



Anti-bacterial and anti-fungal activities of various extracts of *Acanthospermum hispidum* DC

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Abstract

Objective: To examine the 50% aqueous-ethanol, benzene and chloroform extracts of whole plant of *Acanthospermum hispidum* DC for their anti-bacterial and anti-fungal properties against a wide range of microorganisms. **Method:** The anti-bacterial activity was studied by agar dilution method using Muller Hinton (MH) agar media. The results were compared with standard ciprofloxacin (5 µg / disc). The anti-fungal activity of the extracts was investigated by tube dilution method using Sabouraud Dextrose Agar (SDA) medium and the results compared with standard Clotrimazole (125 µg/ml). **Result:** All studied extracts showed potential anti-bacterial and anti-fungal properties. The MIC (Minimum Inhibitory Concentration) was found to be 66.66 µg / ml for benzene extract and of varying range (66.66-200 µg / ml) for chloroform and 50% aqueous-ethanol extracts, with different micro-organisms. The anti-fungal property of all the studied extracts were comparable with that of standard clotrimazole. **Conclusion:** The present study brought to light the scientific data on the anti-infective property of the plant.

Keywords: *Acanthospermum hispidum* DC, Anti-microbial activity, Anti-bacterial activity, Anti-fungal property.

1. Introduction

The genus *Acanthospermum* includes 8 species, which are widely distributed in West Indies, South America and Madagascar. *Acanthospermum hispidum* DC selected for the study gets its name from hispid (rough hair) nature of plant parts. It is an aggressive colonizer of wastelands, hollow field and roadsides and also in some rain forest regions in India. Earlier reports indicated the presence of antiviral property and anti-plasmodial activity [1]. In

addition, the plant was also found to possess diuretic, febrifuge and sudorific activity. It is used for gonorrheal activities in some parts of America [2, 3]. Aqueous extract of both *Acanthospermum hispidum* DC and *Cajanus cajan* mixed together is used as an abortifacient in Brazil [4]. The ethanolic extract of the leaves and flowering tops got is reported for anti-bacterial activity [5]. Terpenoid, polyphenol, sesquiterpene lactones, phenolic compounds,

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sesquiterpenoids, diterpenes, flavones, nevadensin, salvigenin, volatile oil etc are previously isolated classes of constituents. In the present study, an attempt was made to investigate benzene, chloroform and 50% aqueous-ethanol extract of *Acanthospermum hispidum* DC for its anti-bacterial and anti-fungal activities against a wide range of micro-organisms.

2. Material

The plant specimen for the proposed study were collected fresh from the rain forest area, Thirunelveli District, Tamil Nadu on June 5th 2003 and its authenticity was confirmed by survey of Medical plants unit, Siddha C.C.R.A.S., Govt of India, Palayamkottai, Thirunelveli-627 002, Tamil Nadu, India and PARC, Tambaram, Chennai, 600 018, India. Voucher specimen of *A. hispidum* DC (N0.12003) deposited in the herbarium of the Department of Pharmacognosy, College of Pharmacy, Sri Ramachandra Medical College & Research Institute. The whole plant except its fruits was used for the study, as fruits are reported toxic [6].

The following micro-organisms were procured 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 typhi*, *Salmonella paratyphi* A and B, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and Coagulase Negative *Staphylococcus* (CONS).

The medium MH agar, ciprofloxacin discs (5 µg / disc) were obtained from Hi-media Laboratories Limited, Mumbai – 400 086, India.

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

were obtained from Hi-media Laboratory Ltd, Mumbai - 400 086, India.

3. Method

3.1 Preparation of plant extract

Freshly collected plant material excluding fruits were dried in shade, then coarsely powdered and 1 Kg of powder was extracted in an aspirated bottle with 50% aqueous - ethanol, benzene and chloroform by cold maceration for 3-7 days. All extracts was filtered through Whatman filter paper no.41 and evaporated on a water bath and finally dried in vacuum. The residue of all extracts was suitably diluted with DMF (Dimethyl formamide) to get a final concentration of 1000 µg / ml and used for the study.

3.2 Anti - bacterial activity [8]

The plates were prepared by using MH agar and the extracts of various dilutions allowed to solidify and dry. Then a loopful of bacterial cultures was inoculated at 37°C for 24 hours. The results were read by the presence or absence of growth of organisms (Table 1) and the MIC was determined. The same procedure was followed for the investigation of all extracts.

