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Phytochemical and pharmacological analysis of *Lepidagathis cristata*, Willd.

(Acanthaceae)

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Abstract

Medicinal plants are expensive gift from nature to human. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. The herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries; more than 65% of the global population uses medicinal plants as a primary health care modality. India is one of the world's 12 biodiversity centers with the presence of over 45,000 different plant species. Of these, about 15,000 to 20,000 plants have immense medicinal value. Everyday new inspiring information is being added to folklore medicine for the development of drugs. The present study was carried out to determine the vast pharmacological applications of Lepidagathis cristata, Willd (Acanthaceae), a multipurpose medicinal plant. Traditionally this herb is used for the treatment of fever, eczema, psoriasis, epilepsy, skin abscess, burns, mouth ulcer, snake bites, wounds, anti-inflammatory, hypoglycaemic, immunosuppressive, skin itching and other skin diseases. The present article highlights the phytochemical screening and pharmacological properties of Lepidagathis cristata. Taking great concern of the useful benefits of the plant it can be used as safe drug for mankind.

Key words: Lepidagathis cristata, anti-inflammatory, analgesic, immunosuppressive

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INTRODUCTION	medicinal taxa. In the recent past, the Ethno botanical

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicines, food supplements, pharmaceutical and clinical entities for synthetic drugs (Ncube et al., 2008). The World Health Organization reported that about 75%-95% of world population of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involve the use of plant extract products on their active constituents (WHO, 2011). Researchers are increasingly turning their attention to folk medicine and looking for new leads to develop better drugs against cancer, as well as viral and microbial infection (Hoffman et al., 1993).

India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetation and floristic composition. About 2,500 plant species are known to be useful and more than 6,000 manufactures produce about 1,500 Ayurvedic, Unani and Siddha medicinal preparations from plants. South India in particular blessed with diverse

survey has been triggered to gather the medicinal knowledge of tribal as well as non-tribal groups (Madhu et al., 2010). Hence the present study was designed to throw light on a medicinal herb Lepidagathis cristata, Willd, belonged to the family Acanthaceae for its ethnomedicinal uses, phytochemical constituents and pharmacological activities.

L.cristata (Figure-1) is commonly known as 'Nakkapidi', 'Lankapindi' (Yanadi tribal), 'Mullabanthi' (Telugu), 'Karappanpoondu' (Tamil), 'Karappanundu' (Malayalam) and 'Otdhompo' (Santhal tribe) (Purma Aravinda Reddy and Venkatesh war Rao, 2013). It is distributed in the central and eastern peninsular India; Konkan, Deccan North Circars, Carnotic and other regions. Usually it appears in dry places and waste lands. It is a perennial herb with branched - sessile, linear-lanceolate leaves, globose headed flower, crowded at the base of the stem, hairy calx, white with brown or purple spoted corella, didynamous anther two celled and exerted stamens, fruit capsule with seeds. Flowering seasons is January – March (Gamble, 1967).

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Fig. 1.

cristata has a lot of traditional uses. The shade dried powder of *L.cristata* plant mixed with honey in two spoonfuls is administered twice a day for about twenty days for asthma disease (Madhu *et al.*, 2010). Whole plant powder is mixed with coconut oil to treat itchy infections in ethnic groups of Kurnool, Andhra Pradesh (Venkata Subbaiah and Savithramma, 2012), ash of entire plant is boiled with coconut oil and the infusion is applied externally on chronic wounds of pet animals twice a day up to 6-8 days (Salave Ashok Punjaji, 2012), dried shoot ash used for skin infections (Jagtap *et al.*, 2010) and the whole plant paste is used for itching infections (Sinha *et al.*, 2013).

