Original Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF HYDROETHANOLIC LEAF, STEM, ROOT EXTRACT OF *ACALYPHA INDICA* - A COMPARATIVE STUDY

Running title: Antibacterial activity of *Acalypha indica* extract

ABSTRACT:

BACKGROUND AND AIM: *Acalypha indica* is a weedy, short lived and monoecious plant. It has an antioxidant effect and it must be implemented to control the disease where free radicals are involved. Acalypha plant is useful for the bronchitis, pneumonia, pulmonary tuberculosis patients. *A.indica* plant is used in the rejuvenation in the worst conditions which may undergo infections in the microorganisms and it is the chemotherapeutic agent and distributed in the large contributions in human health and well being. The main aim of this study is to assess which part of *A. indica* has an antibacterial activity.

MATERIALS AND METHODS: Agar well diffusion method was used for assessing the antimicrobial activity of the plant extract. The nutrient broth is inoculated with bacterial strains *E. faecalis*. The broth was then incubated at 37°C overnight. Antibacterial activity was determined by measurement of the diameter of zones of inhibition (mm).

RESULT: When compared to root, stem and leaves of hydroethanolic extract of *A.indica*, leaf maximum inhibition against *E. faecalis*. As the concentration of extract increases from 25 µg/ml to 100 µg/ml, the zone of inhibition also increases. This shows the antimicrobial activity is better at higher concentration.

CONCLUSION: *A.indica* has effective antimicrobial activity. It reduces the growth of various human pathogenic bacteria. The root, stem and leaf of *A.indica* possess Antibacterial activity against E. *faecalis.*leaf extract has significant antibacterial activity.

KEYWORDS: Antibacteria, Antimicrobial, phytochemicals, inhibition, Strains, Innovative techniques.

INTRODUCTION:

A.indica Linn was also called as kucinggalak. It is widely distributed in Tropical Africa, South Africa, India and Sri lanka.(1). *A.indica* is a weed, short lived and monoecious plant. *A.indica* Linn is an annual plant and it is an erect herb. This plant is commonly called in Tamil as 'KUPPAIMENI'. It belongs to Euphorbiaceae. This plant can grow up to1.5 to 2.5 m tall. A.indica plant contains the acalyphine which is used to treat deficiency of Vitamin C,Vitamin E patients. Leaf of the acalypha plant has been used for treatment of scabies and the other cutaneous diseases. It is useful to treat rheumatism and several other ailments (2). *A.indica* is most diverse within nearly 450 species. 2/3rd species found in America, 19 found in Venezuela and they are mainly used as an ornamental plant (3)(4).

It has an antioxidant effect and it must be implemented to control the disease where free radicals are involved. Acalypha plant is useful for the bronchitis, pneumonia, pulmonary tuberculosis patients (5). These plants are diuretic, emetic, expectorant, laxative. This plant has beneficial effects on nosocomial infections and the bacterial pathogens (6).This plant also has beneficial effects on asthma (7). It is a herbal plant and grows in the wet temperate and tropical regions. The plant can be used in medicine and in several therapeutic treatments.(6,8) *A.indica* plant is used in the rejuvenation in the worst conditions which may undergo infections in the microorganisms and it is the chemotherapeutic agent and distributed in the large contributions in the human health and the well being (9,10). Nearly 88% of the global population's derived medicines from these plants and it act as the first line of defense for maintaining health and combating the disease (11).

It can be used for anthelmintic, antiulcer, bronchitis (12), antidiabetic, anti hyperlipidemic, anti obesity, antivenom, hepatoprotective and hypoxia (13). Extracts from the stem, root, leaf can make the drugs valuable and it has a high export potential (9). The dried leaves of the *A.indica* were made into a poultice to treat the bedsores and the wounds. (14) The leaves of *A.indica* have also been reported to possess contraceptive activity. The juice of this Euphorbiaceae plant is added to oil or lime and used to treat a variety of skin disorders. These plants possess the bronchodilation and the bronchial hyperreactivity. Essential oil which is anhydroethanolic extract has the positive outcome in the in vitro study and has the minimum inhibitory concentration methods (15). It has acaricidal effects. It is also expectorant against pneumonia

and also as an emmenagogue. This plant is also known to possess respiratory effects on experimental animals. Acalypha is one of the genuses that show a great potential in the world of the scientific advancement due to its chemical and biological results (16).To enrich the knowledge of the antibacterial activity of *A.indica* plant extract against the gram positive and the gram negative bacteria (17).

