

## **Antibacterial and antifungal activities of different organic solvent extracts of *Acalypha indica* (Linn.)**

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### **ABSTRACT**

*The present study is about the evaluation of antibacterial and antifungal activities of crude extracts of leaves of *Acalypha indica* L. using different solvents like petroleum ether, chloroform, ethyl acetate, acetone and methanol. The effect of different extracts were tested on Gram positive bacteria like *Staphylococcus aureus*, *Streptococcus pyogenes* and Gram negative bacteria like *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella flexneri* and *Proteus mirabilis* and on the fungal species *Candida albicans*, *Candida tropicalis* and *Aspergillus flavus* by in vitro disc diffusion method.*

**Key words:** Antibacterial, antifungal activity, *Acalypha indica*, solvent extraction

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### **INTRODUCTION**

*Acalypha indica* Linn. (Euphorbiaceae) is a common weed in many parts of Asia including India, Pakistan, Yemen, Sri Lanka and throughout Tropical Africa and South America [1]. It is an annual herb, about 80 cm high and commonly found in waste places or fields [2]. It is locally known as “kucing galak” or “rumput lis-lis”, “kuppaimeni” in India and “t’ie han tsai” in China [3]. This plant is used as diuretic, anthelmintic and for respiratory problems such as bronchitis, asthma and pneumonia [4]. The roots of *Acalypha indica* is used as laxative and leaves for scabies and other cutaneous diseases [5]. Major phytochemicals identified from *Acalypha indica* are acalyphine, cyanogenic glycoside, inositol, resin, triacetomamine and volatile oils [6]. This plant has been used extensively in herbal medicine in many tropical and sub tropical countries [3,1].

Previous studies on *Acalypha indica* revealed that this plant has antibacterial activity against several gram positive bacteria [7,8]. Others have shown that the species in the same genus has potential anti-microbial properties [9]. Recently, [10] reported that *Acalypha indica* have analgesic and anti-inflammatory effects. In Malaysia, *Acalypha indica* is used for generations for the treatment of superficial fungal and several other bacterial infections [11]. Thus, the objective of this current study was to evaluate the antibacterial and antifungal activities of petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of leaves of *Acalypha indica* and compare the anti-microbial activity with standard antibiotics and antifungal drugs.

### **MATERIALS AND METHODS**

#### **2.1 Collection of plant material**

The fresh leaves of *Acalypha indica* L. were collected in the month of June 2011 from Annamalai nagar (Latitude 11° 23' 17 N, Longitude 79° 42' 57 E), Cuddalore district, Tamilnadu State, India. Herbarium was deposited in

Department of Botany Annamalai University and, the voucher specimen No is AUBOT#249. The leaves of *Acalypha indica*. were washed thoroughly with running tap water followed by rinsing with distilled water. The leaves were shade dried at room temperature then pulverized into powder. Powder was stored in an air tight container till further use.

## 2.2 Preparation of Extracts

The dried powder of sample was successively extracted with petroleum ether, chloroform, ethyl acetate, acetone and methanol in soxhlet apparatus. The extract was stored at 4 °C and used for antimicrobial activity.

## 2.3 Test Organisms:

Gram positive bacterial strains such as *Staphylococcus aureus* and *Streptococcus pyogenes*; Gram negative bacteria strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Shigella flexneri* and *Proteus mirabilis* and fungal materials such as like *Candida albicans*, *Candida tropicalis* and *Aspergillus flavus* were obtained from the Department of Clinical Microbiology, Rajah Muthaiah Medical College and Hospital, Annamalai University, Annamalai nagar. The organisms were sub- cultured on Muller Hinton Agar medium incubated at 37 °C for 24 h and stored at 4 °C in the refrigerator to maintain stock culture for future requirements.

