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Short Communication

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Antimicrobial activity of crude extracts of *Heliotropium marifolium* Retz.

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Abstract

Objective: To investigate the antimicrobial activity of different extracts (chloroform, ethylacetate, methanol, water) of the plant. *Heliotropium marifolium* Retz. Methods: Antimicrobial activity was assessed by standard dilution test using Mueller Hinton agar (MH) medium. The zone of inhibition of the extracts was compared with that of ciprofloxacin (5 μg / disc) by disc diffusion method. Results: The findings showed potential antimicrobial properties of the extracts against the organisms tested. The minimum inhibitory concentration (MIC) of the extracts was 133.33 μg/ml. The zone of inhibition of all extracts was comparable with that of ciprofloxacin. Conclusions: The study suggests that the plant is promising for development of phytomedicine for antimicrobial properties.

Key words : *Heliotropium marifolium* Retz, extracts, antimicrobial activity, ciprofloxacin.

1. Introduction

Several plants have been identified for their antimicrobial properties. The plants like *Clausina anisata* [1], *Semicarpus anacardium* (Phallatak) [2], *Cassia alata* [3] and *Thymus vulgaris* [4] are some which have been well documented for their antimicrobial properties. The hexane extract of the plant *H.marifolium* Retz has been reported to posses antimicrobial property against *Staphylococcus aureus, Escherichia coli* [5]. The literature survey indicates that other extracts of the plant namely chloroform, ethylacetate, methanol and water have not been investigated for their antimicrobial properties and hence the present study focussed on this. The plant *H.marifolium* Retz is belonging to the family Boraginaceae. The plant is usually herb and widely distributed in Pennisula region of Srilanka and also in some regions of waste lands in India.

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Table 1
Preparation of agar plates and antimicrobial activity of different extracts of *H.marifolium* Retz.

Extracts	**Concentration of the Extracts in µg/ml**	**Micro organisms**							
		S. aureus	***E.coli***	***K.pneumonia***	***P.aeruginosa***	***P. mirabilis***	***S. typhii***	***S. paratyphii A***	***S.paratyphii B***
Hexane	33.33	+	+	+	+	+	+	+	+
	66.67	+	+	+	+	+	+	+	+
	133.33	-	-	-	-	-	-	-	-
Chloroform	33.33	+	+	+	+	+	+	+	+
	66.67	+	+	+	+	+	+	+	+
	133.33	-	-	-	-	-	-	-	-
Ethylacetate	33.33	+	+	+	+	+	+	+	+
	66.67	+	+	+	+	+	+	+	+
	133.33	-	-	-	-	-	-	-	-
Methanol	33.33	+	+	+	+	+	+	+	+
	66.67	+	+	+	+	+	+	+	+
	133.33	-	-	-	-	-	-	-	-
Aqueous	33.33	+	+	+	+	+	+	+	+
	66.67	+	+	+	+	+	+	+	+
	133.33	-	-	-	-	-	-	-	-

+ Indicates growth ; - Indicates no growth

Table 2
Microbial inhibition zones (mm) of extracts from *H.marifolium Retz.* [a]

Micro Organisms		Extracts					
	Category	**H**	**C**	**E**	**M**	**W**	**Ref Cip[b]**
S.aureus	Gram (+)	28	28	28	30	27	31
E.coli	Gram (-)	39	37	38	39	37	40
K.pneumonia	Gram (-)	29	27	29	30	28	29
P.aeruginosa	Gram (-)	30	30	28	3	27	29
P.mirabilis	Gram (-)	29	29	27	28	26	25
S.typhi	Gram (-)	30	28	26	28	24	24
S. paratyphii A	Gram (-)	30	29	28	30	25	25
S. paratyphii B	Gram (-)	29	26	28	29	25	36

a Values are an average of triplicate
H - Hexane extract; C - Chloroform extract; E - ethylacetate extract; M - methanol extract;
W - aqueous extract (conc 133.3 µg/disc); G - gram reaction of bacterium.
b Cip - Ciprofloxacin (5 µg/disc) SD060 from Himedia, Laboratory Ltd., Mumbai 400 086, India.

