



STUDIES ON ANTIMICROBIAL ACTIVITY OF *ACALYPHA INDICA* ALONG WITH PRILIMINARY PHYTOCHEMICAL SCREENING

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ABSTRACT

The aim of the present study was to investigate the antibacterial properties and phytochemical evaluation of *Acalypha indica*. The organic solvent (Ethanol, Methanol, Hexane) and water extracts from the whole plant of *Acalypha indica* (Euphorbiaceae) were tested against, *Salmonella typhimurium*, *Proteus vulagaris*, *Shigella dysenteriae* and a fungal pathogen *Candida albicans* by Agar disc diffusion method. The results showed prominent antimicrobial activity against the tested microbial pathogens. Of all those, Methanol extract was found to give a strong antimicrobial effect when compared to the other extracts (Ethanol, Hexane and water). Phytochemicals like tannins, flavonoids, alkaloids, steroids, saponins and terpenoids are found in the tested samples. The Anthraquinones were found to be absent in plant material observed.

KEY WORDS: *Acalypha indica*, Agar Disc Diffusion Method, Phytochemicals, Antimicrobial Properties

INTRODUCTION

Acalypha indica (Euphorbiaceae) is an herb distributed throughout India and other topical regions of the world. The various parts of the plant (leaves, roots, seeds and seed and seed oil) are widely used in a variety of ailments in traditional system of medicine such as Ayurveda and Siddha. The paste of plant leaves is used for the treatment of skin diseases by rural people. The aim of present research is, to determine the preliminary phytochemical constituents, antimicrobial activity of Ethanol, Methanol, Hexane and water extracts of the leaves and stems of *Acalypha indica*. Traditional medicines derived from medicinal plants are used by about 60% of the world's population. Though there are various approaches to control diseases and their secondary complications, herbal formulations are preferred due to lesser side effects and low cost. The use of and search for drugs and dietary supplements derived from plants has been increased in recent years. Botanists, Ethno pharmacologists, microbiologists, and

chemists are combing the earth for phytochemicals and drugs which could be developed for treatment of highly infectious diseases in a natural way. While 30 to 50% of current pharmaceuticals are derived from plants, only a few of them are used as antimicrobials. Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as Terpenoids, Tannins, Alkaloids, Flavonoids, saponins and Anthraquinones which have been found in vitro to have antimicrobial properties. *Acalypha indica* belonging to family Euphorbiaceae, commonly called Indian Copperleaf grows along the sides of the road which is often mistaken as a weed plant inspite of immense medicinal properties. Indian Copperleaf is a small erect herb, growing up to 60 cm or more. The ascending branches are angled and velvet-hairy. Leaves are broadly ovate, nearly triangular, rather coarsely toothed. Leaf stalks are as long as or longer than the 3-5 cm long blades.

Flowers are stalkless, borne on erect axillary spikes longer than the leaves. Male flowers are minute, crowded distally. Female flowers are scattered along the inflorescence axis, each subtended by a conspicuous semicupular leaf-like toothed green bract nearly 7 mm long. Capsule is bristly, 1 mm broad.^[1-3] Some of the medicinal properties used by folk are: Juice of the root and leaves given to children as expectorant and emetic. The leaves, in decoction or powdered form, are used as a laxative. For constipation, an anal suppository of the bruised leaves helps relax the constricted sphincter ani muscle. In Philippines, decoction of leaves used for dysentery. Leaves mixed with common salt applied to scabies. In Indian pharmacopoeia, it is used as an expectorant. Also used for the prevention and reversal of atherosclerotic disease.

Used for pneumonia, asthma and rheumatism. In Tamilnadu, India, the Paliyar tribes of Shenbagathope use the entire plant for bronchitis, a decoction of the herb for tooth- and ear aches and paste of the leaves is applied to burns. Poultice of bruised leaves used for syphilitic ulcers, to maggot-eaten sores and as an emollient to snake bites. Decoction of leaves used as instillation for earaches and for periauricular poultice or compress. Leaves mixed with garlic used as anthelmintic. Root, bruised in water, used as a cathartic. Powdered dried leaves used for bed sores. Juice of, fresh leaves, mixed with oil or lime, are used for rheumatic complaints. Bruised leaves used as "suppository" in constipation, assumed to work through decrease of the sphincter anti contraction.



