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Short Communication

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Anti-bacterial and anti-fungal activities of ethyl acetate extract and the isolated fraction of *Acanthospermum hispidum* DC

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Abstract:

Objective: To examine the anti-bacterial and anti-fungal activities of ethyl acetate extract and the isolated fraction of the plant *Acanthospermum hispidum* DC. Method: Anti-bacterial activity was studied by agar dilution method using Muller Hinton(MH) Agar media. And the MIC of the extract was compared with that of standard ciprofloxacin (5µg/ml). Anti-fungal activity of the extract was investigated by tube dilution method using Sabouraud Dextrose Agar (SDA) medium and the results compared with standard clotrimazole(125µg/ml). An attempt was made to isolate the fraction responsible for the anti microbial property of the extract. Result: The ethyl acetate extract showed potential anti-bacterial and anti-fungal properties comparable with standard ciprofloxacin and clotrimazole respectively against the organisms examined. The minimum inhibitory concentration (MIC) of the extract for anti-bacterial activity was 200µg/ml. The isolated fraction was also found to possess anti microbial properties similar to that of the crude extract. The MIC of the fraction was 133.33µg /ml. And the thin layer chromatographic study of the fraction showed it as diterpene. Conclusion: The plant appears to be promising for isolation of active constituent for development of phytomedicine for anti-microbial properties.

Key words: *Acanthospermum hispidum* DC, Ethyl acetate extract, Anti-microbial activity, Ciprofloxacin, Clotrimazole.

1. Introdction

The plant *Acanthospermum hispidum* DC belongs to the family Asteraceae (N.O. Compositae). The plant is a hispid herb widely distributed in South America, West Indies, Madagaskar and also in some rain forest region in India. Earlier reports indicate that the plant possess anti-viral properties against α-Herpes virus, Pseudo rabbies virus and Bovine Herpes virus-1 [1] and anti-plasmodial activity against Plasmodium falciparum chloroquine resistant w_2 strain [2]. Besides, the

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ethanolic extract of the leaves and flowering tops of the plant has also been reported to have varying degrees of activity against the pathogenic bacteria namely *Bacillus subtilis, Staphylococcus aureus, Streptococcus pyrogenes, Salmonella typhii, Pseudomonas aeruginosa, Escherichia coli, Clostridium histolyticum.*

The activity was attributed to the polar fractions of the alcoholic extract and the non-polar fraction were deficient in the anti-bacterial activity [3]. The antimicrobial properties of certain plants have been attributed to the presence of diterpenes [4]. The presence of diterpenes in *Acanthospermum hispidum* DC has also been reported [5]. Diterpenes are partially soluble in chloroform and freely soluble in ethyl acetate.

Considering this, in the present study an attempt was made to prepare ethyl acetate extract of the plant and to investigate the extract for its antimicrobial activity. Further the fraction contributing to the antimicrobial activity was also isolated and identified.

2. Materials

The plant *Acanthospermum hispidum* DC was collected fresh, during the month of May-2003 from the rain forest area of Thirunelveli, TamilNadu, India and its identity confirmed by Plant Anatomy Research Centre (PARC) Tambaram, Chennai-India, in comparison with the voucher specimen deposited there. The whole plant except its fruits was used for the present study, as the fruits are reported toxic [6].

The following micro-organisms were obtained from standard laboratory maintained in the Institute of Microbiology, Madras Medical College, Chennai-600 003, and used for the study.

Bacteria: Escherichia coli, Staphylococcus aureus, Salmonella typhiii Salmonella paratyphii A, Salmonella paratyphii B, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis and *coagulase negative staphylococcus (CONS).*

Fungi: Aspergillus niger, Penicillium chrysogenum, Microsporum gypseum and *Epidermophyton floccosum.*

The medium MH agar, Ciprofloxacin disc (5µg/ disc) and clotrimazole were obtained from Himedia laboratory Ltd, Mumbai-400 086, India.

3. Method

3.1 Preparation of plant extract

The freshly collected plant material excluding the fruits was dried in shade, then coarsely powdered and 1000 gram of the powder was extracted in an aspirated bottle with ethyl acetate (3000 ml) by cold maceration for 3 to 7 days. The ethyl acetate extract was filtered through whatmann no. 41 filter paper and evaporated on a waterbath and finally dried in vacuum. The residue was suitably diluted with di-methyl formamide (DMF) to get the final concentration of the extract as 1000 µg/ml and used for the study.

Thin layer Chromatography (TLC) of extracts – The TLC plates using Silica gel G were prepared by the standard procedure, the extract was spotted on the plates using a capillary tube 2 cm above the bottom end of the plate. The chromatogram was developed using different solvent systems.The spots identified by different spraying reagents. The R_f for the different spots were recorded.

3.2 Fractionation of the extract

The extract was fractionated in the column packed with silica gel GF(chromatographic grade) and eluted with varying solvents by gradient elution technique. The fraction with

Table 1
Anti-bacterial activity of ethyl acetate extract and isolated fraction of *Acanthospermum hispidum* DC.