2. Materials

The plant *H.marifolium* Retz was collected fresh, during the month of July 2002 from forest research area Kolappakam, Chennai and its authenticity confirmed by Plant Anatomy Research Centre (PARC) Tambaram in comparision with the voucher specimen desposited there.

The following microorganisms were selected for the study.

Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Salmonella paratyphii A, Salmonella paratyphii B. 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 (5 µg/disc) was obtained from Himedia Laboratory Ltd., Mumbai.

3. Methods

3.1 Preparation of plant extracts

The freshly collected plant material was dried in shade, then coarsely powdered and 100 g of the powder was extracted successively with hexane, chloroform, etyhlacetate methanol and water (each 250 ml) in a Soxhlet apparatus for 24 h. The extracts were filtered through Whatman No. 41 filter paper and evaporated on a water bath and finally dried in vacuum. The residues were suitably diluted with dimethyl formamide (DMF) so as to get the final concentration of each extract 1000 µg/ml and used for the study.

3.2 Antibacterial activity [8, 9]

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°C for 24 h. The results were read by the presence or absence of growth of organism (Table 1) and the

minimum inhibitory concentration (MIC) was determined (Table 2).

3.3 Zone of Inhibition [6, 7]

The discs of 6 mm diameter were prepared from Whatman filter paper No.41 and sterilized in hot air oven at 160°C for one hour. The discs were then impregnated with the MIC of the extracts, standard ciprofloxacin (5 µg/disc) and the solvent DMF.

The MH agar plates were inoculated with the bacterial culture by the standard procedure. The plates were allowed to dry. The standard, extract and DMF discs were placed on the agar plates and then the plates were kept at 4°C for 30 min to allow prediffusion of the standard, extracts and DMF. The plates were then incubated at 37°C for 24 h and the results were recorded (Table 2).

4. Results

All the extracts showed inhibitory response against all the organisms examined (Table 1). The MIC of all extracts was 133.33 µg/ml against the bacteria (Table 2). The zone of inhibition against the bacteria produced by all the extracts was comparable with that of standard ciprofloxacin.

5. Discussion

The results in the present study clearly demonstrated antimicrobial effect against *S. aureus, E.coli, K. pneumonia, P. aeruginosa, P. mirabilis, S. typhii, S. paratyphii A,* and *S. paratyphii B.* This study further revealed that besides hexane extract as reported earlier [5], other extracts (chloroform, ethyl acetate, methanol, water) also possess antimicrobial property.

Additionally, the hexane extract which was earlier reported effective against *S.auerus,* and *E.coli,* was also found to be effective against other organisms namely *Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, Salmonella paratyphii A,* and *Salmonella paratyphii B.* In conclusion the present study indicates that the plant contains potential antibacterial components that may be of use for development of phytomedicine for the therapy of infections.

References

1. Chakraborty A, Chowdury BK, Bhattacharya P. (1995) *Indian J.Pharmacol.* 40 : 295-298.

2. Nair A, Bhide SV. (1996) *Indian Drugs* 33 :323-8.

3. Sakharkar PR, Patil AT. (1998) *Indian J. Pharm. Sci.* 60, 311-12.

4. Agnihotri S, Vaidya AD. (1996) *Indian J. Exp. Biol,* 34:712-5.

5. Singh B, Dubey MM. (2001) *Phytotherapy Res.* 15, 231-234.

6. Bauer AW, Kirby WM, Sherrie JC, Turek M. (1966) *Am. J. Clin. Path* 45, 493-495.

7. Agarwal KC. (1974) *Indian J. Path. and Bact.*, 17(3), 149-151.

8. Charkrabothy. (1995) *A textbook of microbiology* 1st edition.

9. *Indian pharmacopoeia* (1998) Vol-II, Govt. of India, Ministry of Health and family welfare, A-53-54.