Figure 1
Acalypha indica with fruits and flower

MATERIALS AND METHODS

Acalypha indica plants were collected from various places in and around the areas of Kurnool. Whole plants were collected and identified by comparing with herbarium specimens. The stems along with leaves of plants were air-dried and powdered. The dry powder was extracted by refluxed in 100 mL methanol for 24 h, using a Soxhlet apparatus (Khan *et al.*, 1988). The extract was filtered using Whatman filter paper, No. 1. The filtrate was then evaporated using rotary evaporator and dried at 55°C. Ethanol, methanol, hexane and distilled water extracts are obtained and all the extracts are preserved. Dried extract was stored at 20°C in labeled, sterile capped bottles. Stock cultures of microbes are maintained at a temperature of 4 degrees centigrade, active cultures are prepared by growing in tubes of

Muller-Hinton (MHB) / Potato dextrose agar (PDA) for bacteria and Sabouraud dextrose broth (SDB) for fungi.

Microorganisms

The bacterial colonies were isolated from hospital samples at Kurnool, their pure cultures were maintained in nutrient agar and stored at 4°C. Three gram negative bacterial species were grown, namely *Salmonella typhimurium*, *Proteus vulgaris*, *Shigella dysenteria* and the fungus *Candida albicans*.

Antimicrobial assay

Sensitivity tests were performed by disc diffusion with standard antibiotics, following Kirby-Bauer method (Bauer *et al.*, 1966). The assessment of antimicrobial activity was done based on measurements of the diameter of inhibition zones

(NCCLS, 1998). Of the four extracts, Methanolic extract has given interesting results and the aqueous extract showed no response.

phytochemical screening

Phytochemical testing is done for the methanolic extracts as it has shown the interesting activity. The details of the tests are as follows:

1. Braemer's test for Tannins: To a 2–3 ml of methanolic extract, 10% alcoholic ferric chloride solution was added. (Dark blue or greenish grey coloration of the solution indicate the presence of tannins in the drug).
2. Liebermann-burchardt test for Steroids: To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (Dark green coloration of the solution indicate the presence of Steroids)
3. Liebermann-burchardt test for Terpinoids: To 1 ml of methanolic extract of drug, 1 ml of chloroform, 2–3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. (dark pink or red coloration of the solution indicate the presence of terpenoids).
 - a) Salkowski Test for Terpenoids: The extract was mixed with 2ml of chloroform and concentrate H₂SO₄ (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terpenoids.
4. Dragendorff's reagent test for Alkaloids: A drop of methanolic extract was spotted on a small piece of pre-coated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicates the presence of alkaloids)
5. Shinoda test for Flavanoids: To 2–3 ml of methanolic extract, a piece of magnesium ribbon and 1 ml of concentrated hydrochloric acid were added. (Pink red or red coloration of the solution indicate the presence of flavonoids in the drug).
6. Bornträger's test for anthraquinones: About 50 mg of methanolic extract was heated with 10% ferric chloride solution and 1 ml of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. (Pink or deep red coloration of aqueous layer indicate the presence of anthraquinones).
7. Keller-Kiliani test for Cardiac glycosides:

Methanol extract was obtained and the extract reduced to dryness. 50 mg of this was dissolved in 2 ml chloroform. H₂SO₄ was added to form a layer and the colour at interphase recorded. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

8. Frothing test for saponins : A small amount of extract was shaken with water and observed for the formation of persistent foam.

Antimicrobial disc diffusion assay

Antibacterial and antifungal activities of the four plant extracts were investigated by the disc diffusion method^[4]. The MHA plates, containing an inoculum size of 10⁶ colony-forming units (CFU)/mL of bacteria or 2x10⁵ CFU/mL yeast cells on SDA were spread on the solid plates with a glass rod. Then discs (4.0-mm diam.) impregnated with 50 µL of each extract at a concentration of 100.0mg/mL were placed on the inoculated plates. Similarly, each plate carried a blank disk by adding solvent control alone in the centre, and antibiotic discs (6.0-mm diam.) of (20 µg/ml, Streptomycin sulphate for bacteria) and Nystatin (20 µg/ml, for fungal) were also used as a positive control. All of the plates were incubated at 37°C for 18 hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the discs were measured after 18 hours of incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones on the agar surface around the discs .