Description	Micro-organisms (MIC in µg/ml)								
	E.coli	***K.pneu-monia***	***P.aeru-ginosa***	***P.mira-bilis***	***S.aureus***	***CONS***	***S.typhii***	***S.paraA***	***S.paraB***
Control	+	+	+	+	+	+	+	+	+
Ciprofloxacin	5	5	5	5	5	5	5	5	5
Ethyl acetate extract	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>133.33 <200	>33.33 <66.66	>66.66 <133.33	>66.66 <133.33	>33.33 <66.66
Isolated fraction	>66.66 <133.33	>66.66 <133.33	>66.66 <133.33	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66	>33.33 <66.66

(+) Indicates growth of the organism: Values are an average of triplicate.
Ciprofloxacin (5µg/ml) SD060 from Hi media laboratory Limited, Mumbai –400 086, India.

similar R_f values were identified by thin layer chromatography using Hexane-Ethyl acetate (17:3) as mobile phase and 50% sulphuric acid as detecting agent for the presence of diterpenes and it was confirmed further at *uv* 254nm [7].

3.3. Anti-bacterial activity [8,9]

The plates were prepared by using MH agar and the extracts of various dilution, allowed to solidify and dry. Then a loopful of the bacterial cultures was inoculated at the labeled spot and the plates were incubated at 37°C for 24 h.The results were read by the presence or absence of growth of organisms (Table1) and the minimum inhibitory concentration (MIC) was determined. The procedure was repeated for the investigation of the isolated fraction of the extract.

3.5 Anti-fungal activity

The slants were prepared by using Sabourauds dextrose agar medium as per standard procedures and allowed to set. The different fungi were inoculated into the slants and then incubated at 37°C for 1-4 weeks.The results were read by noting the presene or absence of growth of the organisms and compared with standard clotrimazole (125 µg/ml) (Table 2).

4. Results

Both the ethyl acetate extract and the isolated fraction of the extract demonstrated anti-bacterial and anti-fungal activity (Table 1&2). The MIC of the extract and the purified fraction against the bacteria tested are as shown in Table 1. The ethyl acetate extract and its fraction showed same pattern of anti-fungal activity. The minimum inhibitory concentration at which the anti-fungal activity observed was found to be >50<125 µg/ml for both the extract as well as the isolated fraction and the activity comparable with that of standard clotrimazole (125µg/ml) (Table 2).

5. Discussion

The results of the present study clearly indicated the anti-bacterial and anti-fungal properties of the ethyl acetate extract of the plant *Acanthospermum hispidum* DC. The anti-microbial activity of the extract was comparable with the standard anti-bacterial agent ciprofloxacin as well as the standard anti-fungal agent clotrimazole. The presence of diterpenes in the plant has been earlier reported [5] and these diterpenes possess anti-microbial properties [4]. The TLC of the isolated fraction

Table 2.
Anti-fungal activity of ethyl acetate extract and isolated fraction of *Acanthospermum hispidum* DC.

Fungi	MIC in µg/ml		
	Clotrimazole	Ethyl acetate extract	Isolated fraction
A.niger	125	>50<125	>50<125
P.chrysogenum	125	>50<125	>50<125
E.floccosum	125	>50<125	>50<125
M.gypseum	125	>50<125	>50<125

of the ethyl acetate extract showed the presence of diterpens with two different R_f values (0.346 and 0.384).

This supports the contention that the anti-microbial property of ethylacetate extract may be attributed to the presence of diterpenes in the extract. A detailed structural elucidation of the diterpenes in relation to the anti-microbial property will throw more light on the presence of the lead molecule in the plant.

Among the organisms tested in the anti-bacterial study *S. typhii* and *S. paratyphii B* appear to be highly susceptible to the effect of ethyl acetate extract and the isolated fraction (diterpenes) when compared with ciprofloxacin 5 µg/ml. These findings support the beneficial effects of the extract as well as the isolated fraction (diterpenes) against the pathogenic organisms *S. typhii* and *S. parathyphii B* (table 1).

Both the extract and the isolated fraction (diterpenes) in concentration 125, 250 and 500 mg/ml were effective against the fungi *A. niger, P. chrysogenum, E. floccosum* and *M. gypseum* and the effect comparable with that of standard Clotrimazole (125 mg/ml) (table 2).

However, the extract as well as the fraction in a lower concentration 50 mg/ml did not show significant anti-fungal property. The proper role of diterpenes in eliciting the anti-fungal activity is reported first time in the present study. The TLC of extract showed the presence of alkaloids, aminoacids, flavones, glycosides, monoterpenoids, titerpenoids, sesquiterpene lactones, saponins, besides diterpenes and the role of these constituents in the anti-microbial property requires a detail investigation.

The most significant observation in the present study is that the degree of anti-microbial activity produced by the extract is comparable with that produced by the isolated fraction (diterpenes). This important finding lead to suggest that the diterpenes may be playing a major role in eliciting the anti-microbial property of the extract.

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