Our team has extensive knowledge and research experience that has translated into high quality publications (18–20)(21–26),(27)(28),(29)(30),(31)(32)(33–37). So the main aim of this study is to assess which part of *A.indica* has a potential antibacterial activity.

MATERIALS AND METHODS:

Test Organisms

The bacterial strains such as *Enterococcus faecalis*, were obtained from the Department of Microbiology, Saveetha Dental College, Saveetha University were used for the research. They were maintained in a nutrient agar slope at 40°C.

Collection of Plant Powder

Leaf, Stem and Root extract of *A.indica*

The leaves stem and root of *A.indica was* obtained in powder form from a registered pharmacy in Arumbakkam, Chennai, India

Preparation of Extract

The powders obtained were subjected to extraction. Soxhlet extractor was used for this purpose. After the completion of the process of extraction, the solvent is distilled and the extracts are kept in a desiccator after being concentrated to dry residue on a water bath.

Assessment of Antimicrobial Activity by Agar Well Diffusion Method

Agar well diffusion method was used for assessing the antimicrobial activity of the plant extract. The nutrient broth was inoculated with E. *faecalis*. The broth was then incubated at 37°C overnight. The culture was then adjusted to 0.5 McFarland turbidity standard. Muller-Hinton agar plates [MHA-HiMedia M1084] were used for the Lawn culture of the test organism. This was done with using sterile cotton q tips. The plates were clean and dry. Then, a 6 mm diameter well was bored by a sterile cork for different concentrations of the extracts (25, 50, 75 and 100 µg/ml). The extracts were introduced into the wells using micropipettes. The culture plates were allowed to stand on the bench for 30 min for pre-diffusion and were then incubated in an upright position for 24 h at 37°C. After 24 h, antibacterial activity was determined by measurement of

the diameter of zones of inhibition (mm). To minimize the test error, all the tests are done in triplicate.

RESULTS:

When comparing with root, stem and leaves of hydroethanolic extract of *A.indica*, leaf extract shown the maximum inhibition against *E. faecalis*. As the concentration of extract increases from 25µg/ml to 100 µg/ml, the zone of inhibition also increases. This shows the antimicrobial activity is better with increase in concentration. According to this study, hydroethanolic extract of stem, root and leaves of *A.indica* was effective against the bacterial strain *E.faecalis.* In the lower concentration 20 µg/ml of extract, the zone of inhibition of root was 15 mm, stem 16 mm, and leaves 17mm (Table 1). When compared, leaf extract had a larger zone of inhibition than root and stem extract. As the concentration of all parts of plant extract increases, the zone of inhibition gradually increases. The *E. faecalis* served as a positive control while distilled water was used as negative control. These were tested using disc diffusion methods. The results show less antibacterial activity on the root extract of *A.indica* plant (Figure 1). Culture plate showed little higher antibacterial activity on the stem extract of *A.indica* plant (Figure 2). Culture plate showed much higher antibacterial activity on the leaf than the root and stem extracts of *A.indica* plant (Figure 3).

