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In vitro antifungal potentials of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *Lepidagathis cristata* Willd. (Acanthaceae) inflorescence

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ABSTRACT

Objective: To identify bioactive compound oleic acid, 3-(octadecyloxy) propyl ester from *Lepidagathis cristata* Willd. (*L. cristata*) and to assess antifungal potentials of the isolated compound.

Methods: Aqueous extracts of *L. cristata* inflorescence were used for this study. The major bioactive compound isolated was tested for antifungal activities.

Results: The major bioactive compound oleic acid, 3-(octadecyloxy) propyl ester was isolated from the inflorescence of *L. cristata*. The bioactive compound was 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.

Conclusions: The results justify the antifungal potentials of both plant and human pathogenic fungi. The plant bioactive compound will be helpful in herbal antifungal formulations.

1. Introduction

Over the centuries, the use of medicinal herbs has become an important part of daily life. Approximately 3 000 plant species are known to have medicinal properties in India[1]. In Rig Veda (3700 BC) use of medicinal plants as traditional system of medicine is well described. It is estimated that 80% of the world population depends directly on the plant-based remedies for their health care[2].

Lepidagathis cristata Willd. (Acanthaceae) (*L. cristata*) is a medicinal herb. The roots, leaves and inflorescence of *L. cristata* are medicinally useful. 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[3,4].

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 crowed at the base of the plant, fruits glucose capsule[5,6]. 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[7].

Antibacterial studies[7,8], pharmacognostical and phytochemical studies[9], analgestic and anti-

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inflammatory activities studies[10], 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[11]. Biological studies are very much essential to substantiate the therapeutic properties of medicinal herbs used in folk medicine on scientific bases[12]. 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.

2. Materials and methods

2.1. Collection and identification of plant material

Fresh plants of *L. cristata*, Willd. (Acanthaceae) were collected from Pachhaimalai Hills, Tiruchirappalli District (Figure 1a). The taxonomic identities of the plant were confirmed by previously described[5]. The plant material was washed under running tap water, air dried in shade and then the inflorescence was homogenized to fine powder and stored in sterile air tight bottles for the experimental use.

2.2. 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, 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.

2.3. Experimental procedure

Different weight of dry inflorescence power (2 mg, 4 mg, 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 inflorescence powder is mixed with the medium in Petri dish (9 cm) and inoculated with 0.5 mL spore suspension of fungi prepared from 10 days old culture. The experimental Petri dishes were incubated for 8 d at (28±2) °C temperature in dark. Three replicates were prepared and inoculated with fungal spores for each treatment.

2.4. Determination of the minimum inhibitory concentration (MIC)

MIC was determined by the liquid dilution method[13]. 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.

2.5. 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[14].

2.6. Preparation of inflorescence extract for bioactive compound

The dry powdered inflorescence (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 mixture 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 inflorescence extract[15].

2.7. 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 28 cm/s was used. A volume of 1 mL of extract mixed with methanol (80%) at a split rate 10:1 was injected[16]. The compound identification was accomplished by comparing the GC relative retention and mass spectra to those of authentic substances analysed under the same conditions, by their retention indices and by comparison to reference compounds.

3. Results

The aqueous extract of dried powder of *L. cristata* inflorescence 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 400 and 800 mg/L concentration respectively. The total inhibition can be comparable to Control-2, a standard antifungal agent bavistin at 100 mg/L. However at 200 mg/L concentration 75% growth inhibition was exhibited by *C. fulcatum* NCBT 146, *C. lunata* MTCC 2030, *F. oxysporum* NCBT 156 and *M. canis* MTCC 2820 and a total inhibition was noticed for *R. solani* NCBT 194. At 100 mg/L concentration 50% growth inhibition was exhibited by *C. fulcatum*, *C. lunata* and *M. canis*. *F. oxysporum* have shown 75% growth inhibition and a total inhibition was noticed for *R. solani* at 100 mg/L concentration (Figure 1b-1f).

The MIC values of the aqueous extract of inflorescence varied from 4.50 mg/mL to 10.50 mg/mL for the fungi tested. The MIC value of *R. solani*, *F. oxysporum*, *C. fulcatum*, *C. lunata* and *M. canis* were 4.50, 6.50, 8.50, 9.50 and 10.50 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 compound olic acid, 3-(octadecyloxy) propyl ester (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.

