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Archives of Applied Science Research, 2010, 2 (5):311-315

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ISSN 0975-508X
CODEN (USA) AASRC9

Screening for Antimicrobial Activity in *Acanthus ilicifolius*

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ABSTRACT

The antimicrobial activity of ethanol, methanol and aqueous extracts of leaf, stem and root of Acanthus ilicifolius were studied. These created an interest to test the possible antimicrobial activity of the different part of this plant, which has not been reported. The cub-plate agar diffusion method was employed to assess the antimicrobial activity of the prepared extract. Eleven test microorganisms were used in this study. Microorganism were grown overnight at 37^0C in the Mueller-Hinton broth at pH 7.4. The data obtained were subjected to ANOVA test to determine the significance extracts in antimicrobial activity of Acanthus ilicifolius. ANOVA test of data on the antimicrobial activity of aqueous, ethanol and methanol extracts on Bacillus megaterium, Lactobacillus plantarum, Salmonella paratyphi B, Shigella dycentreae, Escherichia coli, Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Aspergillus flavus, Staphylococcus albus, Lactobacillus acidophilus, revealed that the solvent used in extraction procedure had significant effect ($P<0.05$) on the level of significant observed. The inhibitory effect of the extracts on Lacto bacillus acidophillus showed no significant difference ($P>0.05$) between extract concentration. The most active antimicrobial parts were aqueous root, ethanol stem and methanol leaf. The ethanol, methanol and aqueous extracts of the different parts of the Acanthus ilicifolius exhibited strong to moderate activity against test microorganism. The effect exhibited by ethanol extract was significantly higher than that produced by methanol and aqueous extract. However, the length of incubation produced significant effect ($P>0.05$).

Keywords: Antimicrobial activity, Crude extract, *Acanthus ilicfolius*, Cub-plate method, *Candida albicans.*

INTRODUCTION

Acanthus ilicifolius Linn, popularly known as “Harkach Kanta” belongs to the family Acanthaceae, has typical spinose margins on its evergreen leaves and stipular spines at stem nodes. The common name of the plant is holy leaved Acanthus [1]. It is a gregarious, sparingly branched, evergreen shrub, 0.6-1.5 meters in height. It is a plant of marshy habitat distributed widely throughout the mangroves of India including west coasts, Meghalaya and the Andamans

and different parts of the Asian countries like Singhal, Burma, China, Thailand *etc*. The plant grows luxuriously by the side of the Ganges in Sunderbans, the shrub is also planted as a sand-binder along the banks of tidal rivers and lakes. *Acanthus ilicifolius* Linn. (Acanthaceae) is a medicinal plant used against rheumatism, paralysis, asthma and snake bite, analgesic, anti inflammatory and this plant against smallpox and ulcer [1,2]. Antioxidant and hepatoprotective activity of *A. ilcifolius* is reported [3]. Phytochemical screening indicates the presence of flavonoids and terpenes [4,5]. The ethanol extract of the plant was found to scavenge superoxide and hydroxyl radicals. The extract was also found to inhibit the generation of nitric oxide radical and lipid peroxides. Recent studies have shown that the plant extract has a remarkable hepatoprotective effect. The flavonoids present in the plant were found to have hepatoprotective and antioxidant activities [3]. The present study describes the antimicrobial activity of *Acanthus ilicifolius*.

MATERIALS AND METHODS

i) Collection of Plant

Fresh leaves, stem and root of *Acanthus ilicifolius* were collected from Parankipettai, cuddalore district, Tamil nadu, in the month of February to march and were botanically identified by the Botanical survey of India, Tamil Nadu Agriculture University, Coimbatore. A voucher specimen of the plant has been deposited at the botanical survey of India herbarium **(**Voucher number-53582 **)** the leaf, stem and root were air-dried, coarsely powdered and were subjected to extraction.

ii) Preparation of the crude extracts

One gram of each of the air-dried and coarsely powdered plant material was exhaustively extracted for overnight with ethanol, methanol and aqueous [6].

iii) Antimicrobial study

The eleven strains of bacteria were used in this study. They are *Escherichia coli, Bacillus megaterium. Lactobacillus plantarum, Salmonella paratyphi B, Shigella dycentreae, Streptococcus mutans, Klebsiella pneumoniae, Aspergillus flavus, Staphylococcus albus, and Lactobacillus acidophillus*. The yeast strain used in this study was *Candida albicans.* The micro-organisms were grown overnight at 37°C in Mueller-Hinton Broth at pH 7.4 [7,8].

iv) Testing for antibacterial activity

The cup-plate agar diffusion method was employed to assess the antibacterial activity of the prepared extracts [9]. 20 ml of the inoculated nutrient agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates, 5 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed [10]. Alternate cups were filled with 20 µl of each extracts using microtiter-pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37ºC for 18 hours. The respective solvents were used as controls. The diameters of the growth inhibition zones were measured at 24, 48 and 72 hours of incubation averaged and the mean values were tabulated.

v) Testing for anti-fungal activity

The inoculated medium was incubated at 25ºC for two days. Yeast and mould extract agar was used for testing anti fungal activity and the same method as for bacteria were adopted [11].

