RESEARCH PAPER

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Antimicrobial Activity of Extracts of *Acalypha indica* Linn

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Dilution method was employed to determine the effect of petroleum ether extract (40-60°) chloroform and methanolic extract of dried leaves of *Acalypha indica* Linn (Euphorbiaceae) against fungi (*Candida albicans) and bacteria (Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhosa, Bacillus substilis, Klebsiella pneumoniae*). Except the petroleum ether extract, all the extracts exhibited a prominent antimicrobial activity. The methanolic extract was further fractionated into acetone soluble and insoluble parts. Both the parts exhibited prominent antimicrobial activity. The acetone insoluble part exhibited MIC of 0.0040 mg/ml against Staphylococcus aureus and both acetone soluble and insoluble parts exhibited MIC of 0.05 mg/ml against Salmonella typhosa.

Acalypha indica is a common annual herb, growing as a weed in garden and road sides, throughout plains of India. The whole plant contains various saponins and alkaloids[1]. Acalyphamide a new amide is isolated from the root. Acalyphin a cyanogenic glucoside is isolated from leaves and twigs[1-4]. Table 1 shows various biological activities of various principles/extracts of Acalpha indica[5,6]. In view of the widest pharmacological activity of *Acalypha indica* the present study has been undertaken to investigate the antimicrobial activity of the leaves.

TABLE 1 : THE BIOLOGICAL ACTIVITY OF VARIOUS EXTRACTS/COMPOUNDS OF *ACALYPHA INDICA*

S. No.	Biological Activity	Preparation/ Active principle
1.	Anthelmintics	Acalyphamide
2.	Emmenagogue	Extracts of whole plant
3.	Antimycotic	Leaf Extracts
4.	Antidote of snake poison	Leaf Juice
5.	Anti-Asthma	Decoction of Lead

EXPERIMENTAL

Preparation of the extract:

The leaves of *Acalypha indica* were collected from Chennai. The plant identity was confirmed[7-9] and a specimen voucher was deposited in the pharmacognosy museum of Sri Ramachandra Medical University. Air-dried leaves were finely ground. The powdered leaves (500 g) were extracted with petroleum ether (40-60°) using a Soxhlelt extractor. The defatted marc was successively extracted with chloroform and methanol. The methanolic extract was further fractionated with acetone. The dried chloroform, acetone soluble and acetone insoluble part of methanol extracts were refrigerated until use. The drug menstrum ratio used was 1:2. Table 2 shows preliminary phytochemical screening[10] on all the extracts and percentage dried weight of each extracts.

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Antimicrobial screening:

The extracts were tested for *in vitro* antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhosa, Bacillus substilis, Klebsiella pneumonia* and fungi that included

TABLE 2 : PHYTOCHEMICAL COMPOSITION OF LEAF EXTRACTS OF *ACALYPHA INDICA*

Constituents and Colour tests	Extracts		
	Petroleum ether	Chloroform	Methanol
Alkaloids (1. Mayer's reagent 2. Dragendroff's reagent	-	+	+
Carbohydrate (1. Molisch's reagent 2. Fehling solution)	-	-	+
Flavonoids (1. Shinoda's test)	-	+	++
Fixed oils (1. Spot test 2. Saponification test)	-	-	-
Phenolic compounds (1. Ferric chloride solution)	-	-	-
Tannins (1. Gelatin precipitation test)	-	-	-
Proteins (1. Biuret test)	-	-	-
Sterols (1. Liebermann-Burchard's test)	+	+	=
% w/w of extracts	1.5	3.0	3.2

Candida albicans. These test microorganisms were obtained from the Department of Microbiology, Sri Ramachandra Deemed University.

The solution of extracts in PEG were used. The antimicrobial activities of the compounds were determined with cup plate and agar diffusion method[11-14]. The extracts were solubilised in PEG-200. The nutrient agar media was prepared and inoculated with different test organisms. The test solutions were introduced (0.2 ml, 1 mg/ml). The entire experiments were carried out under aseptic conditions. The plates were incubated at 37±0.5° for 24 h. The zone of inhibition was observed around each cup and the observations were given in Table 3.

