



**IN VITRO ANTIFUNGAL EFFICACY OF BIOACTIVE COMPOUNDS
HEPTADECANE, 9- HEXYL AND OCTADECANE, 3-ETHYL-5-(2-
ETHYL BUTYL) FROM *LEPIDAGATHIS CRISTATA* WILLD.
(ACANTHACEAE) ROOT EXTRACT.**

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ABSTRACT

To identify bioactive compounds Heptadecane, 9- hexyl and Octadecane, 3-ethyl-5-(2-ethylbutyl) from *Lepidagathis cristata* Willd. (*L. cristata*) root extract to assess antifungal potentials of the isolated compounds. Aqueous extracts of *L. cristata* roots were used for this study. The major bioactive compounds isolated were tested for antifungal activities. The major bioactive compounds Heptadecane, 9-

hexyl and Octadecane, 3-ethyl-5-(2-ethylbutyl) were isolated from the roots of *L. cristata* and these compound were tested for antifungal potentials and found to be highly effective to plant pathogenic fungi *Colletotrichum fulcatum* NCBT 146, *Fusarium oxysporum* NCBT 156 and *Rhizoctonia solani* NCBT 196 as well as for the human pathogenic fungi *Curvularia lunata* MTCC 2030 and *Microsporum canis* MTCC 2820. The results justify the antifungal potentials of both plant and human pathogenic fungi. The plant bioactive compounds will be helpful in herbal antifungal formulations.

KEYWORDS: Bioactive compounds Heptadecane, 9- hexyl, Octadecane, 3-ethyl-5-(2-ethylbutyl), GC-MS analysis.

INTRODUCTION

The relationship between man, plants and drugs derived from plants described the history of mankind. Since ancient times, people have been exploring the nature particularly plants in

search of new drugs. This has resulted in the use of large number of medicinal plants which curative properties to treat various diseases.^[1] WHO encouraging the traditional drugs because of its less side effects and matter of low cost, easy availability hence most of the European countries expanding towards Ayurvedic medicines.^[2] Now-a- days plant based drugs are widely used and many countries contributes 40-50% of their total health budget in the production of novel drugs.^[3]

In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani, Ayurveda, Siddha.^[4] India is one of the worlds 12 biodiversity centers with the presence of over 45,000 different plant species. Of these, about 15,000 to 20,000 plants have gold medicinal value. Everyday new inspiring information is being added to folklore medicine for the development of drugs.^[5]

Lepidagathis cristata Willd. (Acanthaceae) (*L. cristata*) is a medicinal herb and used as bitter tonic in fevers and used in pneumonia, flu, mouth infections.^[6] eczema, psoriasis and other skin infections.^[7] The ash of whole herb is applied externally on chronic wounds of pet animals.^[8] Fumigation of this medicinal herb is used to treat epilepsy.^[9] The roots of the herb are used in stomachic and dyspepsia, leaves are used for fevers and the inflorescence ash is used for itchy affections of skin and burns.^[10, 11]

The plant is a stiff herb, and the branches procumbently arise from a hard central rootstock. Leaves are alternate, elliptic, serrate and usually lineolate. Flowers are sessile, capitate, the heads terminal or axillary densely crowded at the base of the plant, fruits glucose capsule^[12, 13]. This medicinal herb has been exploited tremendously by common people in many ways for various curative purposes. It is necessary to evaluate the herb in a scientific base for its potential use of folk medicine for the treatment of infectious diseases.^[14]

Antibacterial studies,^[14, 15] pharmacognostical and phytochemical studies,^[16] analgesic and anti- inflammatory activities studies,^[17] hypoglycaemic activity in alloxan induced diabetic rats of *L. cristata* have been documented so far but antifungal activity of this herb seems to be lacking.^[18] Biological studies are very much essential to substantiate the therapeutic properties of medicinal herbs used in folk medicine on scientific bases^[19]. Literature survey on *L. cristata* revealed that the therapeutic properties of this herb had not been established so far. Hence an attempt was made in the present study to investigate the feasibility of using *L.cristata* against various fungal isolates of both plant and human pathogens.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh plants of *L. cristata*, Willd. (Acanthaceae) were collected from Pachhaimalai Hills, Tiruchirappalli District, Tamil Nadu, India (Figure 1a). The taxonomic identities of the plant were confirmed by previously described [12]. The plant material was washed under running tap water; air dried in shade and then the root was homogenized to fine powder and stored in sterile air tight bottles for the experimental use.

