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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *PLUMERIA RUBRA* L.

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ABSTRACT

Petroleum ether, carbon tetrachloride, chloroform and ethyl acetate extracts of *Plumeria rubra* leaves were studied for their antimicrobial activities against eleven human pathogenic bacteria, viz., *Shigella dysenteriae, S. sonnei, Salmonella typhi, S. paratyphi, Bacillus subtilis, B. megaterium, B. cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Vibrio cholerae* and four human pathogenic fungi, viz., *Aspergillus niger, A. ochraceus, A. ustus* and *Candida albicans* using disc diffusion and poisoned food method, respectively. Chloroform and ethyl acetate extract exhibited moderate to good antibacterial and antifungal activity against all the pathogens tested. The ethyl acetate extracts exhibited the largest zone of inhibition (25 mm in diameter with 2000 µg/disc extract) against *E. coli.* The highest inhibition of fungal radial mycelial growth (62. 00% with 100 µg extract/ml medium) was recorded against *A. ustus* with ethyl acetate extract. The MICs were determined by broth macrodilution technique. The ethyl acetate extract exhibited the lowest MIC (750 µg/ml) against *E. coli.* However, for fungi the lowest MIC was 500 µg/ml against *A. ustus* with the same extract.

Key words: Antimicrobial activity, crude extract, leaf, *Plumeria rubra.*

INTRODUCTION

Various plant species have been serving as the natural source of drugs and medicines from the beginning of civilization. The use of, and search for drugs and dietary supplements derived from plants have accelerated in recent years. Traditional healers have long used plants to prevent or cure infectious conditions and western medicine is trying to duplicate their successes. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, saponins, glycosides, quinolines, essential oils and flavonoids, which have been found *in vitro* to have antimicrobial properties (Ahmed *et al.* 2002, Aureli *et al*.1992, Rahman *et al*. 1999). They are capable of mitigating and curing human

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sufferings, healing wounds, cuts, burns and other antimicrobial source. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis 1994). This situation forced scientists to search for new antimicrobial substances from various sources. Now-a-days, the natural products have been accepted as important sources of biologically active antimicrobial substances, and the major sources of which are still left undiscovered. But a few works have been done in this field in Bangladesh(Rahman *et al.*1999, Ahmed *et al.*1999).

Plumeria rubra L. is a small tree with crooked trunk, rough bark and pink fragrant flowers, which belongs to the family Apocynaceae. It grows everywhere in Bangladesh as an ornamental plant. Plant principally contains triterpenes, plumeric acid, glycosides, plumieride and methyl ester. Juice of leaves is used as poultice to swelling and stem bark is used in diarrhoea and piles (Ghani 1998). Considering above mentioned facts, the present work has been undertaken to observe antimicrobial activity of extracts of *P. rubra*.

MATERIALS AND METHODS

Collection and extraction of plant material

Leaves of *Plumeria rubra* were collected in fresh condition from the Chittagong University campus, Chittagong, Bangladesh. The collected and cleaned samples were cut into small pieces (1-2 cm), dried in air to make it suitable for grinding. The samples were ground to fine powder mechanically and then 50 g of the dried powder was kept steeped for 72 hours in petroleum ether, chloroform, ethyl acetate and carbon tetrachloride. The extracts thus obtained separately were filtered, centrifuged at 5000 rpm for 20 minutes and concentrated to a gummy material under reduced pressure at 50^0C by rotary vacuum evaporator. The gummy materials were then collected in small vials and dried to obtain the crude extract.

Test organisms

The crude extracts obtained from *Plumeria rubra* were tested for their antibacterial activity against ten human pathogenic bacteria, viz., *Shigella dysenteriae* AE 14396, *S. sonnei* CRL.(ICDDR,B), *Salmonella typhi* AE 14612, *S. paratyphi* AE 14613 , *Bacillus subtilis* BTCC 17, *B. cereus* BTCC 19, *B. megaterium* BTCC 18, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* CRL(ICDDR'B), *Escherichia coli* ATCC 25922 and *Vibrio cholerae* AE 14748, and four human pathogenic fungi viz., *Aspergillus niger* BTCC 504, *A. ochraceus* BTCC 515, *A. ustus* BTCC 503 and *Candida albicans* BTCC 493.

The test organisms were collected from Department of Microbiology, University of Chittagong, Bangladesh.

Determination of antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the crude extract of the plant were determined by disc diffusion method (Bauer *et al.* 1966) and poisoned food technique (Miah *et al.* 1990), respectively. Mueller-Hinton (agar and broth) medium was used for culture of bacteria and Sabouraud (agar and broth) medium was used for fungi. A 10% solution of the crude extract was used as the test material. All the results were compared with the standard antibacterial antibiotic ampicillin [20µg/disc, BEXIMCO Pharma Bangladesh Ltd.] and antifungal antibiotic nystatin [100µg/ml medium, BEXIMCO Pharma Bangladesh Ltd.]. MIC of the crude extract was determined by macro-dilution broth technique (Jones *et al.* 1985).

