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The present work aimed at exploring the antimicrobial activities of flower and leaf extracts of *Ixora coccinea*, belonging to the Rubiaceae family against clinical samples from tertiary health care hospital patients. In this study aqueous, methanolic and ethanolic extracts of both flower and leaf were screened. Antibacterial activity was screened against these samples and also against reference strains by means of agar-well diffusion method. The ethanolic and methanolic extracts of both flowers and leaves of *I. coccinea* showed promising antibacterial activity against both the clinical and reference *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. The phytochemical screening of the organic plant extracts revealed the presence of alkaloids, steroids, flavonoids and tannins.

Key words: Agar-well diffusion method, anti-bacterial activity, bacterial samples, plant extracts

INTRODUCTION

Drug resistant bacterial strains usually develop due to the over use of a number of antibacterial drugs as a result it arises trouble in controlling the growth of infectious disease, even over usage produces side effects (Tomasz and Tomin, 1986). From ancient times in ayurvedic medicinal plants are used for curative purpose for various ailments as a large number of organic compounds secondary metabolites are present in plants. These act as potent bioactive compounds, for various purposes like chemotherapeutic, bactericidal, and bacteriostatic agents (Bohra and Purohit, 1998). The genus *Ixora* contains more than 400 species and is one of the low growing evergreen perennial shrubs through Southeast Asia. It is a popular flowering shrub belongs to the Rubiaceae family. Pharmacologically the leaves are reported for their antimicrobial, antidiarrhoeal and antinociceptive activities (Annapurna *et al*., 2003; Agashikar *et al*., 2010; Ratnasooriya *et al*., 2005). Flowers possess antioxidant, antiinflammatory, wound healing activities (Saha *et al*., 2008; Udupa *et al*. 1999). The present study has been carried out to evaluate the antibacterial activity and phytochemical analysis of various organic extracts of both the flowers and leaves of *Ixora coccinea* against bacterial pathogens isolated from clinical samples.

MATERIALS AND METHODS

Preparation of plant extracts

The leaves and flowers were shade dried. 20 g of both the samples of leaves and flowers were crushed using a mortar pestle then 70% ethanolic, 70% methanolic and aqueous extract of 200 ml each were prepared and kept at 4°C for 48 h. These extracts were then filtered and were kept in

-18ºC for further use.

Microbial strains and culture media

In the current study, sixty two clinical isolates were used for the assay of the antimicrobial property from *Ixora coccinea.* These isolates were obtained from clinical samples from a tertiary health care hospital, Kolkata. Out of these different clinical samples (wound and pus) 19 samples were found to be positive for *Staphylococcus aureus* and 43 samples for *Pseudomonas aeruginosa*. Apart from that two reference strains of *Staphylococcus aureus* 25923 ATCC and *Pseudomonas aeruginosa* 27853 ATCC were taken. All the isolates were maintained on Nutrient Agar (HiMedia) slants at 5°C.

Antibacterial activity tests

Antibacterial activity tests were performed by standardized agar-well diffusion method (Bazerque *et al*.1990). For this technique, bacterial pathogens prepared in saline water (0.85% NaCl) and adjusted to a turbidity of 0.5 McFarland standards (10^8 CFU/ml) were spread on the solid plates with a sterile swab. Wells (6 mm diam.) were punched and the wells were filled with ethanolic, methanolic and aqueous extracts of both leaves and flowers of *Ixora coccinea* were used as positive control and the solvents used for preparing extracts were used as negative control following standard protocol**.** Standard disc of ciprofloxacin (5 mcg/disc), ampicillin/sulbactam (10/10 mcg/disc), levofloxacin (5mcg/disc), chloramphenicol (30 mcg/disc), nitrofurantoin (300 mcg/disc), nalidixic acid (30 mcg/disc), ceftazidime (30 mcg/disc), piperacillin/tazobactam (100/10 mcg/disc), co-trimoxazole (25 mcg/disc) and oxacillin (1 mcg/disc) (HiMedia) were used against all isolates. Plates were incubated at 37°C for 24 h. Antibacterial activities were evaluated by measuring the diameter of zone of inhibition.

RESULTS AND DISCUSSION

In this study two reference strains *Staphylococcus aureus* 25923 ATCC and *Pseudomonas aeruginosa* 27853 ATCC were taken in parallel to 62 clinically isolated samples. In the study of monitoring antimicrobial activities 10 types of antibiotic discs were tested against individual clinical isolates, i.e., ciprofloxacin, levofloxacin, chloramphenicol, ampicillin/sulbactam, nalidixic acid, ceftazidime, nitrofurantoin, piperacillin/tazobactam, co-trimoxazole and oxacillin along with aqueous, 70% ethanolic and 70% methanolic exrtracts of both the leaves and flowers.