RESULTS AND DISCUSSION

The aqueous extracts of plant has shown negligible antimicrobial activity on tested pathogens, whereas the Methanol extract of plant has shown maximum inhibition on *Salmonella typhimurium* (20.1±1.3).and and it has no effect on *Proteus vulgaris*. Ethanol extract of plant has shown maximum inhibition on *Salmonella typhimurium* (15±0.9) and it also has no effect on *Proteus vulgaris* .Of all the extracts Methanolic extracts have shown maximum inhibition ,so it is used for phytochemical screening of secondary metabolites. Hexane extract of plant has shown maximum inhibition on *Salmonella typhimurium*(12.03) and it has no effect on *Proteus vulgaris* and *Candida albicans* . The results of the phytochemical

screening to test the presence of tannin, anthraquinone, alkaloid, saponin, phlobatannin, flavonoid, cardiac glycosides, volatile oils, terpenoids and steroids in the extracts from various parts of *Acalypha indica* are shown in Table I. The preliminary phytochemical screening study revealed that the leaf of *Acalypha indica* has presence of tannins, cardiac glycosides, alkaloids, flavonoid, terpenoid & saponins. Anthraquinones and steroids are absent in leaf. The roots of *Acalypha indica* contain cardiac glycosides, alkaloids, flavonoid, and steroids. Tannins, Anthraquinones and terpenoids are absent in the root and flowers. Anthraquinones were found to be absent in the stem. Treating Gram-negative bacterial infections can be difficult because of several unique features

of these bacteria. For example, the unique nature of their cell wall makes them resistant to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by carbapenems. However, even these drugs have become ineffective against some bacteria, leaving researchers to go for natural resources, which are medicinal plants. New drugs to combat Gram-negative bacterial infections are needed. In addition, researchers are unraveling the molecular mechanisms of drug resistance in Gram-negative bacteria to identify novel strategies to combat these pathogens. This paper helps in formulating natural principles to combat drug resistance of certain gram negative bacteria.

Table I
Antimicrobial activity of Acalypha indica.

Solvent extracts	μL	Zone of inhibition in mm			
		<i>Salmonella typhimurium</i>	<i>Shigella dysenteriae</i>	<i>Proteus vulgaris</i>	<i>Candida albicans</i>
Aqueous	50	-N-	-N-	-N-	-N-
Methanol	50	20.1 \pm 1.3	12 \pm 0.6	-N-	10.1
Ethanol	50	15 \pm 0.9	10.2	-N-	12.36
Hexane	50	12.03	9.5	-N-	-N-
Streptomycin sulphate($\mu\text{g/ml}$)	20	28 \pm 0.7	25 \pm 0.8	21 \pm 0.5	-
Nystatin($\mu\text{g/ml}$)	20	-	-	-	18 \pm 1.8

-N- --No activity

Table II
Phytochemical Screening of Secondary Metabolites from Acalypha indica Methanolic extract

S.NO	Secondary metabolites	Name of the test	Leaf	Stem	Flower	Root
1.	Tannins	Braemer's test	+	--	--	+
2.	Flavonoids	Shinoda test	+	+	+	+
3.	Anthraquinone	Bornträger's test	--	--	--	--
4.	Saponins	Frothing test	+	+	+	+
5.	Cardiac glycosides	Keller-Kilianii test	--	+	+	+
6.	Alkaloid	Dragendorff test	+	+	+	+
7.	Steroids	Lieberman Burchardt test	--	+	+	+
8.	Terpenoids	Lieberman Burchardt test	+	+	--	--
		Salkowski test	+	+	--	--

'+'Present, '--'Absent

CONCLUSION

In conclusion, all the extracts investigated except water possessed activity against at least one strain of bacteria and/or fungi. Further studies aimed at the isolation and identification of active substances from the Methanol extracts of *Acalypha indica* could also evolve compounds with effective natural medicinal values for the cure of microbial disorders. The plant is said to be a source of many bioactive compounds acting

against some human diseases. The present study helps in herbal formulation of *Acalypha indica* for its fight against infectious microbes.

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