RESULTS AND DISCUSSION

The crude extracts (petroleum ether, chloroform, ethyl extract and carbon tetrachloride extracts) obtained from *Plumeria rubra* were screened for their antibacterial activity against eleven human pathogenic bacteria and compared to that of standard antibacterial antibiotic ampicillin. The results of the sensitivity test are presented in Table 1. Among the four solvent extracts, only chloroform and ethyl acetate extracts showed antibacterial activity. The chloroform and ethyl acetate extracts exhibited good antibacterial activity against the test organisms tested. But crude extracts of petroleum ether and carbon tetrachloride did not show antibacterial activity at a concentration of 2000 µg/disc extract. The ethyl acetate extract exhibited the largest zone of inhibition (25 mm in diameter with 2000 µg/disc extract) against *E. coli*. The standard antibiotic ampicillin (20µg/disc) was also found to be active against all the bacteria tested. Similar antibacterial activity of other plant extracts has been reported previously (Sarker *et al*. 1991, Rojas *et al.* 1992, Brantner and Grein 1994, Rahman *et al*. 1998, Ahmed *et al*. 1999).

The antifungal activity of the crude extract (100 µg/ml medium) against four human pathogenic fungi was studied and compared with that of standard antifungal antibiotic nystatin. The results of the inhibition of radial mycelial growth of fungi are summarized in Table 2.

TABLE 1: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS FROM *PLUMERIA RUBRA.*

Name of bacteria	Diameter of inhibition zone in mm. (Crude extract 2000µg/disc)				Ampicillin* 20µg/disc
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethyl actate	
Bacillus subtilis	-	-	10	19	19
B. megaterium	-	-	12	12	16
B. cereus	-	-	14	20	18
Staphylococcus aureus	-	-	18	22	22
E. coli	-	-	19	25	10
Vibrio cholerae	-	-	14	14	15
Shigella dysenteriae	-	-	10	11	22
S. sonnei	-	-	13	16	20
Salmonella typhi	-	-	12	16	20
S. paratyphi	-	-	14	15	17
Pseudomonas aeruginosa	-	-	10	11	15

*Standard antibacterial antibiotic

TABLE 2: ANTIFUNGAL ACTIVITY OF THE CRUDE EXTRACTS FROM *PLUMERIA RUBRA.*

Name of fungi	Percentage inhibition of fungal mycelial growth[a] (100 µg/ml medium)				
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethyl acetate	Nystatin*
Aspergillus niger	-	-	23.00	26.00	62.50
A. ochraceus	-	-	42.00	50.00	68.00
A. ustus	-	-	50.00	62.00	72.00
Candida albicans	-	-	50.00	38.00	78.50

* Standard antifungal antibiotic ; Minus(-) mean no growth;
[a]Growth measured- radial growth in mm.

From Table 2, it appeared that the petroleum ether and carbon tetrachloride extracts have no antifungal activity at a concentration of 100 μg/ml medium. On the other hand, chloroform and ethyl acetate extract of *P. rubra* inhibited the radial mycelial growth of all the test fungi at a concentration of 100 μg/ml medium. The highest inhibition (62.0%) of fungal radial mycelial growth was recorded against *A. ustus* with ethyl acetate extract at a concentration of 100 μg/ml medium. Antifungal antibiotic nystatin (100μg/ml medium) exhibited inhibitions of radial mycelial growth of all the four fungi. Similar antifungal activities on plant extracts of other plants have also been previously reported (Naidu and John 1981, Shetty and Shetty 1987, Miah *et al*. 1990, Stange *et al*. 1993, Anwar *et al*. 1994).

The MIC values of the crude extracts obtained from *P. rubra* leaf are summarized in Table 3. It appeared that the chloroform and ethyl acetate extract exhibited the MIC values from 750 μg/ml to 2000 μg/ml against the bacterial pathogens. But petroleum ether and carbon tetrachloride extract did not show MIC up to the extract concentration of 2500 μg/ml. The lowest MIC (750 μg/ml) was recorded against *E. coli* with ethyl acetate extract. In case of fungi, chloroform and ethyl acetate extracts exhibited MICs from 500 μg/ml to 2000 μg/ml against the fungal pathogens. The lowest MIC (500 μg/ml) was recorded against *A. ustus* with ethyl acetate extract.

TABLE 3: MICS OF CRUDE EXTRACTS FROM *PLUMERIA RUBRA.*

Bacteria / fungi	MIC (Crude extract µg/ml medium)			
	Pet. ether	C. tetrachloride	Chloroform	Ethyl acetate
A. Bacteria:				
Bacillus subtilis	NF	NF	2000	1500
B. megaterium	NF	NF	1500	2000
B. cereus	NF	NF	1500	1000
Staphylococcus aureus	NF	NF	2000	1000
E. coli	NF	NF	2000	750
Vibrio cholerae	NF	NF	1500	1000
Shigella dysenteriae	NF	NF	2000	1500
S. sonnei	NF	NF	1500	1000
Salmonella typhi	NF	NF	1500	1500
S. paratyphi	NF	NF	1500	2000
Pseudomonas aeruginosa	NF	NF	1500	1000
B. Fungi:				
Aspergillus niger	NF	NF	2000	1000
A. ochraceus	NF	NF	1500	750
A. ustus	NF	NF	1000	500
Candida albicans	NF	NF	750	750

NF – not found up to 2500 µg/ml

The present investigation confirms that there are antibacterial and antifungal properties in the crude extract of *Plumeria rubra* leaf. However, it is important to point out that crude extract such as this needs to be further processed to obtain pure compound(s) which can then be tested for antimicrobial activity.

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