The mixture of root paste is mixed with seed powder of Abrus precatorius and karanj oil is applied for leucoderma (Varghese, 1996). The root of the herb also used as antidysenteric and reduces heat in stomach and fumigation of the herb inhaled for the treatment of epilepsy (Nitin Dongarwar et al., 2012). In Chhattisgarh, the leaf extract is used for malarial fever and to clean the cattle in rainy season, and it is also used for skin itchy affection, burns and wounds. The leaf juice with copper sulphate is given during snakebite for gaining consciousness (Sikarwar et al., 2008). The aqueous extract of leaves mixed with Ocimum juice in 10:1 ratio is used to cure fever by 'Yanadi' tribal of Andhra Pradesh (Purma Aravinda Reddy and Venkatesh war Rao, 2013). Leaf extract is externally applied for ring worm and skin diseases (Sathya Bama et al., 2013).

The inflorescence ash is mixed with coconut oil and applied on the affected part for a week to treat inflammation, skin abscess and tumors (Hamambarareddy *et al.*, 2000). The tuberous flower ash mixed with coconut oil is applied externally for burns and wounds (Sudhakar Reddy *et al.*, 2009) and smoke of flower head is used to treat mouth ulcer (Pawar Shubhangi, 2011). Inflorescence ash is mixed with oil applied externally for black batches on face (Vijigri Dinesh, 2013).

Chloroform, ethyl acetate and methanolic flower extract of *L.cristata* showed analgesic activity. This is

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screened by hot plate and tail immersion methods (Aravinda Reddy Purmaand RaoVenkateshwar, 2013). The antiemetic activity was reported in chicks using ethanolic extract of the herb (Rachapalli Sowjanya Kumar Reddy et al., 2014). Methanol, ethyl acetate and chloroform root extracts of L.cristata showed antiinflammatory activity by carrageenan induced paw edema method and formalin induced paw edema method (Vijaya Narasimha Reddy et al., 2014). The hypoglycaemic activity was studied and reported in ethanolic extract of L. cristata in alloxan induced diabetic rats (Srinija et al., 2013). In L. cristata, alkaloid-I (cristain) showed immunosuppressive activity against con-A (2 µg/ml, T-cells) and LPS- induced (Bcells) proliferation of mouse splenic lymphocytes, and con- A and LPS were used as controls and cyclosporine A was used as standard drug (Ravikanth et al., 2001).

MATERIALS AND METHODS

Collection and identification of plant material

Fresh plants of *L. cristata*, Willd. (Acanthaceae) were collected from Pachhaimalai Hills, Tiruchirappalli District. The taxonomic identify of the plant was confirmed (Gamble, 1967). 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.

Preliminary Phytochemical analysis

The active principles of many drugs found in plants are secondary metabolites (Dobelis, 1993; Ghani, 1990). Therefore, basic phytochemical investigation of *L.cristata* extract for their major phytoconstituents is also vital. Hence, a preliminary phytochemical screening of the plants was conducted following the standard protocols (Brindha *et al.*, 1981). In the present investigation, maximum emphasis was given to alkaloids, flavonoids, phenolics, saponins, tannins and terpenoids.

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 iLdf, film thickness, carrier gas helium at a flow rate of 28 cm/s was used. A volume of 1 mL of the extract mixed with methanol (80%) at a split rate 10:1 was injected (Reyes-Chilpa *et al.*, 2004). 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.

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Antifungal activity

Fungal cultures

The fungal cultures tested in this work include *Colletotrichum fulcatum* NCBT 146 (*C. fulcatum*), *Fusarium oxysporum* NCBT 156 (*F. oxysporum*) and *Rhizoctonia solani* NCBT 196 (*R. solani*). They were maintained in immobilized condition in polyurethane foam in the 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.

Experimental procedure

Different weight of dry inflorescence, leaf and root 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 powder was 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 days 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 (Irobi *et al.*, 1996).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 (4×10⁶ spores/mL) were added and incubated at 28±2°C for 24h. The lowest concentration which did not show any growth of the tested fungi after microscopic evaluation was determined as MIC.

