

Antibacterial Potentiality of Ethanol and Ethyl Acetate Extract of *Acalypha indica* against Human Pathogenic Bacteria

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Abstract

Medicinal plants have been used as a source of medicine and in widespread use of herbal remedies and healthcare preparations. Nowadays, several plants have been identified for their antimicrobial properties. The present study was conducted to evaluate the antibacterial potentiality of ethanol and ethyl acetate solvent extracts of mature leaves of *Acalypha indica* against nine pathogenic bacterial isolates viz., *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Pseudomonas aeruginosa*. The turbidity of the bacterial inoculums was compared with 0.5 Mc Farland standards and the antibacterial potential of *Acalypha indica* ethanol extract was tested by using Agar well diffusion method. The ethanol extract of *Acalypha indica* (100 mg/ml) showed maximum zone of inhibition (30 mm) against *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*. *Staphylococcus aureus* showed less zone of inhibition (12 mm). The ethyl acetate extract of *Acalypha indica* (100 mg/ml) showed maximum zone of inhibition (23 mm) against *Escherichia coli*. There was no zone of inhibition against *Pseudomonas aeruginosa*. Phytochemical tests were performed and showed that the antibacterial activity of *Acalypha indica* plant leaves was due to the presence of phytochemical compounds like alkaloids and tannins.

1. Introduction

Infectious disease can become a threat to public health in this world. The use of medicinal plants for the treatment of various diseases is an old practice in most countries and it still offers a enormous potential source of new anti-infective agents. Although ancient civilization recognized the antiseptic or antibacterial potential of many plant extracts, they failed to document the preservative and curative effects of plant extracts (Arumugam *et al.*, 2009).

Plants are the basic sources of medicine. Interest in a large number of traditional natural products has increased (Taylor *et al.*, 1996). Indian biodiversity is known for its richness and variety of medicinal plants. Pathogens are a major threat to major life forms, especially humans, animals and birds. Inhibition of microbial activity using plant extracts has gained an importance due to their efficacy and target specify towards pathogens (Renganathan *et al.*, 2009).

Plants have been an essential part of human society since the start of civilization. Around 250 drugs have been identified from plants during Rig Veda and Atharvana Veda descriptions of the Veda period. The rural population in different parts of the world is more disposed to traditional ways of treatment because of very easy availability and

cheaper cost. It is estimated that 80% of the black population is consulting with traditional healers (Rajasekara Pandiyan *et al.*, 2007).

In recent years, drug resistance to human pathogenic bacteria has been commonly and widely reported in literature (Rubin *et al.*, 1999). Because of the side effects and the resistance that pathogenic microorganisms built against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Essawi *et al.*, 2000).. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of world (Saxena and Sharma, 1999).

The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used. Antimicrobials therefore, may have a significant clinical value in treatment of resistant microbial strains (Eloff, 1998). In particular, the antimicrobial activities of plant oils and extracts have formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Hammer *et al.*, 1999).

Acalypha indica is an annual erect herb commonly called as “Kuppai meni”. It belongs to the family Euphorbiaceae. It is a common shrub in Indian

gardens, backyards of houses and waste places through the plains of India. The root, stem and leaf of *Acalypha indica* possess herbal activity. The present study was aimed to evaluate the antibacterial potentiality of ethanol and ethyl acetate extract of *Acalypha indica* against nine bacterial pathogens and phytochemical analysis of *Acalypha indica*.

2. Materials and Methods

Collection and Drying of plant materials

Mature leaves of *Acalypha indica* were collected from Dhesiga Perumal temple at Chengelpet, Tamil Nadu. The leaves of *Acalypha indica* were washed thoroughly three times with water and once with distilled water. The plant materials were air dried and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

Preparation of plant extract

40 g of powdered leaves were extracted successively with 200 ml of ethanol at 56-60°C and ethyl acetate at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use (Chessbrough, 2000).