Statistical analysis of data

The data obtained were subjected to ANOVA test to determine the significance extracts in antimicrobial activity of *Acanthus ilicifolius*

RESULT AND DISCUSSION

The antimicrobial activity of *Acanthus ilicifolius* leaf, stem and root extract of (aqueous , ethanol and methanol) against *Bacillus megaterium, Lactobacillus plantarum, Salmonella paratyphi B, Shigella dycentreae, Escherichia coli, Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Aspergillus flavus, Staphylococcus albus, Lactobacillus acidophilus* is shown in Table 1. The methanol extract showed considerably more activity than the aqueous extract. Maximum antimicrobial activity was shown against *Staphylococcus albus, Streptococcus mutans, Escherichia coli, and Candida albicans.* The aqueous extract of root showed maximum activity against *Staphylococcus alpus, Lactobacillus acidophilus, Candida alpicans, Klebsiella pneumoniae, Streptococcus mutans, Shigella dycentreae.*

TABLE: 1 **Preliminary screening for antimicrobial activity of different parts against standard organisms Mean diameter inhibition zone (mm)**

S.no	Micro organism	Aqueous			Ethanol			Methanol		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
1	*Escherichia coli*	5	5	5	11	11	10	13	10	10
2	*Bacillus megaterium*	5	5	5	11	12	10	10	N.d	10
3	*Lactobacillus plantarum*	5	5	5	10	8	12	7	5	N.d
4	*Salmonella paratyphi B*	5	5	11	11	9	12	8	7	8
5	*Shigella dycentreae*	5	5	12	11	9	N.d	8	8	7
6	*Streptococcus mutans*	5	5	15	10	15	13	10	8	9
7	*Klebsiella pneumoniae*	5	5	13	10	10	7	9	8	5
8	*Candida albicans*	5	5	12	7	7	5	10	13	9
9	*Aspergillus flavus*	5	5	10	11	10	9	7	5	5
10	*Staphylococcus albus*	5	5	17	9	9	7	17	8	12
11	*Lactobacillus acidophilus*	7	N.d	12	10	12	10	11	10	8

N.d- Not determined as the zone is not clear

The ethanol extract showed inhibitory activity against *Streptococcus mutans, Lactobacillus acidophilus, Salmonella paratyphi B, Lactobacillus platarum, Bacillus megaterium and Escherichia coli* only, and none of the other bacterial strains were affected. ANOVA test of data on the antimicrobial activity of aqueous, ethanol and methanol extracts on *Bacillus megaterium,*

Lactobacillus plantarum, Salmonella paratyphi B, Shigella dycentreae, Escherichia coli, Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Aspergillus flavus, Staphylococcus albus revealed that the solvent used in extraction procedure had significant effect ($P<0.05$) on the level of inhibition observed. Results of the antimicrobial testing on *Salmonella paratyphi B* also showed that the inhibition of the organism is time dependent as the degree of inhibition decreased with increased length of incubation. The inhibitory effect of the extracts on *Lactobacillus acidophillus* showed no significant difference ($P>0.05$) between extract concentration. In the present study, the aqueous, ethanol and methanol extracts of the leaf, stem, and root of *Acanthus ilicifolius* inhibited the growth of all clinical isolates, but their effectiveness varied. The leaf extracts were more effective than the root extracts. This may be attributed to a significantly higher ($P<0.05$). Though methanol extracts exhibited more pronounced inhibition than aqueous extracts, yet the effectiveness of the aqueous extract to inhibit the growth of the clinical isolates in the present study could not be contemned. From the above results, it can be concluded that plant extracts have great potential as antimicrobial compounds gains microorganisms and those they can be used in the treatment of infectious diseases caused by resistant microorganisms. *Staphylococcus albus* and *Streptococcus mutans* showed maximum antimicrobial activity and so this plant can be used to discover bioactive natural products that may serve for the development of new pharmaceuticals that address hither to unmet therapeutic needs. Such screening of various natural organic compounds and identifying active agents is the need of the hour, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. *Acanthus ilicifolius* known to possess antimicrobial properities. Therapeutically, the methanol extract of leaves, ethanol extract of stem and aqueous extract of root found to possess the most active principles against the test organism. ANOVA test of data on the antimicrobial activity of aqueous, ethanol and methanol extracts on *Bacillus megaterium, Lactobacillus plantarum, Salmonella paratyphi B, Shigella dycentreae, Escherichia coli, Streptococcus mutans, Klebsiella pneumoniae, Candida albicans, Aspergillus flavus, Staphylococcus albus, Lactobacillus acidophilus*, revealed that the solvent used in extraction procedure had significant effect ($P<0.05$) on the level of significant observed. The inhibitory effect of the extracts on *Lactobacillus acidophillus* showed no significant difference ($P>0.05$) between extract concentration. Methanolic extract of leaves, ethanolic extract of stem, root and aqueous extract of root considerable antimicrobial proprieties against the resistant strains of *Escherichia coli, Staphylococcus albus, Lactobacillus plantarum, Salmonella paratyphi B, Streptococcus mutans* and *Lactobacillus acidophilus*. However, it was weakly active against *Klebsiella pneumoniae, Aspergillus flavus, Candida albicans* and *Bacillus megaterium.* The rest of the extracts showed moderate activity. Hence the methanol was discovered to be the best solvent among the three.

Acknowledgement

We thank Dr. D.Jaya Sundra Sharmila for her keen interest in the work and constant encouragement.

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