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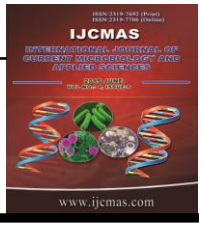

Original Research Article

Antibacterial Activity of *Acalypha indica* Linn

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A B S T R A C T

The present study was subjected to evaluate the antibacterial activity of petroleum ether, chloroform, acetone, methanol and ethanol extract of the medicinal plant *Acalypha indica* Linn using the standard disc diffusion method against four bacterial species, viz., *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* The antibacterial activity revealed that the petroleum ether extract has more effective than the ethanol, acetone, methanol extracts. A preliminary phytochemical screening was conducted on the plant extracts using standard qualitative procedures that revealed the presence of the alkaloids, saponins, phenols, flavonoid and tannin in rich status.

Keywords

Antibacterial Activity, *Acalypha indica* Linn, disc diffusion method

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. At the same time, indigenous people of the rest of the planet were also utilizing plants for curing diseases. Today, nearly 88% of the global populations turn to plant derived medicines as their first line of defense for maintaining health and diseases.

One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs; 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered. Currently, people of Asia and India are utilizing plants as part of their routine health management (Perumal samy, 2008). A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreadful diseases.

Acalypha indica Linn is an annual erect herb commonly called in Tamil as "Kuppai meni". It belongs to the family Euphorbiaceae. is a common weed in many parts of Asia including India, Pakistan, Yemen, Sri Lanka and throughout Tropical Africa and South America (Ramachandran, J., 2008). The root, stem and leaf of *Acalypha indica* possess herbal activity. The plant traditionally used as an expectorant against asthma and pneumonia and also as an emetic, emenagogue and anthelmintic (Shivayogi *et al*.,1999).

Acalypha indica contains acalyphine which is used in the treatment of sore gums and to have a post-coital antifertility effect (Bedon., 1982), anti venom properties (Annie *et al*., 2004), wound healing effects (Suresh reddy., 2002), antioxidant activities (Ruchi *et al*, 2007), anti-inflammatory effects (Mohana vamsi *et al.*, 2008), acaricidal effects (Singh *et al*, 2004), diuretic effects (Das *et al*.,2005) and antibacterial activities (Govindarajan *et al*., 2008), The roots of *Acalypha indica* is used as laxative and leaves for scabies and other cutaneous diseases (Perry, 1980).

It has also been reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments Chopra (1956). The dried leaves of *Acalypha indica* was made into a poultice to treat bedsores and wounds and the juice of *Acalypha indica* is added to oil or lime and used to treat a variety of skin disorders. The leaves of Acalypha *grandis* have also been reported to possess contraceptive activity (Bourdy *et al.*, 1992). Several chemical (Donw *et al.*, 1938) and biological (Bauer *et al.*, 1923) investigations have been carried out on this plant. In the present research study, the experiment was carried out the antimicrobial activities of the leaves *Acalypha indica* Linn. (*Euphorbiaceae)*.

Materials and Methods

Collection and drying of plant materials (crude)

About 5 kg of finely powdered dry stem, bark and leaves were soaked with petroleum ether, chloroform, acetone, methanol and ethanol. The soaking process was repeated three times for each solvent. The solvent extracts were filtered and evaporated under vacuum at 55°C to yield the respective crude extract. The crude extracts were transferred into sample bottles and kept in refrigerator prior to use.

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 flavonoids

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 wad added. Blue colour was observed for gallic tannins and green black for catecholic tannins (Iyengar, 1995).

Antimicrobial screening

Microorganisms

The microorganisms used in this present study were bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*)

Disk diffusion method

An antimicrobial assay was performed by using the disc diffusion agar method (Bauer& kirby, 1966). Petri dishes 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.

The stem, bark and leaves extracts impregnated discs were aseptically transferred on the inoculated agar plates and left to be incubated for 24 hrs to 7 days. The clear zones of inhibition around the extracts disc were measured for any indication of antimicrobial activity. Ampicillin and tetracycline impregnated discs were used as standard reference or positive controls and the solvents were used as negative controls. All assays were carried out in triplicate.

