Antimicrobial Activity of *Plumbago Rosea* Root Extract against Human Pathogens

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Introduction

Plumbago rosea, also known as *Plumbago indica* is an ornamental garden plant. Root of this plant with acrid, vesicant, alterative, digestive, stimulant abortifacient and oral contraceptive properties is used in Ayurvedic medicine (Okeyo, 2006). According to the previous studies root of *P. rosea* contains plumbagin or 5-hydroxy-2-methyl-1,4-naphthoquinone (Mallavadhani 2002). Plumbagin is present in all the varieties of genus plumbago to a maximum of about 0.91%. *Plumbago zeylanicas*, another species belong to genus plumbago has been reported for its antimicrobial properties. (Dhale, 2011).

The emergence of antibiotic resistant strains of human pathogens and side effects of currently available drugs are becoming a serious problem, for which alternative therapies are urgently required. Infections caused by *Staphylococcus aureus* especially due to methicillin–resistant *S. aureus* (MRSA) in immune compromised patients is continue to be a serious problem in worldwide. *Staphylococcus saprophiticus* is a cause for community-acquired urinary tract infections in young women. Opportunistic pathogens such as *Pseudomonas aeruginosa* which causes range of human infections and the *Escherichia coli* are also, being reported for the antibiotic resistance. Drug resistant strains of *Candida albicans*, which causes candidiasis is also a problem with global concern.

Materials and methods

The roots of *Plumbago rosea* were collected around Haldumulla, Badulla, Sri Lanka. The collected plant materials were identified at Herbarium, National Botanic Garden, Peradeniya, Sri Lanka. Pure cultures of human pathogenic bacteria: *Staphylococcus aureus*, (NCTC 6571) *Staphylococcus saprophiticus* (clinical isolate), *Psudomonas aeruginosa* (ACTC 27853), *Escherichia coli*, (NCTC 10418) and fungi *Candida albicans* (clinical isolate) were obtained from Department of Microbiology, Faculty of Medicine, University of Peradeniya, Sri Lanka. Cultures were maintained on Muller-Hinton agar at 4 ^{0}C.

The collected roots were air dried for two weeks and ground. 20 g of root samples were extracted separately with distilled water (100 mL) and ethanol (100 mL) using Soxhlet extractor for a period of 8 h and 48 h, respectively. The aqueous extract was concentrated up to 50 mL by continues maintaining of extract at ambient pressure in the same heating mantel for another 2 h. Ethanol extract was concentrated in vacuum under pressure using rotary evaporator to obtain the crude ethanol extract. Then the extracts were maintained at 4 ^{0}C in capped vials.

Agar well diffusion assay was used to determine the antimicrobial activity of the aqueous extract and the crude ethanol extracts. Plates were inoculated with 0.5 McFarland microbial isolates using pour plate method and well diameter was 8 mm. The experiment was carried out in triplicate. Agar plate dilution method based on the BSAC standard (Andrews, 2001) was performed to determine the Minimum Inhibitory Concentration (MIC), lowest concentration which did not show any visible growth, with the dilution series: 8.192, 4.096, 2.048, 1.024, 0.512, 0.256, 0.128, 0.064, 0.032 mg/ml. The experiment was carried out in

duplicate. All the plates were incubated aerobically at 36-37 °C and read at 24 h. Samples were dissolved in sterilized distilled water mixed with Tween 20 (5:0.5) and same solution was used as the negative control.

Statistical analyses were performed using Minitab Statistical Package 14.0 version.

Results and discussion

The aqueous root extract of *P. rosea* (Table 01) showed high activity against *S. aureus* and for *S. saprophiticus* and moderate activity against *E. coli*, *P. aeruginosa* and *C.albicans* in agar well diffusion assay.

Agar well diffusion assays results of crude ethanol extracts with six months old and two weeks old samples (Table 1) indicates there is a considerable reduction of the activity with the age of the sample. With six month old samples at the concentration of 1 mg/ml, it did not show any antimicrobial activity. At the concentration of 5 mg/ml, it showed activity only against *S. aureus* (24.93 ± 0.08 mm) and at the concentration of 10 mg/ml only against *S. aureus* (25.13 ± 0.09 mm) and *S. saprophyticus* (13.03 ± 0.02 mm). With two weeks old extracts at 1 mg/ml concentration *S. aureus* and *S. saprophyticus* shows activity and at 10 mg/ml concentration E. *coli* shows activity where as *P. aeruginosa* was resistant even with the new extracts. Activity on two *Staphylococcus* species has significantly increased with new samples *(P = 0.002,* and *0.000).* Observations after 72 h with two weeks old crude ethanol extracts indicates that *C. albicans* also showed considerable sensitivity to root extracts of *P. rosea.* These results suggest that the antimicrobial active compounds in *P. rosea* may decompose with time at 4 ^{0}C.

Table 1: Diameter of the inhibition zone in agar plate dilution assays with aqueous extract and six month old and two weeks old crude ethanol extracts

Organism	Inhibition zone diameter /mm						
	Aqueous extract	Crude ethanol extract					
		Six month old samples			Two weeks old samples		
		1 mg/ml	5 mg/ml	10 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml
S. aureus	27.83 ±0.35	-	24.93 ± 0.08	25.13 ± 0.09	24.93 ± 0.11	30.40 ± 0.69	34.93 ± 0.11
S. saprophyticus	25.60 ± 0.52	-	-	13.03 ± 0.02	15.43 ± 0.40	29.66 ± 0.15	33.93 ± 0.05
E.coli	14.13 ±0.41	-	-	-	-	-	12.93 ± 0.11
P. aeruginosa	15.16 ±0.76	-	-	-	-	-	-
C. albicans	11.16 ±0.15	-	-	-	23.33 ± 0.57*	31.33 ± 0.57*	35.00 ± 0.10*

(-) Absence of an inhibition zone
*Observations after 72 h

Minimum inhibitory concentration values resulted from agar plate dilution assay (Table 2) with two weeks old crude ethanol extracts of *P. rosea* indicates these is high antimicrobial

activity in root extracts of *P. rosea against S. aureus, S. saprophyticus* and *C. albicans (MIC:* 0.128 – 0.064 mg/ml). However, the sensitivity of *E.coli* and *P. aeruginosa* is less *(MIC:* 4.096 mg/ml). On the basis of the result obtained in present investigation, it implied that the gram-positive bacteria were more susceptible to the root extract of *P. rosea* than the gram-negative bacteria. Possibly because of the presence of outer membrane that serves as an effective barrier in gram-negative species (Girish, 2008).

Table 2: Minimum inhibitory concentration of crude ethanol extract on different organisms

Organism	Minimum Inhibitory concentration /mg/ml	
	Trial 1	Trial 2
S. aureus	0.128	0.064
S. saprophyticus	0.128	0.064
C. albicans	0.128	0.128
P. aeruginosa	4.096	4.096
E.coli	4.096	4.096

Further, root extract of plant P. rosea has potential to use as a chemotherapeutical agent against bacterial and fungal infectious diseases, indicating possible industrial applications with value added products.

Conclusions

Root extracts of *P. rosea* has high antimicrobial activity in against gram positive bacteria: *S. aureus* and *S. saprophyticus* and fungi: *C. albicans*. However, the activity against the gram negative bacteria: *E.coli* and *P. aeruginosa* is less. *P. rosea* has potential to use for value added products with antimicrobial properties.

References

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