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The antibacterial activity of *Acalypha indica* L.

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Abstract: The antibacterial activity of *Acalypha indica* was investigated against three strains of human pathogenic bacteria viz., *Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae* using ethylacetate, hexane and methanol extracts of leaves, stem and roots of *Acalypha indica*. The ethylacetate extracts of leaves and roots inhibited the growth of all the three selected bacterial species. The *in vitro* assay may open way for complementary future investigations in identifying potentially useful properties of chemical and pharmacological importance.

Keywords: *Acalypha indica*, antimicrobial activity, human pathogenic bacteria, medicinal plant.

Introduction

The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Today, as much as 80% of the world's population depends on traditional medicine as primary health care needs. With the recent advancement of research in the field, it has become apparent that many of the species utilized by indigenous people as well as the knowledge of traditional healers has begun to make its mark on society as a possible avenue for curing diseases. Medicinal plants include those that can be put to culinary and or medicinal use and those associated with orthodox drugs such as fox glove and opium poppy, as well as everyday plants such as garlic (Serrentino, 1991). Recently, most of the research conducted in the traditional medicines has shown that some remedies obtained from traditional healers are very effective in spite of the fact that there is no scientific justification. The greater part of traditional therapy involves the use of plant extracts on their active principle (WHO, 1993).

Antibacterial resistance especially among gram-negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search for alternative drugs of natural anti-bacterials (Jensen *et al*., 1996). One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Fabricant & Farnsworth, 2001). This has forced scientists to search for new antimicrobial substances from various medicinal plants.

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem. Progress in antimicrobial drugs, has introduced many antibiotics most of which are nontoxic, though all have side effects (Osler, 1983; Shafiei & Ghanbarpour, 1992). Hence, despite significant value of antibiotics, the increase of bacterial resistance has restricted their clinical application (Neu, 1992; Niekel, 1993; Yurdakok *et al*., 1997). 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 (Reminton, 1991; Samsam & Moatar, 1991).

A. indica is a common annual shrub in Indian gardens, backyards of houses and waste place throughout the plains of India. Leaves possess laxative properties (a substitute for senega) used in the form of powder or decoction. *Acalypha* cures diseases of the teeth and gums, burns, toxins of plant and mixed origin, stomach pain, diseases due to pitha, bleeding piles, irritations, stabbing pain, wheezing, sinusitis and neutralizes predominance of the Kabha factor. The ethanolic extracts of *Heliotropium indicum*, *Plumbago zeylanicum* and *Acalypha indica* were evaluated for their wound healing activity in rats (Suresh Reddy *et al*., 2002). Evidently, there are not sufficient scientific studies that confirm the antimicrobial properties of these plants.

Hence, *A. indica* was chosen for this study to investigate the bactericidal activity against pathogenic bacteria like, *Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae*

which cause the most common cases of infectious diseases. This work is an attempt to screen the antibacterial activity of different parts of the plant such as leaf, stem and root by agar disc diffusion method and possibly to isolate the active principle(s).

Materials and methods

A. indica, which occurs as common weed, was collected for its different parts in and around the town of Chengalpattu, Tambaram and Chrompet, Kancheepuram Dt., of Tamil Nadu, India. The collected plant materials were thoroughly washed with running tap water, rinsed with distilled water and air dried under shade for 30 days.

Preparation of plant extract

The different parts of dried plant materials were powdered in blender. About 50 g of leaves, stem and root powder were extracted separately with three different solvents viz., ethyl acetate, hexane and methanol for 24 h. The extraction was continued until the powder was free of extracts by passing through Whattman No.1 filter paper and further concentrated to dryness under reduced pressure with a vacuum evaporator at 40°C, and stored at 4°C until further use. Before use, each crude extract was re-suspended in their respective solvent to yield 50 mg extract residue per ml solvent.

Screening for antibacterial activity of crude extracts

The crude extracts of *A. indica* were screened initially for their antibacterial activity against the 3 bacterial species by employing agar-well-diffusion and agar-disc-diffusion methods.

Bacterial strains

Three bacterial cultures obtained from Madras University Botany Laboratory (MUBL) Culture collection, Centre for Advanced studies in Botany, University of Madras, Guindy Campus, Chennai-600 025 were used for this study. It includes two Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* and a Gram Negative bacterium: *Klebsiella pneumoniae*. The test bacterial cultures were sub-cultured periodically and maintained on Nutrient Agar (NA) medium for further experiments at room temperature at 30±2°C.

Effect of crude extract on growth of bacteria by agar well diffusion method

In the preliminary screening, the effect of crude extracts of leaf, stem and root on bacterial growth was determined by agar well diffusion method (Patel *et. al*., 2007). The molten nutrient agar medium was poured on petriplates and allowed to solidify under laminar airflow chamber. About 1 ml of each bacterial inoculum was spread on the agar surface using sterile glass spreader or cotton swab. Then a well of 0.5 cm was made in the agar medium using a sterile cork borer. About 100 µl of each sample of crude extract was transferred into separate wells and plates were incubated at 37°C for 24 h. A well with respective solvent served as control. The development of inhibition zone around the sample loaded well was recorded.