Table 1

Antifungal potentials of bioactive compound olic acid, 3-(octadecyloxy) propyl ester isolated from inflorescence of *L. cristata*.

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

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

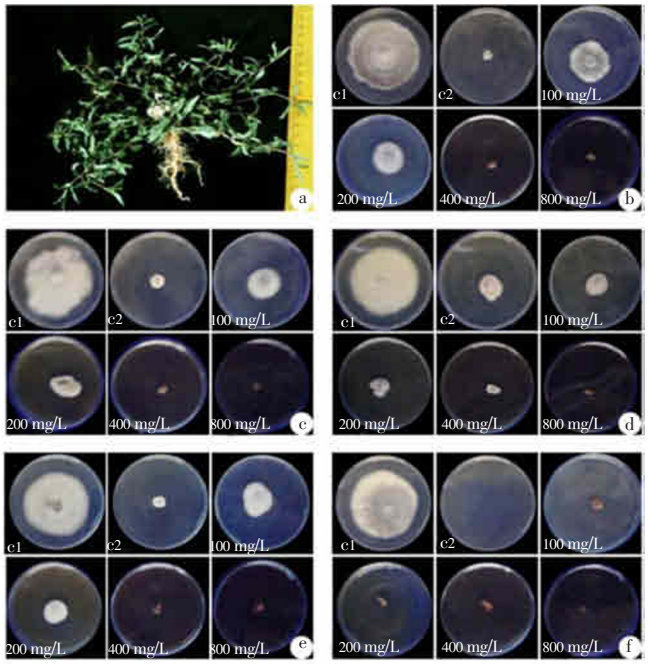

Figure 1. Antifungal activity of bioactive compound oleic acid, 3-(octadecyloxy) propyl ester isolated from *L. cristata* Willd. (Acanthaceae) inflorescence.
a: *L. cristata* plant habit; b: *C. fulcatum* NCBT 146; c: *C. lunata* MTCC 2030; d: *F. oxysporum* NCBT 156; e: *M. canis* MTCC 2820; f: *R. solani* NCBT 194; C1: Control-1 (without inflorescence extract); C2: Control-2 (Bavistin 100 mg/L). Concentrations of inflorescence extract ranged from 100 mg/L to 800 mg/L.

Table 2

Components identified in *L. cristata*.

RT	Name of the compound	Molecular formula	MW	Peak area (%)
4.95	Heptadecane,9-hexyl-	$C_{23}H_{48}$	324	34.48
5.54	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	34.48
7.63	Oleic acid, 3-(octadecyloxy) propyl ester	$C_{39}H_{76}O_3$	592	31.03

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

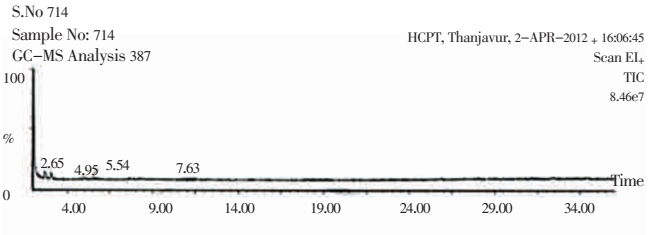

Figure 2. GC-MS chromatogram of *L. cristata*.

4. Discussion

The results of earlier work with *L. cristata* reveal that the plant extract is significantly effective against Gram-positive bacteria[7,17,18]. *Lepidagathis trinervis* plant ashes were used to cure eczema[9]. *L. cristata*, inflorescence ash with oil applied externally to cure black patches on face[19]. Review of literature reveals lack of information on the antifungal potential of *L. cristata* extract. In the present investigation the antifungal activity of its inflorescence has been demonstrated for the first time. *L. cristata* inflorescence extract was investigated for its potential bioactive compound, olic acid, 3-(octadecyloxy) propyl ester ($C_{39}H_{76}O_3$), an effective plant extract and as antifungal agent for plant and human pathogenic fungi.

Conflict of interest statement

We declare that we have no conflict of interest.

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