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Antibacterial activity of *Acalypha indica L.*

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Abstract. – Hexane, chloroform, ethyl acetate and methanol extracts from the leaves of *Acalypha indica* were tested against Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis*) and Gram-negative (*Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa)* bacteria. All the extracts exhibited antibacterial activity against Gram-positive organisms with minimum inhibitory concentrations (MIC) between 0.156 to 2.5 mg/ml. Among the Gram-negative bacteria, only the *Pseudomonas aeruginosa* was susceptible to the extracts.

Key Words:

Acalypha indica, Leaf extracts, Antibacterial activity.

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. Scientific experiments on the antimicrobial properties of plants and their components have been documented in the late 19th century[1]. 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. Plant based drugs were commonly used in India and China[2]. 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 combacting 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[3].

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[4]. 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[5]. Several chemical[6] and biological[7] investigations have been carried out on this plant.

In the present study, an attempt has been made to enrich the knowledge of antibacterial activity of *Acalypha indica* plant extract against Gram-positive and Gram-negative bacteria.

Materials and Methods

Plant Materials

The fresh leaves of *Acalypha indica* were collected from Vittaloor, Kumbakonam, Thanjavur District, Tamilnadu, India, and authenticated by botanist Dr. V. Venkatesalu, Reader in Botany, Annamalai University, Annamalainagar, Tamil Nadu, India. A herbarium specimen was deposit-

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ed in the Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu, India.

Preparation of Plant Extract

The shade dried coarsely powdered leaves were subjected to Soxhlet extraction using hexane, chloroform, ethyl acetate and methanol. The solvent was removed in vacuo and the extract was used for antibacterial assay.

Bacterial Strains

The test organisms were supplied by the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalainagar, Tamil Nadu, India. Four Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Streptococcus faecalis*) and four Gram-negative (*Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa*) bacterial strains were used in the study. The organisms were sub-cultured on Mueller Hinton Agar medium, incubated at 37°C for 24 h and stored at 4°C in the refrigerator to maintain stock culture.

Antibacterial Assay

Diffusion Method

Antimicrobial activity was carried out using disc-diffusion method[8]. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (HIMEDIA, Mumbai, India). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of the crude extract (5, 2.5 and 1.25 mg per disc) with three replicates. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10 µg/disc) was used as positive control. The plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in millimeters and the experiment was repeated three replicates.

Dilution Method

For determination of minimum inhibitory concentration (*MIC*)[9] and minimum microbicidial concentration (*MMC*), the broth twofold macrodilution method in Muller-Hinton broth (Hi-media, Mumbai) was applied. Briefly, test strains were grown in a nutrition medium containing progressively lower dilutions of the test extract and incubated at 37°C. The last two tubes were free of test extract and served as growth control in broth and respective solvent. After incubation, approximately the 10 µl of content of each test-tube was transfered with a loop onto Muller Hinton agar. Agar plates were incubated for an appropriate time under aerobic conditions at 37°C. MIC was defined as the lowest concentration of extract that allows no more than 20% bacterial growth, and MMC as the lowest extract concentration from which the microorganisms did not recover and grow when transferred to fresh medium.

Statistical Analyses

Data from the experiments were subjected to one-way analysis of variance (ANOVA) using SPSS using the SPSS 10.0 software package.

Results

Hexane, chloroform, ethyl acetate and methanol extracts of *Acalypha indica* leaves were showed significant zone of inhibition against "Gram-positive" bacteria, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Streptococcus faecalis* and one "Gram-negative" bacterium *Pseudomonas aeruginosa* (Table I). Other "Gram-negative" bacteria were not inhibited. The results indicated that the tested crude extracts showed antibacterial activity towards the "Gram-positive" bacteria. Among the four extracts tested at three different doses, the methanol and ethyl acetate extracts at 5 mg/disc dose were more potent in their antibacterial activity. Minimum inhibitory concentrations and Minimum microbicidial concentration of different extracts of *Acalypha indica* were tested against bacteria and the results revealed all four extracts showed highest activity against four bacterial organisms (Table II).

