

## Phytochemical Analysis And *In Vitro* Antibacterial Activity Of Leaf

### Extract Of *Acalypha Indica* Linn.

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#### ABSTRACT

*Acalypha indica* is herb found in tropical countries. This is used traditionally for treating various diseases for centuries, including anti-bacterial and anti-fungal activities. Thus, the objective of this present investigation was to perform qualitative analysis of phytochemical compounds and also evaluate *in vitro* anti-bacterial activity of *Acalypha indica*. Crude ethanol and water extract of leaves from *Acalypha indica* were tested for anti-bacterial activity against five bacterial species - *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* (Gram-negative bacteria) and *Staphylococcus aureus* (Gram-positive bacteria). It is inferred that ethanolic extract exhibited strong antibacterial activity with a maximum activity recorded against *Staphylococcus aureus* (20mm). The qualitative phytochemical screening indicated the presence of alkaloids, phenols saponins, steroids, flavonoids and catechol.

**KEYWORDS:** *Acalypha indica*, antimicrobial activity, phytochemical constituents

## INTRODUCTION

India has a rich flora that is widely distributed throughout the country. Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Medicinal components from plants play an important role in conventional as well as western medicine. Plant derived drugs have been a part of the evolution of human, healthcare for thousands of years. Plant based drugs were commonly used in India and China (Duraipandiyan and Ignacimuthu, 2007).

Medicinal plants constitute an effective source of both traditional and modern medicine. Herbal medicine has been shown to have genuine utility and about 80% of rural populations depend on it as their primary health care. The world health organization recently compiled an inventory of more than 20,000 species of medicinal plants. Indian medicinal plants and their products are used to control diverse diseases such as catarrh, bronchitis, pneumonias, ulcers and diarrhea. Traditional medical treatments in daily life are now being used with empirical methods (Tanaka *et al.*, 1999). It was also reported by Gupta (2003) that contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infections and diseases.

*Acalypha indica* L. (family: *Euphorbiaceae*) is a weed widely distributed throughout the plains of India. It has been reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments (Chopra *et al.*, 1956). The plant is used as expectorant as a substitute for senega. It's also has many uses as diuretic action, useful for bronchitis, asthma, pneumonia, rheumatism, purgative, antiparasiticide, antibacterial and antihelminthic (Varies *et al.* 1996). Activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Candida albicans* (yeast) is discussed from 35 plants available in the Turkish flora were as for their *in vitro* antibacterial activities against pathogenic bacterial and yeast (Sokmen *et al.* 1999). This plant contains acalyphe, cynogenic glycoside, inositol methylether, resin, triacetomamine and volatile oils.

## MATERIALS AND METHODS

### Plant Collection

*Acalypha indica* leaves were taken from the gardens of Hindusthan college campus located in Coimbatore, Tamilnadu, India. The leaves were separated and oven dried at 45°C overnight. The plants were grounded into powder form and used for further investigations.

### Solvents Used

Organic solvents like ethanol and acetone were used to prepare different extracts. All the solvents used were of analytical grade.

### Qualitative Analysis

**Test for Alkaloids:** To 2ml of test solution, added 2 N HCl, aqueous layer formed was decanted and to that added few drops of Mayer's reagent. The test result was observed.

**Test for Anthroquinone:** To 2ml of test solution, added magnesium and acetate solution. The result was observed.

**Test for Catachol:** To 2ml of test solution in alcohol added Erlich's reagent and few drops of concentrated HCL. The result was observed.

**Test for Flavonoids:** To 2ml of test solution, added alcohol and a bit of magnesium. Then few drops of concentrated hydrochloric acid was added and boiled. The test result was observed.

**Test for Phenols:** To 2ml of test solution, added alcohol and then few drops of neutral ferric chloride solution was added. The test result was observed.

**Test for Saponins:** To 2ml of test solution, added 2ml of water and shake well. The result was observed.

**Test for Steriods:** To 2ml test solution, added minimum quantity of chloroform. Then 3-4 drops of acetic anhydride and 3 drops of concentrated sulphuric acid were added. The test result was observed.

**Test for Tri Terpenoids:** To 2ml of test solution, added pieces of tin and 2 drops of thionyl chloride. The test result was observed.