Fungal cultures

The fungal cultures tested in this work *Colletotrichum fulcatum* NCBT 146 (*C. fulcatum*), *Fusarium oxysporum* NCBT 156 (*F. oxysporum*) and *Rhizoctonia solani* NCBT 196 (*R. solani*) were maintained in immobilized condition in polyurethane foam in Microbiology Lab, Department of Biotechnology, National College, Tiruchirappalli, Tamil Nadu, India, whereas *Curvularia lunata* MTCC 2030 (*C. lunata*) and *Microsporum canis* MTCC 2820 (*M. canis*) were obtained from Microbial Type Culture Collection and Gene Bank MTCC, Chandigarh, India.

Experimental procedure

Different weight of dry root powder (2 mg, 4mg, 6 mg and 12 mg) were mixed with different volume of Sabourand dextrose agar (SDA) medium (HI media M063) to form different concentrations (100 mg/L, 200 mg/L, 400 mg/L and 800 mg/L). The Control-1 contained only 20 ml of SDA medium and Control-2 contained 2 mg of bavistin fungicide added to 20 ml of SDA medium at 100 mg/L concentration. The leaf powder is mixed with the medium in Petridish (9 cm) and inoculated with 0.5 ml spore suspension of fungi prepared from 10 days old culture. The experimental Petridishes were incubated for 8 d at (28±2) °C temperature in dark. Three replicates were prepared and inoculated with fungal spores for each treatment.

Determination of the minimum inhibitory concentration (MIC)

MIC was determined by the liquid dilution method [20]. Dilution series were prepared with 0.25 to 15.00 mg/ml of Sabourand dextrose broth medium. To each tube 0.1 ml of standardized suspension of fungal spores (4×10^6 spores/ml) were added and incubated at (28±2) °C for 24 h. The lowest concentration which did not show any growth of the tested fungi after microscopic evaluation was determined as MIC.

Isolation of bioactive compound-Thin Layer Chromatography (TLC)

Glass plates (4 cm×12 cm) were used in which 30 g silica gel mixed with 60 ml distilled water and slurry was prepared and coated on the glass plate to 0.25 cm thickness dried for an hour at 110 °C in an air oven.^[21]

Preparation of root extract for bioactive compound

The dry powdered root (500 mg) of *L. cristata* was mixed with 5.0 ml of chloroform and ground into a paste, dried at room temperature. About 1 ml of chloroform was added to the dried samples and spotted on the TLC plates. The TLC plates were kept in several eluent mixtures with different polarities to separate the bioactive chemical compounds. The eluent used were chloroform: n-hexane (8:2), chloroform: ethyl acetate (8:2), chloroform: acetone (8:2), n-hexane: acetone (9:1), and chloroform: acetone (9:1). Samples spotting on the TLC plate were done by using a micropipette in which the dot diameter was 0.5 mm. The chloroform: acetone (9:1) was the best eluent since it was able to separate the compounds contained in root extract^[22].

Gas Chromatography and Mass Spectroscopy (GC-MS)

GC-MS analyses were performed using a GC Clarus 500 Perkin Elmer equipment, equipped with a flame ionization detector and injector MS transfer line temperature of 230 °C, fused silica capillary column Elite-5 MS (5% diphenyl/95% dimethyl polysiloxane), 30.00×0.25 µl df, film thickness, carrier gas helium at a flow rate of 28cm/s was used. A volume of 1 ml of extract mixed with methanol (80%) at a split rate 10:1 was injected^[23]. The compound identification was accomplished by comparing the GC relative retention and mass spectra to those of authentic substances analyzed under the same conditions, by their retention indices and by comparison to reference compounds.

RESULTS AND DISCUSSION

The aqueous extract of dried powder of *L. cristata* root has shown varied antifungal properties against both plant pathogenic as well as human pathogenic fungi tested in this work (Table 1). The growth of both plant and human pathogenic fungal strains were totally inhibited at 800 mg/L concentration except for *C. fulcatum* NCBT 146. The total inhibition can be comparable to Control-2, a standard antifungal agent bavistin at 100 mg/L. However at 400 mg/L concentration total inhibition was noticed by *C. lunata* MTCC 2030, *F. oxysporum* NCBT 156, *R. solani* NCBT 194 and 75% growth inhibition by *C. fulcatum* NCBT 146, and *M. canis* MTCC 2820. In 200mg/L concentration 75% inhibition by

F.oxysporum NCBT 156, *M. canis* MTCC 2820, *R. solani* NCBT 194 and 50% growth inhibition by *C. fulcatum* NCBT 146, *C. lunata* MTCC 2030 . All fungal strains have shown 50% growth inhibition at 100 mg/L concentration (Figure 1b-1f).

Table 1-Antifungal potentials of bioactive compounds Heptadecane, 9- hexyl and Octadecane, 3-ethyl-5-(2-ethylbutyl) isolated from root of *L. cristata*.

