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Antimicrobial potential of *Plumeria rubra* Syn *Plumeria acutifolia* bark

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ABSTRACT

Successive extracts of Plumeria rubra Syn Plumeria acutifolia were prepared using petroleum ether (60-80^{O}C), chloroform, methanol and water. The stock solution of 10 mg/ml of the extracts were screened for antimicrobial activity by using Cup plate method and Minimum inhibitory concentration (Turbidity method) against Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Candida albicans and Aspergillus niger. The result showed that a methanol extract exhibited a significant activity against the bacterial strains when compared with Ciprofloxacin as a standard and aqueous extract was active against the fungal strains when compared to Fluoconazole. Hence this study proves that Plumeria acutifolia Syn Plumeria rubra possess antimicrobial activity.

Keywords: *Plumeria rubra*, Apocynaceae, antimicrobial, minimum inhibitory concentration

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INTRODUCTION

Plumeria is a small genus belonging to family Apocynaceae, which comprises of lactiferous trees and deciduous shrubs. Its origin is Central America, but it is now native to warm tropical areas of Pacific islands, Caribbean, South America, and Mexico. Various species are now found widely and distributed in the warmer regions of the world [1]. The plant *Plumeria acutifolia* Poir syn *Plumeria rubra* L. is a native of Mexico and cultivated in gardens throughout India as an ornamental tree. Its leaves fall during the month of March and new foliage is produced in April. Different parts of the plant are used traditionally in medicine. The root bark is bitter, pungent, heating, carminative, laxative and useful in leprosy and ulcers. In Indonesia, a decoction of *Plumeria rubra* bark is used to treat gonorrhoea, while in the Philippines, bark extracts are employed for their purgative, emmanogogue and febrifuge effects. In Mumbai (India), it is used in intermittent fever, like cinchona. In Ayurveda system of medicine it is used in malaria, fever, antiseptic and as a stimulant [2,3,4].

MATERIALS AND METHODS

The bark of *Plumeria acutifolia* Poir Syn *Plumeria rubra* L. was collected from University campus in June 2010 and authenticated by Dr. H.B. Singh, Head Raw Material Herbarium & Museum, New Delhi vide Ref. NISCAIR/RHMD/Consult-2010-11/11/1413/11. A voucher specimen has been retained in Department of Pharmaceutical Science, Guru Jambheshwar University of Science &Technology, Hisar. The plant material (1kg) was air-dried at room temperature (30-40^{o}C) and then powdered to pass through a sieve of 1mm.

Micro-organisms used

The strains of bacteria used were *Escherichia coli* (MTCC1652), *Bacillus subtilus* (MTCC 2063) and *Staphylococcus aureus* (MTCC 2901). The fungal strains used in this study were *Candida albican* (MTCC 227) and *Aspergillus niger* (MTCC 8189).

Preparation of test inoculums

The various strains of micro-organism were inoculated in sterile nutrient broth (Hi media). This medium was incubated at $37^{O}C \pm 1^{O}C$ for 24 hours and sterilized. The inoculums were used for antimicrobial assay.

Antimicrobial assay

Agar well diffusion method

The antimicrobial activity of bark extract (petroleum ether, methanol, chloroform and aqueous extract) of *P. rubra* was evaluated. About 15 to 20 ml of Nutrient agar and Sabourad Dextrose agar medium were poured in the sterilized petri dishes and allowed to solidify. One drop of bacterial and fungal strains was spread over the medium by a rod. Wells of 6 mm in diameter and about 2 cm apart were punctured in the culture medium using sterile cork borers. The standard and test compounds (extracts of petroleum ether, chloroform, methanol and water) solution were prepared in dimethyl sulphoxide (20 % v/v) at the concentration of 10 mg/ml respectively. The plant extract solutions were added to the wells. Plates were incubated at 37°C for 24 h. Standard drugs used in the study were Ciprofloxacin for bacterial assay and Fluoconazole for assay of fungi. Antimicrobial activities were evaluated by measuring the inhibition zone diameters[5,6,7].