Test microorganisms

Nine pathogenic bacteria, viz., *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Pseudomonas aeruginosa* were used during the present study and were obtained from SGS India laboratories – Thoraipakkam, Chennai – 96. The cultures were sub-cultured and maintained on Nutrient agar slants and stored at 4°C.

Determination of antibacterial activity

Preparation of Mc Farland Nephelometer standards

10 test tubes of equal size and good quality have been thoroughly cleaned and arranged in the test tube stand. 1% chemically pure Sulphuric acid and 1.175% aqueous solution of Barium chloride was prepared. Slowly and with constant agitation, the designated amounts of two solutions were added to the tubes as shown in Table-1 to make a total volume of 10 ml per tube. The tubes were sealed. The suspended Barium chloride precipitate corresponds approximately to homogenous cell densities per ml throughout the range of standards as shown in table. Store the Mc Farland standard tubes in the dark at room temperature. They should stable for 6 months.

Table-1. Mc Farland Nephelometer standard

Chemical Name / std	0.5	1	2	3	4	5	6	7	8	9	10
Barium chloride (ml)	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Sulfuric acid (ml)	10	9.9	9.8	9.7	9.6	9.5	9.4	9.3	9.2	9.1	9
Approximate cell density (x 10 ⁸ /ml)	1.5	3	6	9	12	15	18	21	24	27	30

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards.

Determination of antibacterial activity (Agar well Diffusion Method or Cup Plate Method)

Muller Hinton agar plates were inoculated with test organisms by spreading the bacterial inoculum on the surface of the media. Wells (8 mm in

diameter) were punched in the agar. Ethanol and ethyl acetate extracts with different concentrations (25 mg/ml, 50mg/ml, 75mg/ml and 100 mg/ml) were mixed with 1 ml of Dimethyl sulfoxide (DMSO) and added into the well. Well containing DMSO alone act as a negative control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (in mm).

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the following procedure.

Test for Alkaloids (Meyer's Test)

The extract of *Acalypha indica* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent (Siddiq and Ali, 1997). The samples were then observed for the presence of turbidity or yellow precipitation (Evans, 2002).

Test for Glycoside

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer (Siddiq and Ali, 1997).

Test for Tripenoid and Steroid

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids (Siddiq and Ali, 1997).

Test for Flavonoid

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was

warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones (Siddiq and Ali, 1997).

Test for Reducing sugars

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Test for Triterpenes

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation.

Test for Phenolic Compounds (Ferric chloride test)

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

Test for Tannins

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

3. Results and Discussion

Table:1. Antibacterial activity of *Acalypha indica* ethanol extract against bacterial pathogens

S. No.	Organisms	Concentration of extract and zone of inhibition			
		25 mg	50 mg	75 mg	100 mg
1	<i>Pseudomonas aeruginosa</i>	NZ	15 mm	25 mm	30 mm
2	<i>Escherichia coli</i>	9 mm	18 mm	26 mm	30 mm
3	<i>Shigella flexneri</i>	NZ	13 mm	20 mm	25 mm
4	<i>Staphylococcus aureus</i>	NZ	3 mm	7 mm	12 mm
5	<i>Klebsiella pneumoniae</i>	NZ	9 mm	12 mm	20 mm
6	<i>Salmonella typhi</i>	8 mm	16 mm	21 mm	25 mm
7	<i>Vibrio cholerae</i>	NZ	7 mm	13 mm	20 mm
8	<i>Bacillus cereus</i>	NZ	4 mm	9 mm	15 mm
9	<i>Bacillus subtilis</i>	10 mm	18 mm	26 mm	30 mm

NZ – No zone

Table 2: Antibacterial activity of *Acalypha indica* ethyl acetate extract against bacterial pathogens