Table. 1. Presence of the phytochemical components

 of the plant extract of *L. Cristata.*

Phytochemicals	Result
Alkaloids	+
Flavonoids	+
Glycosides	-
Phenolics,	+
Saponins	+
Tannins	+
Terpenoids.	+
Volatile oil	-

+ - present; - -absent

RESULTS AND DISCUSSION

The plant extract was tested for phytochemical constituents and the results are tabulated (Table 1 and 5).

The aqueous extract of dried powder of *L. cristata* in florescence (Fig.2 and Table 2), leaf (Fig.3 and Table 3) and root (Fig.4 and Table 4) showed varied antifungal properties against both plant pathogenic as well as human pathogenic fungi tested in this work. The growth of both plant and human pathogenic fungal strains was 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.

MIC values of the plant extracts 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

Table. 2. Inhibitory effect of the extract of the inflorescence of *L. cristata* on the growth of test fungi.

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Fungus	Contr	ol	Concentration of extract					
	1	2	100 mg/L	200 mg/L	400 mg/L	800 mg/L		
C. fulc atum NCBT 146	++++	-	++	+				
C. hunata MTCC 2030	++++	+	++	+	122	<u>,</u> 12		
F.oxysporum NCBT 156	++++	+	++	+	-	(H)		
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.

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of growth was possibly due to the interference by active principles. Therefore, the MIC determination is important in giving a guideline of the choice of an appropriate and effective concentration of antifungal

S.No 714

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
l.	4.95	Heptadecane, 9-hexyl-	C23H48	324	34.48
2.	5.54	Octadecane, 3-ethyl-5-[2-ethylbutyl]-	C26H54	366	34.48
3.	7.63	Oleic acid, 3-(octadecyloxy)propyl ester	C39H76O3	592	31.03

Note: *Parameters tested are not covered under the scope of NABL accreditation

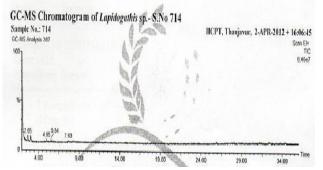


Fig. 2. GC MS Analysis of L. cristata Inflorescence shown the presence of some major phytochemical constituents

therapeutic substance.

DISCUSSION

The results of the earlier work with *L. cristata* revealed that the plant extract was significantly effective against Gram-positive bacteria (Vlietinck *et al.*, 1995). *L. cristata*, inflorescence ash with oil was applied externally to

Table. 3. Inhibitory effect of the leaf extract of L.

 cristata on the growth of test fungi

			LEAF				
Fungus	Cont	rol Ion	Concentration of extract				
-	1	2	100 mg/L	200 mg/L	400 mg/L	900 mg/L	
C. fulcatum NCBT 146	****		++	++	+		
C. lunata MICC 2030	9999	÷	**	++	÷	7	
Forysporum NCBT 156	****	÷	**	*	×	*	
M. canis MICC 2820	****	÷	**	*	*	+	
R solani NCBT 194	****	*	**	.*	*	*	
and find a state of the	and a				t sie aa	A 101 AR 814	14.14

Control-3: Medium without leaf extract; Control-2: Medium with Bavistin (100 mg/L). ++++: Normal growth: +++: 25% growth inhibition; ++: 50% growth inhibition; +: 75% growth inhibition; -: Total (100%) growth inhibition.