Effect of active compound on the growth of bacteria by agar disc diffusion method

The antibacterial activity of the active compound isolated from the ethyl acetate extracts of leaf, stem and root of *A. indica* was performed using a modification of agar disc diffusion method (Bauer *et. al*, 1966). About 100 mg of partially purified active compound of Ethyl acetate extract was loaded on a sterile filter paper disc of 6 mm diameter. The petriplates containing nutrient agar medium were spread with 100 µl of actively growing broth culture of the test bacteria using sterile cotton swab and allowed to dry for 10 min. Then the impregnated discs were placed on the surface of inoculated agar medium and incubated

Table 1. Diameter of Inhibition zone (cm) of bacterial growth by solvents extracts of different plant parts of A. indica.

Bacterial strains	Solvents	Leaf (cm)	Stem (cm)	Root (cm)
B. subtilis	Ethyl acetate	1.9	1.2	1.7
	Hexane	1.0	1.1	1.2
	Methanol	1.3	1.2	1.5
	Control	-	-	-
K. pneumoniae	Ethyl acetate	3.5	1.2	3.3
	Hexane	0.9	0.7	0.8
	Methanol	1	0.4	1.2
	Control	-	-	-
S. aureus	Ethyl acetate	2.7	1	2.1
	Hexane	0.8	0.8	1.1
	Methanol	1.1	0.8	1.2
	Control	-	-	-

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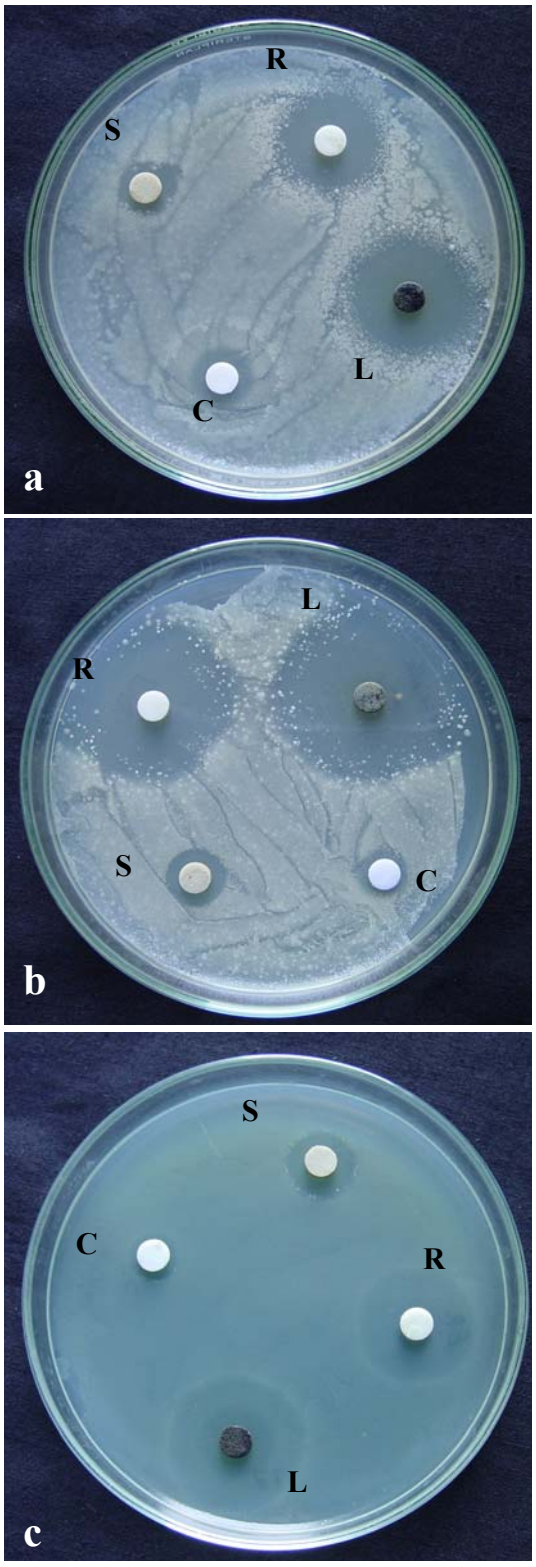
Fig. 1. Inhibition of bacterial growth by ethyl acetate extracts of leaf, stem and root by "Agar Disc Diffusion" method.



a - Bacillus subtilis; b - Klebsiella pneumoniae; c - Staphylococcus aureus; C - Control; L - Leaf extract; S - Stem extract; R - Root extract.

for 37°C for 24 h. Discs loaded with small volume of ethyl acetate served as control. The development of inhibition zone around the active compound loaded disc was recorded.