S. No.	Organisms	Concentration of extract and zone of inhibition			
		25 mg	50 mg	75 mg	100 mg
1	<i>Pseudomonas aeruginosa</i>	NZ	NZ	NZ	NZ
2	<i>Escherichia coli</i>	7 mm	13 mm	18 mm	23 mm
3	<i>Shigella flexneri</i>	4 mm	8 mm	11 mm	15 mm
4	<i>Staphylococcus aureus</i>	6 mm	9 mm	13 mm	17 mm
5	<i>Klebsiella pneumoniae</i>	9 mm	14 mm	17 mm	20 mm
6	<i>Salmonella typhi</i>	7 mm	11 mm	15 mm	18 mm
7	<i>Vibrio cholerae</i>	NZ	NZ	8 mm	13 mm
8	<i>Bacillus cereus</i>	NZ	NZ	5 mm	11 mm
9	<i>Bacillus subtilis</i>	NZ	7 mm	12 mm	13 mm

NZ – No zone

Table 3: Phytochemical analysis of *Acalypha indica* extracts

S. No.	Test	Result
1	Alkaloids	+
2	Glycosides	-
3	Triprenoid and steroid	-
4	Flavonoid	-
5	Reducing sugars	-
6	Triterpenes	-
7	Phenolic compounds	-
8	Tannins	+

Antibacterial activities of ethanol and ethyl acetate extract of *Acalypha indica* was assayed against various bacterial pathogens. The zone of inhibition against ethanol extract was *Staphylococcus aureus* (12 mm), *Bacillus cereus* (15 mm), *Bacillus subtilis* (30 mm), *Salmonella typhi* (25 mm), *Shigella flexneri* (25 mm), *Escherichia coli* (30 mm), *Klebsiella pneumoniae* (20 mm), *Vibrio cholerae* (20 mm) and *Pseudomonas aeruginosa* (30 mm). The zone of inhibition against ethyl acetate extract was *Staphylococcus aureus* (17 mm), *Bacillus cereus* (11 mm), *Bacillus subtilis* (13 mm), *Salmonella typhi* (18 mm), *Shigella flexneri* (15 mm), *Escherichia coli* (23 mm), *Klebsiella pneumoniae* (20 mm), *Vibrio cholerae* (13 mm) and *Pseudomonas aeruginosa* showed no zone of inhibition.

Sumathi and Pushpa, (2007) evaluated the antibacterial activity of some Indian medicinal plants. The aqueous extract of *Acalypha indica* was tested against different bacterial pathogens. The aqueous extract of *Acalypha indica* showed 9 mm inhibition zone to *Escherichia coli* and no zone was

showed against *Staphylococcus aureus*, *Salmonella typhi* and *Shigella flexneri*. Alcoholic extract of *Acalypha indica* showed 10 mm inhibition zone towards *Staphylococcus aureus* and *Salmonella typhi*.

In this study ethanol was best solution for extracting the effective anti microbial substances from the medicinal plant *Acalypha indica* than ethyl acetate. The ethanol extract of *Acalypha indica* showed effective results against all test organisms but the ethyl acetate extract of *Acalypha indica* was low effective against all the microorganisms. This could be related to the presence of bioactive metabolites present in *Acalypha indica* which are not soluble in ethyl acetate but they can be soluble in ethanol.

Some studies concerning the effectiveness of extraction methods highlight that methanol extract yields higher antibacterial activity than n-hexane and ethyl acetate (Sastry and Rao, 1994). Whereas other report that chloroform is better than methanol and benzene (Febles et al., 1995). It is

clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method (Lima-Filo *et al.*, 2002).

Phytochemical analysis of *Acalypha indica* extract was showed in Table-3. It showed the presence of alkaloids and tannins. Antibacterial activity of *Acalypha indica* was due to the presence of alkaloids and tannins.

4. Conclusion

The study of antibacterial activity of herbal plant extract of *Acalypha indica* showed that the ethanol extract shows promising antibacterial activity against bacterial human pathogens when compared to ethyl acetate extract. Phytochemical analysis showed that the antibacterial activity of *Acalypha indica* was due to the presence of phytochemical compounds like tannins and alkaloids. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. These plants could serve as useful source of new antimicrobial agents.

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