Table 1 : Sensitivity of reference strains with standard antibiotics discs and plant extracts in (mm)

Antibiotics (mcg/disc) and extracts (mg)	*Pseudomona saeruginosa* ATCC 27853	*Staphylococcus aureus* ATCC 25923
Leaf ethanolic extract (10)	19	21
Leaf methanolic extract (10)	17	18
Leaf aqueous extract (15)	9	8
Flower ethanolic extract (10)	23	22
Flower methanolic extract (10)	17	15
Flower aqueous extract (15)	9	9
Ciprofloxacin (5)	30	33
Levofloxacin (5)	24	33
Chloramphenicol (30)	18	30
Ampicillin/Sulbactam (10/10)	13	31
Nalidixic Acid (30)	24	15
Ceftazidime (30)	27	21
Nitrofurantoin (300)	20	21
Piperacillin/Tazobactam (100/10)	28	37
Co-Trimoxazole (25)	38	35
Oxacillin (1)	24	25

All the reference strains showed sensitivity to all the ten antibiotics and in all the plant extracts (Table 1). Among the 43 *Pseudomonas aeruginosa* clinical isolates they were found to be maximum resistant to 69.8%, 67.4%, and 65.1% with the following antibiotics ampicillin/sulbactam, oxacillin, and nitrofurantoin respectively; in other words 30, 29 and 28 strains were resistance to ampicillin/sulbactam, oxacillin and nitrofurantoin respectively. Nalidixic acid, chloramphenicol, ceftazidime showed moderate resistance (Table 3). In case of plant extracts, ethanolic extracts of both leaves and flowers showed antibacterial activity of 65.1% and 58% respectively followed by 53.5% and 48.8% of methanolic extracts of both leaves and flowers (Table 2). The nineteen *Staphylococcus aureus*

Table 2 : Resistant patterns of clinical isolates with plant extracts

Leaf and flower extracts (mg)	Sensitivity pattern of 62 isolates			
	Pseudomonas aeruginosa		*Staphylococcus aureus*	
	No of sensitive isolates	No of resistant isolates	No of sensitive isolates	No of resistant isolates
Leaf ethanolic extract (10)	28	15	18	1
Leaf methanolic extract (10)	23	20	17	2
Leaf aqueous extract (15)	3	40	2	17
Flower ethanolic extract (10)	25	18	16	3
Flower methanolic extract (10)	21	22	14	5
Flower aqueous extract (15)	3	40	2	17

Table 3 : Resistant patterns of clinical isolates with standard antibiotics

Antibiotics (mcg/disc)	Antibiotic sensitivity pattern of 62 isolates			
	Pseudomonas aeruginosa		*Staphylococcus aureus*	
	Number of sensitive isolates	Number of resistant isolates	Number of sensitive isolates	Number of resistant isolates
Oxacillin (1)	14	29	10	9
Ampicillin/Sulbactam (10/10)	13	30	16	3
Nitrofurantoin (300)	15	28	19	0
Co-Trimoxazole (25)	21	22	17	2
Nalidixic Acid (30)	28	15	16	3
Chloramphenicol (30)	26	17	19	0
Ceftazidime (30)	29	14	17	2
Piperacillin/Tazobactam (100/10)	35	8	17	2
Ciprofloxacin (5)	39	4	19	0
Levofloxacin (5)	39	4	19	0

Table 4 : Phytochemical properties of *Ixora coccinea* leaf and flower extracts

Test	*Ixora coccinea* leaf extracts			*Ixora coccinea* flower extracts		
	n-butanol	ethanol	Acidic methanol	n-butanol	ethanol	Acidic methanol
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+

+ = presence, - = absence

clinical isolates showed moderate resistance to oxacilin that is 9 strains (Table 3). In case of plant extracts, both the leaves and flowers extracts showed very good antibacterial activity, the ethanolic and methanolic extracts of leaves showed 94.7% and 89.5% respectively followed by 84.2% and 73.7% of ethanolic and methanolic extracts flowers (Table 2). Phytochemical analysis revealed the presence of flavonoids, tannins, steroids and alkaloids in all the organic extracts of leaves and

flowers of *I. coccinea* (Table 4).

The current study states that both ethanolic and methanolic extracts of leaves and flowers show good antibacterial activity against clinical sample than aqueous extracts. Against *Staphylococcus aureus* both ethanolic and methanolic extracts of leaves and flowers of *Ixora coccinea* showed higher antibacterial activity and for *Pseudomonas aeruginosa* it is moderate. So from current study, these plant parts show more antistaphylococcal activity than antipseudomonal activity. The result shows that this plant shows more antibacterial activity against *S. aureus* which is gram positive bacteria than *P.aureginosa* which is gram negative bacteria (Table 2).There are numerous workers who found related finding in different medicinal plants like in *Ocimum sanctum*, *Cinnamomum zeylanicum* (Joshi *et al*., 2009) and in leaf extracts of neem (Maragathavalli *et al*., 2012). Thus, the study suggests this plant can be used in the treatment of wound and pus infections caused by resistant bacteria. Additionally, the potential of these plants must be explored to a greater extent, in turn to develop an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria.

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