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Antibacterial activity and Phytochemical Analysis of *Acalypha indica L.*

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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 ethyl acetate, petroleum ether and Toleune extract of leaves from *Acalypha indica* were tested for anti-bacterial activity against four bacterial species - *Klebsiella pneumoniae, Salmonella typhi. Bacillus subtilis and Pseudomonas putida,* It is inferred that ethyl acetate extract exhibited strong antibacterial activity with a maximum activity recorded against *Klebsiella pneumoniae* (22.67±0.33mm). The qualitative phytochemical screening indicated the presence of alkaloids, phenols saponins, steroids, flavonoids and catechol.

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

Contents

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1. Introduction

Many of the plants used today were known to the people of ancient cultures throughout the world and were highly considered their preservative and medicinal powers. The exploitation of plants by man for the treatment of diseases has been in practice for a very long time. Herbal drugs constitute a major part in all the traditional system of medicines (Higa *et al.,* 1994). According to World Health Organization, medicinal plants are the best source to

obtain a variety of newer herbal drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. *Acalypha indica* is an annual erect herb commonly called as "Kuppai meni" in Siddha literature. It belongs to the family Euphorbiaceae. It is a common shrub in Indian gardens, backyards of houses and waste places throughout the plains of India.

The root, stem and leaf of *Acalypha indica* possess herbal activity (Ali Rehman *et al.,* 2002). Plants are used as emetic, expectorant, laxative, diuretic bronchitis, pneumonia, asthma and pulmonary tuberculosis. In homeopathy, the plant is used in severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis (Ghani, 2003). The plant is traditionally used as an expectorant against asthma and pneumonia, and also as an emetic and anthelminthic (Nameirakpam *et al*., 2002, Zahir Hussain and Kumaresan (2013). Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. A variety of compounds from plant sources like polysaccharides flavanoids, alkaloid terepenoids, lectins, proteins, peptides etc., are responsible for antimicrobial activity (Parekh *et al.,* 2005, Bisht *et al.,* 2011). The main objectives of this study were to screen antibacterial activity of selected plant species against pathogenic microorganisms. *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). In the present study, an attempt has been made to enrich the knowledge of antibacterial activity of *Acalypha indica* plant extract against bacterial cultures.

2. Materials and Method

Microbial cultures

The test micro-organisms used for the antimicrobial activity screening were

1. *Klebsiella pneumonia* (NCIM -2719)
2. *Salmonella typhi* (Clinical isolate)
3. *Bacillus subtilis* (NCIM- 2063)
4. *Pseudomonas putida*

2.2 Plant Material

The full plant *Acalypha indica* Linn. were harvested from outfield near Courtallam, Tirunelveli District, Tamil Nadu and South India in the month of October. Botanical identification was carried out by the ICAR, Sri Parasakthi College, for Women, Courtallam and South India. Fresh leaves and roots were rinsed severally with clean tap water to make it dust and debris free. Then the leaves and roots were dried in the shady condition at the room temperature for 15 days until they become crispy while still retaining the brownish coloration. Dried leaves and roots were ground in electric chopper and made into coarse powder, stored in an air tight container in a refrigerator prior to subsequent analysis.

2.3 Preparation of the plant extract

The full plants were cleaned and coarsely powdered after shade drying. The powder (100g) was extraction using Ethyl acetate, Petroleum ether and Toleune as solvent. For extraction soxhlet apparatus was used. The extracts were concentrated under reduced pressure in a rotary evaporator. The solvent was removed in vacuo and the extract was used for antibacterial assay. For these fractions antibacterial assay was carried out using four types of NICM bacterial cultures and four types of fungal strains. Using the extracts, stock solutions for each type of organic solvent was prepared by mixing well an appropriate amount of dried extracts with Dimethyl sulphoxide (DMSO) to obtain a final concentration of 100mg/ml. This was used for the evaluation of antibacterial and antifungal activities. Each solution was stored at 4°C after collecting in sterilized bottles until further use.

