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IN VITRO DETERMINATION OF ANTIBACTERIAL ACTIVITY OF LEPIDAGATHIS CRISTATA WILLD

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ABSTRACT

This investigation is carried out to determine the antibacterial activity of different solvent extracts of *Lepidagathis cristata* Willd. Two different sets of solvents i.e. polar (methanol and ethanol) and non polar (toluene and acetone) were selected for the extraction of plant material using Soxhlet apparatus. The extracts were tested against human pathogenic bacteria namely, *Bacillus subtilis*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Among the extracts tested, polar solvent extract showed significant activity than the non polar solvent extracts. It was further observed that the extracts of *L.cristata* seemed to be more effective against Gram positive bacteria than Gram negative bacteria tested.

Keywords: *Lepidagathis cristata*, antibacterial activity, solvent extract

INTRODUCTION

The use of natural products with therapeutic properties has a long history (Kao, 1980). Nearly all cultures from ancient times to the present day have used plants as one of the sources of medicines (Lino and Deogracious, 2006). A considerable percentage of the peoples in both developed and developing nations use medicinal plant remedies. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs many of which have adverse side effects (Shariff, 2001; Parekh and Sumitra, 2006). Many approaches have been made to discover new biologically active principles higher plants (Farnsworth and Loub, 1983). One such approach is systematic screening of plants which may result in the discovery of novel effective biologically active compounds (Farnsworth *et al.*, 2002; Janovska *et al.*, 2003).

In India herbalists traditionally use various herbal preparations to treat a variety of diseases. In this line of herbal usage people in remote places of Chhattisgarh have been traditionally exploited the herb,

Lepidagathis cristata Willd. for various curative treatments particularly malarial fever. They also use the species for the treatment of itching. Cattle owners of this region use the decoction of this herb to wash their cattles in order to keep away the flies. Since the herb has been exploited tremendously in this region in many ways for various curative purposes, it is necessary to evaluate in a scientific base, the potential use of folk medicine for the treatment of infectious diseases.

Biological studies are very much essential to substantiate the therapeutic properties of medicinal herbs used in folk medicine on scientific line (Girish, 2008). Literature survey on *L.cristata* revealed that the therapeutic properties of this herb have not been established so far. Hence an attempt was made in the present study to investigate the feasibility of using *L.cristata* against various human pathogenic bacterial strains. *L.cristata* (Acanthaceae) is a perennial herb with almost no stem. Branches arise out of a globose head on the ground and spread out.

MATERIALS AND METHODS

Plant material Preparation

L.cristata was collected from Chhattisgarh, washed, shade dried, packed properly and transported to the Department of Botany, St. Joseph’s College, Tiruchirappalli, Tamil Nadu, India. The name was authenticated with the help of expert from the Rapinat Herbarium of St. Joseph’s College, Tiruchirappalli and a voucher specimen (Collection no: URT-001) was deposited.

Preparation of extracts

The dried plant materials were pulverized by a mechanical grinder. The powdered plant material (500 g) was successively extracted with acetone, toluene, ethanol and methanol up to 48 hrs at room temperature using a Soxhlet apparatus. The extracts were filtered and concentrated at 35°C.

Test Microorganisms

Authentic pure cultures of human pathogenic bacteria like *Bacillus subtilis*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi* were obtained from National College, Tiruchirappalli, Tamil Nadu, India.

Antibacterial activity assay

Antibacterial activity of solvent extracts of the entire plant was determined

by disc diffusion method. Standard discs (6 mm diameter) were autoclaved and soaked separately in different solvent extract over night. The organism to be tested was uniformly spread on the sterile nutrient agar medium. Three solvent extract soaked discs were carefully placed on the inoculated medium aseptically. The plates were incubated for 24 hrs at 37°C and zone of inhibition if any around the disc was measured in millimeter (mm). The treatment also includes the antibacterial agent Gentamicin as the positive control and the respective solvents as the negative controls. For each treatment three replicates were maintained. The data was subjected to statistical analysis.

RESULTS AND DISCUSSION

The observations and the results of antibacterial activity of various solvent extracts of *L.cristata* against human pathogenic bacterial strains are shown in the Table. The results revealed that toluene, acetone, ethanol and methanol solvent extracts were active against all the tested five pathogenic bacterial strains. The literature indicates that the antimicrobial activity is due to different chemical agents in the extract. These are classified as active

antimicrobial compounds (Rojas, *et al.*, 1992). The extraction of the active antimicrobial compound from the plant material is largely dependent on the type of solvent used. The traditional healers make use of water primarily as a solvent to extract the active compound from the plant material. However in the present investigation four different solvents were used with different polarity. Because the solubility of the active components of the plant material may be vary from solvent to solvent (Boer, *et al.*, 2005). Therefore polar components of the plant material can be extracted using polar solvent and vice versa. When the results are compared between the two different solvent systems used in the present investigation the extracts of polar solvents seemed to be greater activity than the extracts of non polar solvents.

Review of literature reveals lack of information on the antibacterial potential of *L.cristata* extracts. In the present investigation the antibacterial activity of this whole plant extracts has been demonstrated for the first time. The results reveal that the extracts of *L.cristata* were significantly effective against tested Gram-positive organism than Gram-negative organisms.

This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria (Vlietinck, *et al.*, 1995; Rabe and Van Staden, 1997). Among the Gram-negative bacterial strains tested *P.aeruginosa* seemed to be lesser susceptible to the solvent extracts. This could be attributed to the fact that this bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane. Also its tendency to colonize in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Since its natural habitat is the soil, living in association with bacilli, actinomycetes and molds it develops resistance to a variety of their naturally occurring antibiotics.

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Table 1 Antibacterial activity of different solvent extracts of *Lepidagathis cristata* against human pathogenic bacteria

Sl. No	Pathogens	Toluene extract	‘-‘ control	Acetone extract	‘-‘ control	Methanol extract	‘-‘ control	Ethanol extract	‘-‘ control	‘+’ control
1	<i>B.sublitis</i>	0.5±0.2	0±0.00	0.6±0.1 8	0±0.00	11.6±0.2 4	0±0.00	12.4±0.1 4	0±0.00	24±0.0 0
2	<i>K. pneumoniae</i>	0.6±0.2	0±0.00	0.6±0.1 7	0±0.00	7.54±0.3 2	0±0.00	8.5±0.36	0±0.00	20.27± 0.27
3	<i>P.vulgaris</i>	0.72±0. 12	0±0.00	0.75±0. 26	0±0.00	6.33±0.1 4	0±0.00	7±0.00	0±0.00	19.33± 0.14
4	<i>P.aeruginosa</i>	0.25±0. 16	0±0.00	0.36±0. 17	0±0.00	3.5.38±0. 27	0±0.00	4.7.83±0 .14	0±0.00	12.37± 0.12
5	<i>S.typhi</i>	0.53±0. 16	0±0.00	0.67±0. 11	0±0.00	6.5.12±0. 24	0±0.00	7.8.37±0 .15	0±0.00	20.17± 0.27