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S.No 715 [GC MS study]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
ĺ.	4.96	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	24.41
2.	5.55-	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	9.45
3.	7.63	Oleic acid, 3-(octodecyloxy)propyl ester	C39H76O3	592	3.94
4.	11.61	Ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436	22.05
5.	14.96	Rhodopin	C40H58O	554	8.66
6.	24.67	Lycopene	C40H56	536	7.09
7.	32.32	Sligmasterol	C29H48O	412	7.87
8.	34.29	Tetrahydrospirilloxanthin	C42H64O2	600	16.54

Note: *Parameters tested are not covered under the scope of NABE accreditation

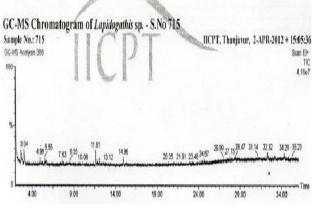


Fig. 3. GC MS Analysis of L.cristata Leaf shows the presence of phytochemical constitutent.

cure black patches on face (Dinesh *et al.*, 2013). Aqueous extract of the leaves mixed with *Ocimum* juice in 10:1 ratio was used to cure fever (Rabe and Van Staden, 1997) and the leaf paste with coconut oil was applied externally on old wounds (Purma Aravinda Reddy and Venkateshwar Rao, 2013). Root extract of *L. cristata* was significantly effective in antiemetic (Rachapalli Sowjanya Kumar Reddy *et al.*, 2014) and anti-inflammatory activities (VijayaNarasimha Reddy Peddireddy *et al.*, 2014). Bioactive compounds oleic acid, 3-(octadecyloxy) propyl ester from inflorescence (Abubacker and Kamala Devi, 2014), Heptadecane,

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Table. 4. Inhibitory effect of the root extract of L.cristata	on the growth of test fungi
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ROOT								
Fungus	Contr	ol	Concentration of extract					
	1	2	100 mg/L	200 mg/L	400 mg/L	800 mg/L		
C. fulcatum NCBT 146	++++	-	++	++	+	-		
C. hunata MTCC 2030	++++	+	++	++	-			
F.oxysporum NCBT 156	++++	+	++	+				
M. canis MTCC 2820	++++	+	++	+	+	222		
R. solani NCBT 194	++++	-	++	+	8	-		

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.

Table. 5 GC MS Analysis of L. cristata Root

S.	No	71	3
GC	MS	st	udy]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	4.96	Heptadecane, 9-hexyl-	C ₂₃ H ₄₈	324	34.18
2.	5.54	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	25.32
3.	7.63	Oleic acid, 3-(octadecyloxy)propyl ester	C39H76O3	592	17.72
4.	8.23	Tetracycline	C22H24N2O8	444	18.99
5.	10.10	Docosanoic acid, 1.2,3-propanetriyl ester	C69H134O6	1058	3.80

Note: *Parameters tested are not covered under the scope of NABL accreditation

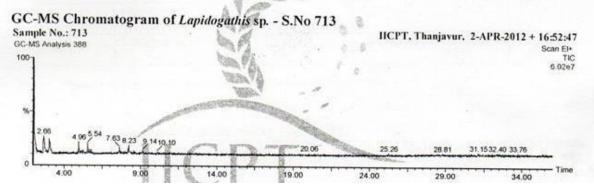


Fig. 4. GC MS Analysis of L. cristata root shows the presence of phytochemical constituents

9- hexyl, Ethyl iso-allocholate from leaf (Abubacker and Kamala Devi, 2015) and Heptadecane, 9- hexyl, Octadecane, 3-ethyl-5-(2-ethylbutyl)from root (Abubacker and Kamala Devi, 2015) were analyzed from L.cristata by using GCMS procedure, and these compounds are 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.

CONCLUSION

On the basis of the perusal of literature and experimental results, it can be concluded that

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www.stetjournals.com Scientific Transactions in Environment and Technovation Lepidagathis cristata is a traditional remedy for fever, eczema, psoriasis, epilepsy, skin abscess, burns, mouth ulcer, snake bites, wounds, skin itching and other skin diseases. The various bioactive compounds present in this herb are highly responsible for its antifungal activities against both plant and human pathogens. It has also various pharmacological activities like analgesic, antimicrobial, antiemetic, antiinflammatory and hypoglycaemia, and immunes up presents. Taking great concern of the useful benefits on the plant, it can be fortified as a safe and highly important medicinal plant for human beings and pet animals.

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