Sterile nutrient broth double strength (peptone broth and Sabouraud's Dextrose Agar media) was used to determine the minimum inhibitory concentration (MIC) of the extracts. Two-fold serial dilution technique[15] was used. Plant extract (2mg/ml) in PEG 200 was used for testing MIC. This solution (0.2 ml) was added to 1.8 ml of nutrient broth formed the first dilution. All culture tubes were incubated at 37° for 24 h. Following incubation the tubes were examined for sign of microbial growth. The lowest concentration inhibiting microbial growth was considered to be MIC and mentioned in Table 4.

RESULTS AND DISCUSSION

Table 3 shows the antimicrobial activity of the chloroform extract and the acetone soluble and insoluble fractions of the methanolic extract. The chloroform extract as well as methanolic extract fractions exhibited prominent antimicrobial activity against all the microorganisms used in the study. From the zone of inhibition produced by the extracts it was observed that *Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhosa, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* were most sensitive to the chloroform extract whereas the acetone soluble fraction of the methanolic

TABLE 3 : ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *ACALYPHA INDICA* LEAVES

Strains of Microorganism	Zone of inhibition in cm		
	Chloroform Extract	Methanol Extract	
		Acetone soluble	Acetone insoluble
Escherichia coli	0.8 ± 0.1	1.0 ± 0.2	1.2 ± 0.1
Staphylococcus aureus	1.0 ± 0.2	1.4 ± 0.1	2.0 ± 0.1
Pseudomonas aeruginosa	0.8 ± 0.2	1.5 ± 0.1	1.2 ± 0.1
Salmonella typhosa	0.8 ± 0.2	1.0 ± 0.1	1.8 ± 0.1
Bacillus substilis	0.7 ± 0.2	1.2 ± 0.1	1.3 ± 0.1
Klebsiella pneumoniae	0.9 ± 0.1	1.2 ± 0.2	1.0 ± 0.2
Candida albicans	0.8 ± 0.2	1.9 ± 0.1	1.5 ± 0.2

Each value is a mean±standard error mean of 5 determinations. Statistical significance is determined at $P \leq 0.01$.

extract exhibited prominent antimicrobial activity against *Candida albicans, Salmonella typhosa, Staphyloccus aureus, Pseudomonas aeruginosa* and also *Bacillus substilis, Klebsiella pneumoniae* were moderately sensitive to this fraction. The acetone insoluble fraction of methanolic extract exhibited prominent antimicrobial activity. Table 4 gives the averages values of MIC in mg/ml of various extracts of *Acalypha indica* used in the study. The MIC of chloroform extract against all microorganisms was found to be 0.4 mg/ml. The acetone soluble fraction of methanolic extract exhibited MIC of 0.05 mg/ml against *Salmonella typhosa* and *Candida albicans*. The acetone insoluble fraction of methanolic extract exhibited MIC of 0.004 mg/ml against *Staphylococcus aureus*.

Thus the chloroform as well as methanolic extract fractions exhibited potent antimicrobial activity. The methanolic extract fractions were significantly more potent compared to the chloroform extract. The petroleum ether extract was devoid of any antimicrobial activity.

The preliminary phytochemical screening Table 2 reveals the presence of flavanoids, alkaloids which may be responsible for the said activity and is confirmed from the above experimentations. Work is in progress on separation and structure elucidation of the compounds responsible for antimicrobial action.

TABLE 4 : MIC VALUES OF LEAF EXTRACTS OF *ACALYPHA INDICA*

Microorganism	Extracts		
	Chloroform mg/ml	Methanol	
		Acetone soluble mg/ml	Acetone insoluble mg/ml
Escherichia coli	0.4 ± 0.1	0.25 ± 0.02	0.125 ± 0.02
Staphylococcus aureus	0.4 ± 0.1	0.15 ± 0.02	0.004 ± 0.03
Pseudomonas aeruginosa	0.4 ± 0.1	0.15 ± 0.02	0.15 ± 0.02
Salmonella typhosa	0.4 ± 0.1	0.05 ± 0.01	0.05 ± 0.02
Bacillus substilis	0.4 ± 0.1	0.15 ± 0.02	0.15 ± 0.02
Klebsiella pneumoniae	0.4 ± 0.1	0.25 ± 0.02	0.125 ± 0.02
Candida albicans	0.4 ± 0.1	0.05 ± 0.02	0.125 ± 0.02

Each value is a mean±standard error of 5 determinations. Statistical significance is determined at $P \leq 0.01$

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