3.3 Anti-fungal activity [7, 8, 9]

For anti-fungal activity a stock solution of extract was serially diluted suitably with Dimethyl formamide (DMF) to get the final concentration of 1000µg/ml and used for the study. A volume of 0.5 ml of micro-organism suspensions containing approximately 4×10^6 cells were used to inoculate the surface of the solidified media (slants) prepared by using Sabouraud Dextrose Agar (SDA) medium and allowed to set and then incubated at 37°C for 1-4 weeks. The results were read by noting the presence or absence of growth of the organisms and compared with standard Clotrimazole (125µg/ml) (Table 2).

Table 1: Anti-bacterial activity of various extracts of *Acanthospermum hispidum* DC

Description	Micro-organisms (MIC in µg/ml)								
	<i>E.coli</i>	<i>K.pneu-</i> <i>monia</i>	<i>Paeru</i> <i>ginosa</i>	<i>P.mira</i> <i>bilis</i>	<i>S.aureus</i>	<i>CONS</i>	<i>S.typhii</i>	<i>S.para</i> <i>typhii A</i>	<i>S.para</i> <i>typhii B</i>
Control	+	+	+	+	+	+	+	+	+
Cipro- floxacin	5	5	5	5	5	5	5	5	5
Benzene	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	-	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66
Chloroform	> 66.66 < 133.33	> 33.33 < 66.66	> 66.66 < 133.33	> 33.33 < 66.66	> 33.33 < 200	> 66.66 < 133.33	> 33.33 < 66.66	> 66.66 < 133.33	> 66.66 < 133.33
50% Aqueous ethanol	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	> 33.33 < 66.66	> 66.66 < 133.33	> 66.66 < 133.33	> 33.33 < 66.66

Table 2: Anti-fungal activity of various extracts of *A. hispidum* DC.

Fungi	MIC in µg/ml			
	Clotrimazole	Benzene extract	Chloroform extract	50% Aqueous- ethanol extract
<i>Aspergillus niger</i>	125	> 50<125	> 50<125	> 50<125
<i>Penicillium chrysogenum</i>	125	> 50<125	> 50<125	> 50<125
<i>Microsporum gypseum</i>	125	> 50<125	> 50<125	> 50<125
<i>Epidermophyton floccosum</i>	125	> 50<125	> 50<125	> 50<125

Clotrimazole (125µg/ml) from Hi-media Laboratory Ltd, Mumbai – 400 086, India.
Values are an average of triplicate.

4. Results

All extracts demonstrated anti-bacterial activity as shown in Table 1 against the bacteria tested. The results of all extracts were comparable with that of the standard ciprofloxacin (5 µg / disc). All the extracts tested inhibited the growth of fungi *Aspergillus niger*, *Penicillium chrysogenum*, *Microsporum gypseum* and *Epidermophyton floccosum*. In concentrations 125, 250 and 500 µg/ml. In lower concentration (50µg/ml) all extracts were found to be ineffective against the fungi tested. The results of the extracts are 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 Benzene, Chloroform and 50% Aqueous-ethanol of *Acanthospermum hispidum* DC. The Anti-bacterial activity was comparable with that of the standard anti-bacterial agent Ciprofloxacin (5 µg/ml) against the organisms tested. The anti-fungal activity was comparable with the standard Clotrimazole. All extracts showed activity against the tested fungi in concentration 125, 250 and 500µg/ml. In lower concentration (50µg/ml) all extracts were ineffective against the tested fungi. The presence of diterpenes in genus

Acanthospermum has been earlier reported [10, 11] and these diterpenes in other plants possess anti-microbial and anti-fungal properties. The TLC of all extracts except 50% aqueous ethanolic extract showed the presence of diterpenes and possibly the anti-bacterial property of the extracts may be attributed to the presence of diterpenes in those extracts. Besides diterpenes, the presence of alkaloids, aminoacids, flavones, glycosides, monoterpenoids, triterpenoids, sesquiterpene lactones and saponins was also noticed in all these extracts. And possibly either or all of these constituents may be contributing to the anti-bacterial property to 50% aqueous-ethanol extract. However, the role of these phyto-constituents other than diterpenes in the anti-

bacterial and anti-fungal properties of benzene and chloroform extracts cannot be ruled out. Among the organisms tested for activity *Salmonella typhii* and *Salmonella paratyphii* B appear to be highly susceptible to the effect of all extracts.

These findings support the beneficial effects of the extracts against the pathogenic organisms. Further studies on the isolation and characterization of the diterpenes from benzene and chloroform extracts and elucidation of constituents responsible for activity in 50% aqueous-ethanol extract should throw light for the future development of phyto- medicine with anti-bacterial and anti-fungal properties.

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