Results and Discussion

Phytochemical compounds present in various extracts of petroleum ether, chloroform, acetone, methanol and ethanol of *Acalypha indica* extracts were analyzed and the results were showed in (Table 1). The *Acalypha indica* showed the presence of alkaloids and tannins. Antibacterial activities of petroleum ether, chloroform, acetone, methanol and ethanol and extracts of *Acalypha indica* was assayed against various bacterial pathogens shown in (Table 2).

Table 1 : Phytochemical screening of *Acalypha indica*

	Pet-Et	chloroform	Acetone	methanol	EtoH
Alkaloids	+	-	+	+	-
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Saponins	-	-	-	-	+
Glycosides	+	-	+	+	+
Phenols	+	-	-	-	-

Table.2 Antimicrobial activity of different solvent extracts of *Acalypha indica*

Extracts	Concentration (mg/ml)	Inhibition (mm)		
		E.coli	***Pseudomonas***	***K.pneumoniae***
Pet-Et	50	16±0.23	14±0. 25	17±0.26
	100	22±0.33	16 ±0.29	18±0.35
	150	28±0.57	20±0.31	21±0.54
Chloroform	50	12±0.16	-	-
	100	14±0.21	-	-
	150	15±0.32	-	-
Acetone	50	13±0.17	-	12±0.20
	100	16±0.23	-	18±0.34
	150	17±0.29	-	25±0.56
Methanol	50	-	-	12±0.30
	100	17±0.11	-	15±0.45
	150	17±0.21	-	17±0.67
EtOH	50	14±0.19	-	15±0.10
	100	16±0.32	-	20±0.33
	150	20±0.39	-	28±0.57
Ampicilin	-	18±34	-	25±0.10
Tetracyclin	-	-	32±	-

Values are mean inhibition zone (mm) ±SD of tree replicates, - No inhibition

The Petroleum ether extract of *Acalypha indica* showed maximum zone of inhbition in *E.coli* (28mm) and *K.pneumoniae* (21mm) *Pseudomonas* (20mm). The Chloroform extract showed maximum zone of inhibition to *E.coli* (15mm), where as *K.pneumoniae*, *Pseudomonas* shows no susceptibility. The acetone extract showed maximum inhibition zone in *K. pneumoniae* (25 mm). *Pseudomonas* showed no zone which indicates its resistance towards the extract. The methanol extract of *Acalypha indica* showed maximum inhibition zone in *K. pneumoniae* (28 mm), *E.Coli* (20mm) and *Pseudomonas* has no activity. The ethanol extract showed maximum inhibition zone in *E. coli* (22mm), *K.pneumoniae* (17mm) and *Pseudomonas* shows no activity. Both *K.pneumoniae* and *E.coli* were susceptible to ampicillin while *P.aeruginosa*

was susceptible to its positive control, tetracycline.

According to a study conducted by (Govindarajan *et al*., 2008), *A. indica* extracts produced active results against all the Gram-positive bacteria tested while one of the Gram-negative bacteria, *Pseudomonas aeruginosa* was only susceptible towards the extracts at a higher concentration. This result could be attributed to the difference in wall compositions that exist in both Gram-positive and Gram-negative bacteria. While the Gram-negative bacteria possess wall that consists of lipo-polysaccharide layer along with proteins and phospholipids that may impede the entry of active compounds of *A. indica* crude extracts, the Gram-positive bacteria contains a very active area of cell metabolism called periplasmic space that carry many digestive enzymes and transport proteins which could attribute to the susceptibility of the microorganisms.

Some studies concerning the effectiveness of extraction methods highlight that methanol yeilds 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 (Reminton,1991; Lima Filo *et al*., 2002).

These plants could serve as useful source of new antimicrobial agents. *A.indica* possessed thermolabile antimicrobial factors that reduced the growth of pathogenic bacteria. Various pathogenic bacteria have developed resistance to many of the currently available antibiotics. The root, stem and leaf of *Acalypha indica* possess antibacterial activity against human pathogens. Historically, plants have provided a good source of anti-infective agents and many of them remain highly effective in the fight against microbial infections. Besides, they are cost- effective and have fewer side effects.

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