TABLE 1: Zone of inhibition(diameter mm). Effect of antibacterial activity of leaf, stem and root extract of *A.indica* against *E.faecalis*

Extract	**25 µg/ml**	**50 µg/ml**	**75 µg/ml**	**100 µg/ml**
A.indica root	15	16	18	21
A.indica stem	16	18	18	20
A.indica leaf	17	19	19	21

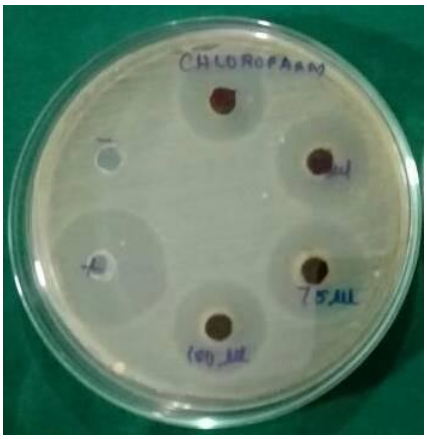

Figure 1: Culture plate showing efficacy of antibacterial activity of *A.indica r*oot extract was tested with four different concentrations which were 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml.

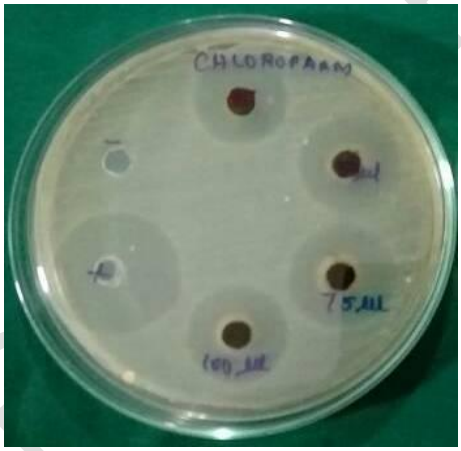

Figure 2: Culture plate showing efficacy of antibacterial activity of *A.indica* stem extract was tested with four different concentrations which were 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml.

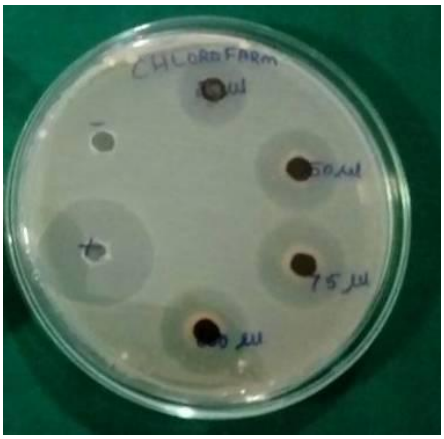

Figure 3: Culture plate showing efficacy of antibacterial activity of *A.indica* leaf extract was tested with four different concentrations which were 25 µg/ml, 50 µg/ml, 75 µg/ml, 100 µg/ml.

DISCUSSION:

A.indica species destroys the growth of all gram positive bacteria but it didn't act on gram negative bacteria (38). Due to its antioxidant activity, it fights against many diseases (39). When compared to ethanol, methanol and acetone extract of root, stem and leaf extract of *A.indica*, ethanol leaf extract has potential antimicrobial activity with the zone of inhibition 20cm against *K.pneumoniae(40)*. Antibacterial activity of the hydroethanolic root extract of the *A.indica* carried out by a maceration method (41). Phytochemical quantitative analysis of the total flavonoid content and the alkaloid content (42,43). Aqueous and ethanolic extract of *A.indica* manifested moderately antibacterial activity against gut pathogens (44). *A.indica* has effective antimicrobial activity (45). Antimicrobial activity is due to the presence of phytochemicals like tannins, flavonoids and phenolics(46). The presence of bioactive compounds such as alkaloids, tannins, steroids, saponins, flavonoids, glycosides and the phenolic compounds was also detected during the phytochemical testing (47). *A.indica* needs more extensive laboratory and clinical work in order to know preferable antibacterial principles (41).

As it stated earlier, the antibacterial activity of leaf stem and root extract is worthy of investigation. But, in the *A.indica* plant for further laboratory and clinical studies of this plant

was required in order to understand better in antimicrobial principles which will allow the scientific community to recommend their uses.

CONCLUSION:

A.indica has effective antibacterial activity. It reduces the growth of various human pathogenic bacteria. The root, stem and leaf of *A.indica* possess Antibacterial activity against E..*faecalis.* Leaf has significant antibacterial activity. Even though there are many drugs, still there is no complete cure. No side effects as they are natural.

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