Fungus	Control		Concentration of the extract			
	1	2	100 mg/L	200 mg/L	400 mg/L	800 mg/L
<i>C. fulcatum</i> NCBT 146	++++	-	++	++	+	+
<i>C. lunata</i> MTCC 2030	++++	+	++	++	-	-
<i>F.oxysporum</i> NCBT 156	++++	+	++	+	-	-
<i>M. canis</i> MTCC 2820	++++	+	++	+	+	-
<i>R. solani</i> NCBT 194	++++	-	++	+	-	-

Control-1: Medium without root extract; Control-2: Medium with Bavistin (100 mg/L). +++++: Normal growth; +++: 25% growth inhibition; ++: 50% growth inhibition; +: 75% growth inhibition; -: Total (100%) growth inhibition.

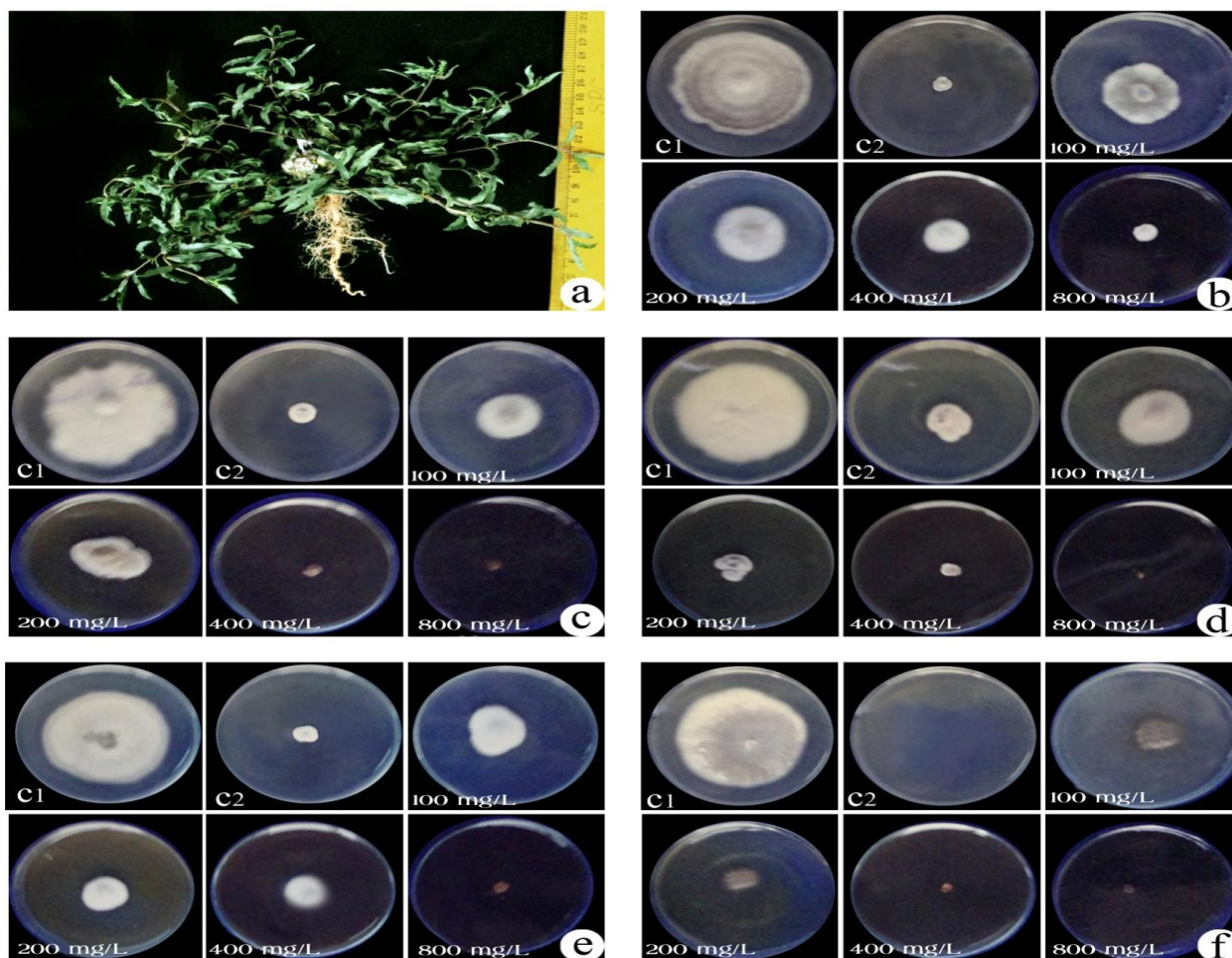


Figure 1-Antifungal potentials of bioactive compounds Heptadecane, 9- hexyl- and Octadecane, 3-ethyl-5-(2-ethylbutyl) isolated from *Lepidagathis cristata* Willd. (Acanthaceae) root. a: *L. cristata* plant habit; b: *C. fulcatum* NCBT 146; c: *C. lunata*

MTCC 2030; d: *F.oxysporum* NCBT 156; e: *M. canis* MTCC 2820; f: *R. solani* NCBT 194; C1: Control-1(without root extract); C2: Control -2(Bavistin 100 mg/L). Concentrations of root extract ranged from 100mg/L to 800 mg/L.