Discussion

Our data show that, in general, the plant antibacterial extracts substances appear to be more inhibitory to Gram-positive organisms than to the Gram-negative types. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bac-

Table I. Antibacterial activity of different solvent extracts of Acalypha indica leaves.

Extracts	Concentration (mg/disk)	Disk diffusion method (inhibition zone, mm)[a]							
		S.a	*S.e*	*B.c*	*S.f*	*K.p*	*E.c*	*P.v*	*P.a*
Hexane	1.25	9.5	8.4	10.6	9.6	–	–	–	–
	2.5	10.7	10.0	11.0	10.5	–	–	–	–
	5	12.8	11.0	13.8	12.0	–	–	–	9.7
Chloroform	1.25	9.6	10.6	8.5	11.5	–			
	2.5	11.5	12.5	10.0	12.6	–	–	–	–
	5	12.3	14.8	12.4	14.8	–	–	–	11.5
Ethyl acetate	1.25	11.8	9.0	11.6	10.7	–	–	–	–
	2.5	12.8	11.5	13.5	12.8	–	–	–	–
	5	14.6	13.8	15.0	15.0	–	–	–	10.0
Methanol	1.25	12.5	11.7	9.7	13.8	–	–	–	–
	2.5	13.6	12.5	11.8	15.4	–	–	–	–
	5	15.8	14.0	14.0	16.5	–	–	–	12.7
Streptomycin	10 µg	14	–	12	13	–	12	-	11

–, No activity; S.a, *Staphylococcus aureus*; S.e, *S. epidermidis*; B.c, *Bacillus cereus*; S.f, *Streptococcus faecalis*; K.p, *Klebsiella pneumoniae*; E.c, *Escherichia coli*; P.v, *Proteus vulgaris*; P.a, *Pseudomonas aeruginosa*; streptomycin, control antibiotic.
[a]Zones are mean diameter of three replicates.

teria[10]. Eight plant extracts showed complete inhibition whereas five plant extracts showed moderate inhibition against the Gram-positive bacteria tested. The negative results obtained against Gram-negative bacteria were not unexpected since this class of bacteria is usually more resistant than Gram-positive bacteria[11]. Antimicrobial activity of *Cassia fistula* leaves, stem bark, and pods was carried out against 14 pathogenic bacteria and 6 fungi at 400 µg/disc[12]. *Cassia fistula* leaf extracts showed antibacterial activity against a wide spectrum of bacteria such as *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris*[13]. The methanol extract from the leaves of *Cassia fistula* had 100% antifungal activity at 10 mg/ml against *Trichophyton rubrum*, *Microsporum gypseum* and *Penicillium marneffei*[14]. The observed antibacterial and antifungal activities of Solanum species are well known and probably caused by the alkaloids[15]. Further laboratory and clinical studies of this plant was required in order to understand better antibacterial principles which will allow the scientific community to recommend their use as an accessible alternative to other synthetic drugs (antibacterials, antibiotics).

Table II. MIC and MMC of Acalypha indica leaf extracts.

Microorganism	MIC[a] (mg/ml)				MMC[b] (mg/ml)			
	He	*Ch*	*E.a*	*Me*	*He*	*Ch*	*E.a*	*Me*
Staphylococcus aureus	0.312	0.625	0.312	0.156	0.625	1.25	0.625	0.312
Staphylococcus epidermidis	1.25	1.25	1.25	0.625	5	5	5	1.25
Bacillus cereus	0.312	0.625	0.625	0.156	0.625	1.25	1.25	0.312
Streptococcus faecalis	0.156	0.312	0.312	0.156	0.312	0.625	0.625	0.312
Klebsiella pneumoniae	> 5	> 5	> 5	> 5	> 10	> 10	> 10	> 10
Escherichia coli	> 5	> 5	> 5	> 5	> 10	> 10	> 10	> 10
Proteus vulgaris	> 5	> 5	> 5	> 5	> 10	> 10	> 10	> 10
Pseudomonas aeruginosa	5	2.5	5	2.5	10	5	10	5

[a]Minimum inhibitory concentration.[b]Minimum microbicidal concentration. He, hexane extract; Ch, chloroform extract; E.a, ethylacetate extract; Me, methanol extract.

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