## 2.4 Antimicrobial activity assay

### 2.4.1 Disc Diffusion Method

An antimicrobial assay was performed by using the disc diffusion agar method [12]. Petridishes were first inoculated with microbes by pipetting the microbial suspension onto the agar. The standardized microbial suspension was applied onto the solidified agars by using sterile cotton swabs and allowed to dry for 10 minutes. Crude extract impregnated discs were aseptically transferred on the inoculated agar plates and left for incubation for 24 hrs to 7 days. The clear zones of inhibition around the test crude extract disc were measured for any indication of antimicrobial activity. Ciprofloxacin, Amphotercin and Ketoconazole impregnated discs were used as standard reference or positive controls and the solvents were used as negative controls. All assays were carried out in triplicates.

## RESULTS AND DISCUSSION

The presence of antibacterial and antifungal substances in the higher plants is well established as they have provided a source of inspiration for novel drug compound as plant derived medicines have made significant contribution towards human for the treatment of diseases as is done in cases of Unani and Ayurvedic systems of medicines.

The antimicrobial activity of different concentrations of petroleum ether, chloroform, ethyl acetate, acetone and methanol extracts of the leaves of *Acalypha indica* against bacterial and fungal pathogens and the results are presented in Table-1. The mean zones of inhibition ranged from 8.5 to 20.1 mm. The acetone extract of leaves of *Acalypha indica* exhibited the highest inhibitory activity against *Staphylococcus aureus* (20.1 mm/1000 µg/disc). Ciprofloxacin (5 µg/disc) antibacterial positive control produced mean zone of inhibition ranged from 18.1 to 25.3. Amphotericin-B (100 units/disc) antifungal positive control produced mean zone of inhibition ranged from 14.5 to 15.3 and Ketoconazole (10 µg/disc) antifungal positive control produced mean zone of inhibition ranged from 16.5. The blind control (5%) DMSO did not show any zone of inhibition. The methanol extract of leaves showed better inhibitory activity over all the microorganism studied. On the other hand, for ethyl acetate extract the mean zone of inhibition were ranged from 7.8 to 16.5 against *Pseudomonas aeruginosa*. The chloroform extract the mean zones of inhibition were ranged from 7.5 to 14.1 mm against *Staphylococcus aureus*. The petroleum ether extracts of *Acalypha indica* leaves exhibited the lowest activity against all the tested pathogens. Petroleum ether extract the mean zone of better inhibitory activity of over all the mean zone of inhibition ranged from 7.3 to 13.8 mm.

Bioactive compounds from plants serve as a novel source for infectious disease management as an alternate to synthetic drugs and several phytochemicals have been derived from the plant materials like bark, stem, leaves, roots, fruits, seeds, fruit rind, flowers and whole plants [13]. Similarly [14] is reported from *Moringa oleifera* Lam. Leaves extracts the pre-dominant antibacterial activity in the organic solvent (acetone) as compared to water, which indicates that the active compounds responsible for the bactericidal activity are more soluble in the organic solvents. Similar to this, in the present study, acetone extract had the maximum inhibitory activity but the water extracts did not show antibacterial activity. Thus, the present study establishes that the organic solvents are having more powerful antibacterial activity than the water extracts, as opined by [15].

Table 1. Antibacterial and Antifungal activities of different crude extracts from the leaves of *Acalypha indica* (L.)