Minimum inhibitory concentration (MIC)

The MIC was evaluated according to two fold serial dilution method. The stock solutions of test solutions (extracts) were prepared at concentration of 100 µg/ml in nutrient broth serially diluted at up to five times. Six assay tubes were taken for screening minimum inhibitory concentration of each strain. In the 1st tube, 1ml of the seeded broth was added followed by addition of 1ml of the test compound solution and thoroughly mixed to obtain a concentration of 50µg/ml. To make further dilution of the solution, 1 ml volume from first tube was inoculated into 2nd assay tube serially. The procedures were conducted under aseptic conditions. The inoculated tubes were kept at 37°C ± 1°C at 24 hours for bacterial assay, 7 days for *Aspergillus niger* and 3 days for *Candida albicans*. After incubation period, tubes were removed and observed for deposits or turbidity in the solution[8,9,10].

RESULTS

In the present study, methanol extract of *P. rubra* bark exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table I and Table II that the methanol extract possesses antimicrobial activity in cup plate method and minimum inhibitory concentration study (Turbidity method). The cup plate method result showed the zone of inhibition to be 16mm, 18 mm and 18 mm for methanol extract against *S. aureus, B. subtilus* and *E. coli* when compared with standard drug ciprofloxacin showing 26 mm, 28 mm and 24 mm zone of inhibition respectively. The aqueous extract showed 8mm 10mm diameter against *S. aureus* and *B. subtilus*. The aqueous extract showed 12 mm diameter against *A. niger* and 10 mm against *C. albicans* when compared with fluoconazole having 20mm and 22mm diameter respectively.

The Minimum inhibitory concentration is reported according to the liquid dilution screening of antimicrobial activity of higher plants. The methanol extract possess a MIC 25 µg/ml in bacterial strain and 50 µg/ml in fungal strain when compared with standard drug Ciprofloxacin and Fluoconazole of MIC 0.I56 µg/ml, 0.156 µg/ml and 0.312 µg/ml against bacterial and fungal strain used. Hence the present study proves that methanol extract gave highest activity against bacteria and aqueous extract against fungi. The antimicrobial evaluation of this plant is drawn by using a standard procedure which is helpful to authenticate the potential of such plant species. This is first such report on this plant using these strains.

Table I: Zone of inhibition of bacteria and fungi (in mm) of *Plumeria acutifolia* bark

Sr. No.	Extract	Micro-organisms used				
		S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
1	Petroleum ether	_	_	_	_	_
2	Chloroform	_	_	_	_	_
3	Methanol	16	18	18	_	_
4	Aqueous	_	_	_	12	10
5	Ciprofloxacin (10 µg/ml)	26	28	24	_	_
6	Fluconazole (10 µg/ml)	_	_	_	20	22

Table II: Minimum Iinhibitory Concentration (MIC) of *Plumeria acutifolia* bark

Microorgansm	MIC of Standard Drug	Extract	Serial dilution (µg/ml)				
			50	25	12.5	6.25	3.2
E. coli	Ciprofloxacin 0.156 µg/ml	Pet ether	_	_	_	_	_
		Chloroform	_	_	_	_	_
		Methanol	+	+	_	_	_
		Aqueous	_	_	_	_	_
B. subtilis	Ciprofloxacin 0.156 µg/ml	Pet ether	_	_	_	_	_
		Chloroform	_	_	_	_	_
		Methanol	+	_	_	_	_
		Aqueous	_	_	_	_	_
S. aureus	Ciprofloxacin 0.156 µg/ml	Pet ether	_	_	_	_	_
		Chloroform	_	_	_	_	_
		Methanol	+	+	_	_	_
		Aqueous	_	_	_	_	_
A. niger	Fluconazole 0.312 µg/ml	Pet ether	_	_	_	_	_
		Chloroform	_	_	_	_	_
		Methanol	_	_	_	_	_
		Aqueous	+	_	_	_	_
C. albicans	Fluconazole 0.156 µg/ml	Pet ether	_	_	_	_	_
		Chloroform	_	_	_	_	_
		Methanol	_	_	_	_	_
		Aqueous	+	_	_	_	_

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