed same pattern of antifungal activity. The minimum inhibitory concentration at which the anti-fungal activity observed was found to be >50<125mcg/ml for both the extract as well as the isolated fraction and the activity comparable with that of standard standard Clotimazole (125mcg/ml) (TABLE 2)

5. DISCUSSION

The results of the present study clearly indicated the anti-bacterial and anti-fungal properties of the methanol extract of the plant *Plumeria alba* Linn. The antimicrobial activity of the extract was comparable with the standard anti-bacterial agent ciprofloxacin as well as standard antifungal agent clotrimazole. The presence of triterpenes in the plant has been earlier reported (3) and these terpenes possess anti-microbial properties (4). The TLC of the isolated fraction of the methanol extract showed the presence of triterpenes with two different Rf values (0.75 and 0.78).

This support the contention that anti-microbial activity of the methanol extract may be attributed to the presence of triterpenes in the extract. A detailed structural elucidation of the triterpenes in relation to the antimicrobial property will throw more light on the presence of the lead molecule in a plant.

Among the organism tested in the anti-bacterial study *S.typhii and S.paratyphii B* to be highly susceptible to the effect of methanol extract and the isolated fractions (triterpenes) when compared with ciprofloxacin 5mcg/ml. These findings support the beneficial effects of the extract as well as the isolated fraction (triterpenes) against the pathogenic organisms *S.typhii and S.paratyphii* B (TABLE 1)

Both the extract and the isolated fraction (triterpenes) in concentration 125,250 and 500mcg/ml were effective against the fungi *A.niger, Penicillin chrysogenum, Microsporum gypseum, Epidermatophytes flocossum* and the effective comparable with that of the standard clotrimazole (125mcg/ml) (TABLE 2).

However the extract as well as the fraction in a lower concentration 50 mg/ml did not show significant antifungal activity. The proper role of triterpenes in eliciting the antifungal activity is reported first time in the present study. The TLC extract showed the presence of alkaloids, aminoacids, flavonoids, glycosides, terpenoids. Besides triterpenes and the role of these constituents in the antimicrobial activity requires a detail investigation.

The most significant observation in the present study is that the degree of antimicrobial activity produced by the extract is comparable with that produced by the isolated fraction(triterpenes). This important findings lead to suggest that the triterpenes may be playing a major role in eliciting the anti-microbial property of the extract.

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