MIC values of the aqueous extract of root varied from 5.50 mg/ml to 11.50 mg/ml for the fungi tested. The MIC value of *R. solani*, *F. oxysporum*, *C. fulcatum*, *C. lunata* and *M. canis* were 5.50, 7.0, 9.0, 10.50 and 11.50 mg/ml respectively. Further investigation was performed to demonstrate the action of the extract on these fungi at different concentrations. The growth of these fungi correspondingly decreased with increasing concentration of the extract and the growth was completely inhibited at their MIC values. The reduction of growth was possibly due to the interference by active principles, i.e., bioactive compounds Heptadecane, 9- hexyl and Octadecane, 3-ethyl-5-(2-ethylbutyl) (Table 2 and Figure 2). Therefore, the MIC determination is important in giving a guideline of the choice of an appropriate and effective concentration of antifungal therapeutic substance. L

Table 2- Components identified in *L.cristata* root.

RT	Name of the compound	Molecular formula	MW	Peak area %
4.96	Heptadecane, 9- hexyl-	C ₂₃ H ₄₈	324	34.18
5.55	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C ₂₆ H ₅₄	366	25.32
7.63	Oleic acid, 3-(octadecyloxy) propyl ester	C ₃₉ H ₇₆ O ₃	592	17.72
8.23	Tetracycline	C ₂₂ H ₂₄ N ₂ O ₈	444	18.99
10.10	Docosanoic acid, 1,2,3-propanetriyl ester	C ₆₉ H ₁₃₄ O ₆	1058	3.80

MW: Molecular weight. Parameters tested are not covered under the scope of NABL accreditation.

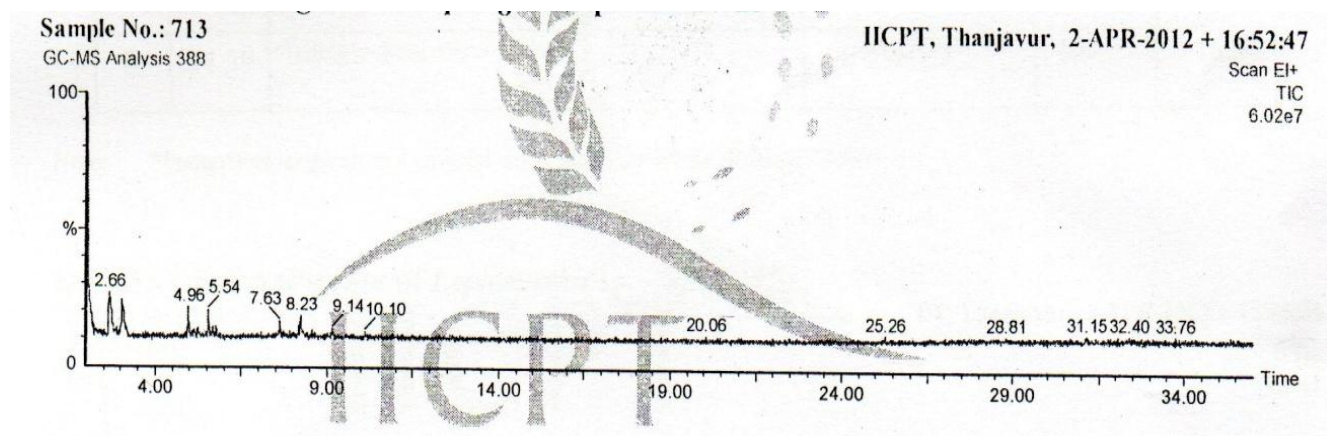


Figure 2- GC-MS Chromatogram of *L. cristata* root.

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The results of earlier work with *L. cristata* reveal that the plant extract is significantly effective against Gram-positive bacteria.^[14, 24, 25] Root extract of *L. cristata* is significantly effective in antiemetic,^[6] analgesic,^[26] anti-inflammatory activities.^[27] Root paste is used as antidysentric and reduces heat in stomach.^[9] Review of literature reveals the information on the antifungal potential of *L. cristata* inflorescence^[28] and leaf extracts.^[29]

CONCLUSION

In the present investigation the antifungal activity of its root has been demonstrated for the first time. *L. cristata* root extract was investigated for its potential bioactive compounds Heptadecane, 9-hexyl(C₂₃H₄₈) and Octadecane, 3-ethyl-5-(2-ethylbutyl) (C₂₆H₅₄) are effective plant extract and as antifungal agent for plant and human pathogenic fungi.

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