2.4 Antibacterial Assay

In vitro antibacterial activities of all crude extracts of Ethyl acetate, Petroleum ether, and Toleune fractions were determined by standard agar disc diffusion assay. Petridishes (100mm) containing 25 ml of MHA were seeded with 100 µl inoculums of the test strain (inoculums size was adjusted so as to deliver a final inoculums of approximately (10^6 CFU/ml) and allowed to solidify. Discs of 6 mm diameter were placed on the solidified agar media with a help of a sterilized forceps. From the stock concentration of crude and fraction of extracts (100mg/ml), three different volumes 50 µl, 75 µl and 100 µl were used to load the sterile disc under aseptic conditions. The plates were incubated at the plates were incubated at 37°C 24h. DMSO and sterilized distilled water was used as positive control. Streptomycin (30 mg/disc/ was used as standard antibiotic disc for crude extract testing .The experiment was performed in triplicate under strict aseptic conditions and the antibacterial activity of each extract was expressed in terms of mean diameter of zone of inhibition (mm) produced by respective *Acalypha indica* extracts.

2.5 Phytochemical Screening

2.5.1 Preparation of the plant extract

Ethyl acetate, Petroleum ether, and Toleune extracts were obtained by using soxhlet apparatus. The three types of extract, with different polarities were concentrated by evaporating it to dryness under reduced pressure by rotary vaccum evaporator to obtain the respective extracts and each residue was stored at 4^0C. These three extracts were resuspended in Dimethyl sulfoxide. The extracts were then stored at -18^0C until further analysis phytochemical screening of eluted fractions was tested for the presence of various phytochemical constituents. The analyses were carried out by following techniques.

2.5.2 Preliminary screening

The various fractions of ethyl acetate, Petroleum ether and Toleune extracts of *Acalypha indica L.*were used to screen for the following the phytochemicals like Catachol, triterpenoids, alkaloids, tannins, flavonoids, saponin and steroids small quantities of all the fractions were dissolved separated in distilled water and filtered. The filtrate was subjected to further analysis.

(i) Detection of triterpenoids

To the filtrate with one or two pieces of tin and three drops of thionyl chloride was added slowly, a violet or purple colour solution indicates the presence of triterpenoids.

(ii) Detection of Alkaloids

Small fraction of various filtrates were sepatately stirred with few ml and dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents such as Mayer's, Hager'S, Wagner's and Dragendorff's reagent.

(iii) Mayer's Test

To small quantity of the various filtrates add Mayer's reagents the formation of cream coloured precipitate indicates the presence of alkaloids.

(iv) Detection of Anthroquinone

To 2ml of test solution, added magnesium and acetate solution. The result was observed.

(v) Detection of Catachol

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

(vi) Detection of saponins

Small portion of the various filtrates were diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. Formation of foamy layer was recorde, it indicated the presence of saponin.

(vii) Detection of Tannins

To the filtrate, add 2ml of solution of gelatin white precipitate was seen which indicates the presence of tannins.

(viii) Detection of Flavonoids

Small portion of the various filtrates were dissolved in alcohol and treated with magnesium metal followed by concentrated hydrochloric acid. Formation of Magenta colour indicates the presence of flavonoids

3. Results and Discussion

Antibacterial Activity

Table 1: Antibacterial activity of ethyl acetate, Petroleum ether and Toleune extract of Acalypha indica *L.* (Disc diffusion method- mm)

S.No	Bacterial Culture	Concentration	Ethyl acetate extract	Petroleum ether extract	Toleune extract	Streptomycin
1.	*Klebsiella pneumonia*	50 µl	18.33±0.33	18.67±0.33	15.67±0.33	21.67±0.33
		75 µl	21.67±0.33	19.67±0.33	17.33±0.33	22.67±0.33
		100 µl	22.67±0.33	21.67±0.33	19.33±0.33	23.67±0.33
2.	*S.typhi*	50 µl	17.33±0.33	13.67±0.33	17.33±0.33	15.67±0.33
		75 µl	18.00±0.00	16.67±0.33	18.67±0.33	16.67±0.33
		100 µl	19.67±0.33	20.33±0.67	19.67±0.67	17.33±0.33
3.	*B.subtilis*	50 µl	17.33±0.33	16.33±0.33	13.67±0.33	17.67±0.33
		75 µl	18.67±0.33	19.00±0.00	15.67±0.33	18.33±0.33
		100 µl	20.67±0.67	19.67±0.33	17.67±0.33	20.33±0.33
4.	*P.putida*	50 µl	13.67±0.33	15.33±0.33	12.33±0.33	13.67±0.33
		75 µl	15.67±0.33	17.67±0.33	14.67±0.33	15.33±0.33
		100 µl	18.67±0.33	18.33±0.67	17.67±0.33	17.33±0.33