Partial purification by Thin Layer Chromatography

The crude ethyl acetate extract of leaf was loaded on to the pre-coated aluminum thin layer chromatogram (Silica gel 60) and the chromatogram plate was air-dried for 15 min. The extract loaded TLC plate was run in a developing chamber with solvent system of Chloroform: Ethyl acetate: Water in the ratio of 6:4:0.5. The different bands appeared in the thin layer chromatogram plates were again tested for its antibacterial activity by agar disc diffusion method. The band showing the potential antibacterial activity was scraped and eluted with ethyl acetate and stored for further purification.

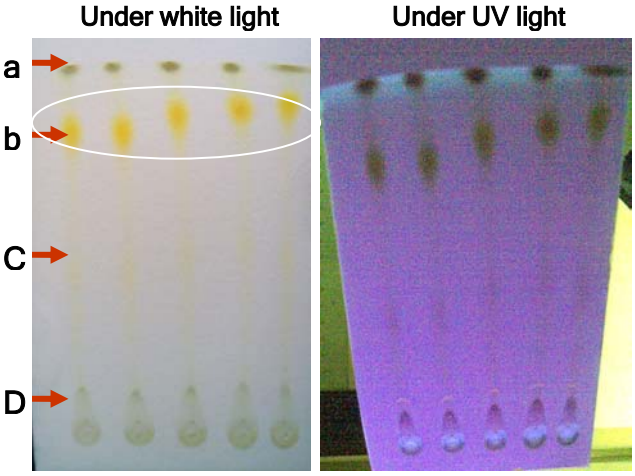
Results and discussion

The results of the present study show that *A. indica* has antibacterial activity against gram positive and gram negative pathogenic bacteria. The diameters of inhibition zone of crude extracts of *A. indica* were presented in Table 1. These results corroborate earlier investigations by Gopalakrishnan *et al.* (2000) and Solomon *et al.*, (2005).

The ethyl acetate extracts have a greater effect than that of methanol and hexane. It indicates that the active plant compounds can readily be dissolved or extracted in ethyl acetate when compared to other solvents. Our results also reveal that the extracts, in general, are more effective on gram positive bacteria than on gram negative bacterium. It may be due to difference in bacterial cell wall composition, the fact need to be checked by employing more bacterial strains. The permeability, entrance and reaction of the most antibiotics and/or antimicrobial agents through cell envelope (the outer and cytoplasmic membrane) are highly efficient for gram positive bacteria depending on reaction with the protein layer (mucopolysaccharides or peptidoglycans) (Baron *et al.*, 1994; Lennette *et al.*, 1985).

It was observed that the type of solvent extraction affected the degree of antibacterial activity. The ethyl acetate extract of leaf gave the widest zone of inhibition (3.5 cm) while the root and stem gave 3.3 and 1.2 cm in diameter respectively (Table 1). Among the different parts of the plant materials, the leaf extracts exhibited more prominent antibacterial activity against all the three bacteria followed by the root and the stem extractions. Since the ethyl acetate extract showed the higher growth inhibitory activity, it was further subjected to the test of agar disc diffusion which also revealed similar pattern. Comparatively, the

Fig. 2 Thin Layer Chromatogram of ethyl acetate extract of leaf.



a: Dark greenish yellow band; b: yellowish orange band; c: pale yellow band; d: pale olive green band.

minimum activity against all the three bacteria was recorded with the stem extract (Fig. 1). The more potent growth inhibitory nature of ethyl acetate extraction of leaf was supposed to the fact that ethyl acetate, an organic solvent better liberate the active component required for antimicrobial activity.

The ethyl acetate extract of leaf was further subjected to thin layer chromatography. The bands appeared on the developed chromatogram were individually eluted with ethyl acetate and screened for their antibacterial activity. Of the four bands, the yellowish orange coloured band showed antibacterial activity, which can be purified (Fig. 2).

The result of this work indicates that the differences in the zones of inhibition may be directly related to the susceptibility of each test organisms to the leaf extracts. The factors responsible for this high susceptibility of the bacteria to the extracts are not exactly known but may be attributed to the presence of secondary plant metabolites which is soluble in ethyl acetate. It is noteworthy that the antibacterial activities of these plants extracts were dependent on the concentration of the extracts as reported by Ekwenye and Elegalam (2005). Also, if the extract has high molecular weight, the rate of diffusion is always slow, reduced and also takes longer time, whereas an extract of low molecular weight diffuses faster and at a quicker rate.

In vitro antimicrobial screening methods could provide the needed preliminary observations necessary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigation. Gangadevi *et al*. (2008) earlier reported that the endophytic fungi isolated from the leaves of *A. indica* elicited promising antibacterial activity against the three human pathogenic bacteria. Further studies are needed for the clarification of the precise functioning of the active ingredient (s) of the plant extracts in purified form for *in vitro* and *in vivo* antimicrobial activities.

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Conclusion

The evidences presented here indicate that the antibacterial compound present in the leaves and root of *A. indica* is potent and have shown this plant to hold excellent potential as an antimicrobial agent.

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