**Test for Tannins:** To 2ml of test solution, added lead acetate solution. The result was observed.

### Leaf Extract Preparation

Surface sterilized leaves were subjected to ethanol and acetone solvent extraction. Samples were extracted with solvent one after another by Soxhlet apparatus for about 24 hours (Brantner and Grain, 1994).

### Bacterial Strains Used

The bacterial strains viz *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus sp* were obtained from MTCC, Chandigarh, India. The bacterial stock cultures were maintained on nutrient agar.

### Antimicrobial activity

Antibacterial assay of the crude leaf extracts of ethanol and distilled water were performed on nutrient agar plate by well diffusion method enriched with various concentrations (20, 40, 60, 80 and 100 $\mu$ l) of extracts. Petri plates were prepared with 20 ml of sterile Nutrient Agar (HIMEDIA, Mumbai, India). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Wells are punctured in the culture medium at 2cm apart from each other using sterile Cork borers. The tests were conducted at five different concentration of the leaf extract. Negative control was prepared using respective solvent. Streptomycin was used as positive control solvent. The plates were incubated for 24 h at 37°C. The antibacterial activity was measured as the zone of inhibition recorded in millimeters.

## RESULTS AND DISCUSSION

The qualitative analysis of the extracts from leaf sample of *Acalypha indica* showed the presence of phytochemical constituents such *alkaloids*, *catechols*, *flavonoids*, *phenolic compounds*, *saponins* and *steroids*. At the same time, the phytochemical constituents like *anthroquinone*, *tannins* and *triterpenoids* were absent.

The plant products over synthetic compound in the treatment of diseases are needed, because it does not have a deleterious effect in higher plants and animals including man. In India we have a variety of traditional medicine systems that rely to a very large extent on native plant species for their raw drug materials. So, now we have to work on traditional medicinal plants which can serve as therapeutic agent. The qualitative analysis of *Acalypha indica* revealed the presence of biomolecules such as *alkaloids*, *catechols*, *flavonoids*, *phenolic compounds*, *saponins* and *steroids* respectively.

Medicinally important plant species *Acalypha indica* was selected for screening of secondary metabolites. During this investigation, an attempt has been made to decipher the effect of these secondary metabolites towards its antibacterial activities. Hexane, chloroform, ethyl acetate and methanol extracts from the leaf and of *Acalypha indica* exhibit antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus faecalis*, *Pseudomonas aeruginosa*.

Hexane, chloroform, ethyl acetate and methanol extracts of *Acalypha indica* leaves showed significant zone of inhibition against "Gram-positive" bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus faecalis* and one "Gram-negative" bacterium *Pseudomonas aeruginosa* (Azmahani A., 2002).

Antibacterial activity of *A. indica* is listed in Table 2. The extract of *A. indica* showed varying degrees of antibacterial activity against all microorganisms tested. The gram positive bacteria are more susceptible than the gram negative bacteria. These different antibacterial activities could be due to the nature and concentration of antibacterial compounds plus its/their mode of action (Tortora et al., 2001).

**TABLE 1 Qualitative analysis of leaf samples of *Acalypha indica***

Sl.No	Phytochemicals	Quantity of test solution	Chemicals & Reagents used	Results
1	Alkaloids	2 ml	2N HCl, Mayer's reagent	Positive
2	Anthroquinone	2 ml	Magnesium and Acetate Solution	Negative
3	Catechol	2 ml	Alcohol, Erlich's reagent and Conc.HCl	Positive
4	Flavonoids	2 ml	Alcohol, Magnesium and Conc.HCl	Positive
5	Phenols	2 ml	Neutral Ferric Chloride	Positive
6	Saponins	2 ml	Water	Positive
7	Steroids	2 ml	Chloroform, Acetic Anhydride, Sulphuric acid	Positive
8	Tri Terpenoids	2 ml	Thionyl chloride	Negative
9	Tannins	2 ml	Lead acetate solution	Negative

**TABLE 2 Antibacterial Activity of *Acalypha indica***

Sl.No	Sample	Concentration ( $\mu$ l)	Bacteria		
			<i>E.coli</i>	<i>S.aureus</i>	<i>B.subtilis</i>
1	Ethanol Extract	20	-	6.5	-
2	Ethanol Extract	40	7.1	10.7	11.0
3	Ethanol Extract	60	12.7	14.3	12.3
4	Ethanol Extract	80	15.6	17.5	13.2
5	Ethanol Extract	100	18.3	20.7	15.6

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