The presence of antibacterial substances in the higher plants is well established (Srinivasan, 2001). Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant

contribution towards human health. Sumathi and Pushpa, (2007) evaluated the antibacterial activity of some Indian medicinal plants. Ethyl acetate extract, Petroleum ether extract and Toleune extract of *A. indica* were tested for antibacterial activity and four strains of pathogenic bacteria cultures were tested. Streptomycin was used as standard drug to compare the inhibitory effect of the *A. indica* extract of the four bacterial strains tested. The sensitivity of all the bacterial strains to ethyl acetate extract was more than that of Petroleum ether extract except for the *S.typhi* cultures the findings of the present investigation were showed in (Table 1&2). For Ethyl acetate extract the degree of inhibition of bacterial growth was increasing in a dose dependent manner. , *B.subtils, K.pneumoniae* and *P.putida* responded well and the diameter of zone of inhibition for this bacterial culture was above 22.67±0.33 mm at 100 µl dose.

Petroleum ether extract also inhibited the growth of the bacterial strains efficiently. The diameter of zone of inhibition for *K.pneumoniae* and *B.subtilis* was 21.67±0.33mm and 19.67±0.33 at 100 µl doses. The differential effects of the three solvents indicate that the ethyl acetate, was able to extract effective bioactive metabolites more than the solvent Petroleum ether .The present study indicates that under the experimental conditions, ethyl aectate is an ideal solvent to extract antimicrobial compounds from full plant. 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 relay 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.

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993). The qualitative analysis of *Acalypha indica* revealed the presence of biomolecules such as *alkaloids, catechols, flavonoids, phenolic compounds, saponins* and *steroids* respectively.

Table 2: Phytochemical screening of ethyl acetate, petroleum ether, Toleune extract fractions of *Acalypha indica* L.

S.No	Phyto constituent	**Ethyl aectate extract fractions of *Acalypha indica* L.**		
		Ethyl acetate : petroleum ether : Toleune		
		Ethyl aectate	**Petroleum ether**	**Toleune**
1	Triterpenoids	-	+	-
2	Anthraquinones	+	-	-
3	Alkaloids	+	+	+
4	Phenolic compounds	+	-	+
5	Saponins	+	+	+
6	Steroids	-	-	-
7	Tannins	+	-	-
8	Aromatic acid	-	-	-
9	Flavonoids	+	+	+
10	Catachols	+	+	+

(+) Presence (-) Absence

Phytochemical screening of various fractions of ethyl acetate reveals the presence of -and absence. Phytochemical screening of various fractions of ethyl acetate extract of *Acalypha indica .L* reveals the presence of, alkaloids, phenolic compounds, Saponins, tannins, flavanoids, Catechols and absence of Triterpenoids, Steroids and aromatic acids. (Table -2). Phytochemical studies revealed the presence of flavanoid compounds and other phytochemical components. As ethyl acetate, petroleum ether are different polar grade solvents, it was found that the polarity of the solvents seems to play a role in the extraction of natural products which influences the antibacterial activity of the extracts (Negi *et al*., 2012). From the present study it is clear that the extracts of the *Acalypha indica* contain an effective antibiotic agent. The extracts of ethanol are able to bring out that agent effectively. Recent literature survey in this plant indicate the bioactive potential of this plant *Acalypha indica* the plant extract is found to certain the phytochemical.

4. Conclusion

The study of antibacterial activity of herbal plant extract of *Acalypha indica* shown that ethyl acetate extract shows promising antibacterial activity against *Klebsiella pneumonia, Bacillus subtilis* and *Pseudomonas putida* when compared to petroleum ether and Toleune extract .The results revealed the presence of medicinally important constituents in the plants studied. Many evidences gathered in earlier studies which confirmed the identified

phytochemicals to be bioactive. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties to the plants studied in the treatment of different ailments. Therefore, extracts from these plants could be seen as a good source for useful drugs. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

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