S. No	Name of the organism	Mean zone of inhibition <sup>a</sup> (mm) <sup>b</sup> (Concentrations µg/ml)															Control
		Petroleum ether			Chloroform			Ethyl acetate			Acetone			Methanol			(Cip)/ (Amp)/ (Ket)
		1000	500	250	1000	500	250	1000	500	250	1000	500	250	1000	500	250	
1	<i>S. aureus</i>	11.1±0.2	11.0±0.3	9.0±0.2	14.1±0.2	11.0±0.5	10.0±0.2	15.4±0.3	14.5±0.2	10.2±0.2	20.1±0.6**	17.5±0.2	14.2±0.1	18.0±0.1	15.1±0.1	13.0±0.3	18.1±0.6
2	<i>S. pyogenes</i>	12.5±0.1	10.0±0.2	8.5±0.2	12.5±0.2	10.0±0.2	8.1±0.3	13.5±0.2	10.5±0.2	9.6±0.1	15.8±0.3**	12.1±0.1	10.0±0.2	14.0±0.2	11.0±0.3	9.8±0.3	18.3±0.4
3	<i>E. coli</i>	12.0±0.1	8.0±0.1	7.5±0.2	12.3±0.3	9.5±0.2	7.8±0.2	12.8±0.3	9.0±0.1	8.0±0.1	14.0±0.5	10.0±0.1	9.3±0.1	13.5±0.1	9.5±0.1	8.8±0.2	20.8±0.6
4	<i>P. aeruginosa</i>	12.5±0.2	10.1±0.1	8.3±0.1	13.8±0.3	10.8±0.2	8.8±0.2	16.5±0.1	9.0±0.2	9.0±0.3	16.8±0.2	13.0±0.1	10.0±0.2	15.3±0.1	11.0±0.1	9.0±0.2	25.3±0.4
5	<i>P. vulgaris</i>	10.5±0.2	9.5±0.1	7.3±0.1	11.5±0.2	9.5±0.1	7.5±0.1	12.0±0.1	10.3±0.1	9.0±0.1	13.0±0.2	11.0±0.1	9.5±0.1	12.5±0.5	10.5±0.1	9.0±0.15	24.1±0.4
6	<i>S. flexneri</i>	11±0.1	9.8±0.1	7.8±0.2	12.3±0.1	9.8±0.1	8.0±0.2	12.5±0.1	10.5±0.1	9.1±0.1	13.5±0.1	11.3±0.1	9.5±0.1	13.1±0.1	10.6±0.2	9.5±0.1	24.3±0.6
7	<i>P. mirabilis</i>	11.0±0.4	9.3±0.1	7.5±0.1	11.5±0.1	9.5±0.1	7.5±0.1	12.0±0.2	9.8±0.2	8.0±0.1	12.8±0.2	12.5±0.1	9.0±0.2	12.5±0.1	10.0±0.2	8.8±0.3	21.3±0.3
8	<i>C. albicans</i>	13.8±0.3	9.1±0.1	7.5±0.1	12.0±0.1	9.5±0.1	8.0±0.2	12.3±0.1	10.1±0.1	9.0±0.1	14.0±0.2	11.0±0.1	9.8±0.3	12.8±0.3	10.5±0.1	9.3±0.1	15.3±0.3
9	<i>C. tropicalis</i>	11.0±0.2	8.8±0.2	7.3±0.1	11.3±0.1	9.0±0.1	7.5±0.1	11.8±0.2	9.3±0.1	7.8±0.3	12.5±0.2	10.11±0.2	8.5±0.1	12.1±0.2	9.5±0.1	8.0±0.2	14.5±0.5
10	<i>A. flavus</i>	12.1±0.2	10.0±0.2	7.5±0.1	12.8±0.2	10.0±0.2	8.5±0.1	13.5±0.2	10.8±0.2	9.3±0.6	16.3±0.2	12.3±0.2	10.1±0.1	14.1±0.1	11.5±0.1	9.5±0.2	16.5±0.5

\*\* mean±= Standard deviation; Including disc (6mm) diameter; bacterial = Cip- Ciprofloxacin (5µg/disc); Candida =Amphotericin – (100 units/disc); Ket- Ketoconazole for Aspergillus (10 µg/disc)

In this study, different extracts of *Acalypha indica* leaves have potent antimicrobial activity against Gram positive and Gram negative bacteria, and fungal pathogens were equally affected by the leaf extract of *Acalypha indica* indicating the presence of broad spectrum of antibacterial substance in the plant.

### CONCLUSION

The plant extracts studied could be an answer to the people seeking for better therapeutic agents from natural sources which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. In the present study, acetone extract of leaves of *Acalypha indica*. can be effectively used for curing the bacterial diseases. So further study is needed to more about acetone extract of leaves of *Acalypha indica*.

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