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Bioactivity of root hexane extract of *Coleus forskohlii* Briq. Labiatae : GC/MS/MS Characterization and identification

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ABSTRACT

Coleus forskohlii belongs to the Natural Order Labiatae (Lamiaceae), a family of mints and lavenders. This species is a perennial herb with fleshy, fibrous roots that grows wild in the warm sub-tropical temperate areas in India, Burma and Thailand. In Thailand, tubers of this plant have been used in cooking as one of the ingredients in Thai curry. In India, it is cultivated for use as a condiment. The root hexane extract of C.forskohlii was analyzed using Varian 4000 gas chromatography mass spectrometry (GC-MS-MS). Six major components α-cedrene, β- cadinene, citronellal, two labdane derivatives and ß-citronellol were identified. α - Cedrene constituted 92.4% area followed by a Labdane Derivative which comprised 86.2%. These molecules have been characterized and identified as a rich source of medical and other biological properties.

Keywords: *Coleus forskohlii*, GC–MS, Labdane, Cedrene.

INTRODUCTION

The genus Plectranthus (Lamiaceae) consists of some 350 species of perennial plants largely occurred in tropical Africa, Asia, Australia, the East Indies, the Malay Archipelago and the Philippines. Several species are grown as ornamental plants, leaf vegetables, root vegetables for their edible tubers [1,2], it is also of economic, medicinal and other biological interest [3]. *Coleus forskohlii*, has a rich diversity of ethnobotanical applications and is used as a condiment for heart ailments and stomach cramps [4,5]. It has been reported for its effect on intra-ocular pressure [6-8] and hypotensive [9,10]. *C. forskohlii* has also been reported to have antianaphylactic [11], amoebicidal [12], antiplatelet [13,14], gastroprotective [15], bronchodilating [16], anti cancer [17] activity and prevents hair graying [18]. Forskolin is used as a research tool assessing the adenylate cyclase & cyclic AMP in cellular physiology [19]. Identification of individual components of complex mixtures such as terpenes/terpenoids in root hexane extracts requires the use of several techniques. GC-MS-MS is a useful tool in medicine and biological research aiming for the identification of mixtures and this method has already been applied successfully for the analysis of terpenoids, especially mono- and sesquiterpenes, in various extracts. Identification of the biomolecules found in an extracts by comparing their relative retention times/indices and their mass spectra. Therefore the identified chemical constituents are used in folk medicine for a variety of diseases including infectious conditions. Other constituents of *C.forskohlii* like alkaloids (forskolin and its derivatives), phenols and tannins have been reported to exhibit some biological activities like stimulating adenyl cyclase, inhibition of platelet aggregation, mast cell degranulation,

relaxation of the arteries, increasing the insulin secretion and thyroid function, decreasing adipose accumulation, reduction of body weight, treating skin diseases, cardiovascular disease, asthma, stimulating the secretion of digestive enzymes and absorption of nutrients in small intestine etc. [20-22]. The roots hexane extracts of *C. forskohlii* was found to contain 3-decanone, bornyl acetate, sesquiterpene hydrocarbons and sesquiterpene alcohols, ß-sesquiphellandrene and γ-eudesmol which are effective in inhibiting the growth of skin pathogens than the better known tea tree oil [23]. The diterpenoids in Coleus have attracted interest on account of their antibacterial activity [24-26]. Therefore, our study is focused on the chemical composition of root hexane extract of *C.forskohlii* and confirmed their biological activity with reference to antimicrobial and insecticidal properties. The roots were extracted and the obtained extract was investigated by GC-MS-MS for the Identification of the biologically active compounds were made by a typical library search (NIST, WILEY) and literature comparison.

MATERIALS AND METHODS

Plant material:

C.forskohlii plants were collected from in and around Coimbatore District, Tamilnadu.

Method of extraction

The roots of *C.forskohlii* were extracted with hexane and other organic solvents in cold condition. Solvents were removed using rotary evaporator and dried, stored at -20°C for further analysis. The hexane extract which contains the oil fraction was analyzed using GC-MS-MS.

GC-MS-MS analysis:

Analysis was carried out in a Varian 4000 gas chromatograph with Mass spectrophotometer operating in EI mode with VF 5MS (30 m × 0.25 mm i.d., 0.25 m film thickness), coupled to a detector interfaced with the GC/MS/MS Chemstation with Wiley and NIST mass spectral library. Column temperature was programmed from 100°C to 260°C. Injection was performed at 250°C. Helium was used as the carrier gas at a flow rate of 0.5 ml/min. Mass spectra was recorded in the scan mode at 70 eV (35-350 amu). The data obtained was compared with the mass spectral compounds available in the Wiley and NIST library. The set temperature program used is presented in Table 1.

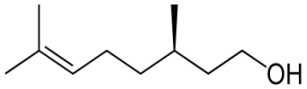
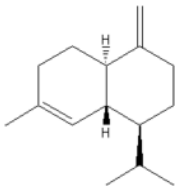
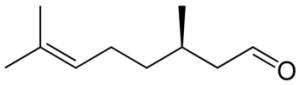
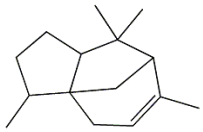
Table 1 GC-MS-MS analytical program for the study of the hexane extract of *C.forskohlii* root

S.No.	Standby Time (min)	Temperature (°C)	Rate Of Increase In Temperature (°C/ min)
1.	0	100	6.0
2.	0	190	6.0
3.	1	235	4.0
4.	10	260	-

Table 2 GC–MS-MS analysis of hexane extract *C. forskohlii* root and identified compounds

S.No	Compound	Chemical Synonym	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)
1.	α- Cedrene	(3R-(3alpha,3abeta,7beta,8aalpha))-2,3,4,7,8,8a hexahydro-3,6,8,8-tetramethyl-1H-3a,7-methanoazulene	$C_{15}H_{24}$	204	15.108	92
2.	γ- Cadinene	(1*S*,4a*R*,8a*R*)-1-isopropyl-7-methyl-4-methylene-1,2,3,4,4a, 5,6,8a-octahydronaphthalene	$C_{15}H_{24}$	204.35	15.507	67
3.	Citronellal	3,7-Dimethyl-6-octenal	$C_{10}H_{18}O$	154	25.255	6
4.	Labdane Derivative	8-α,13-ß- Dihydroxy labd 14-en-3-one	$C_{20}H_{34}O_3$	322	27.580,28.43, 32.897	3.9, 5, 86
5.	Labdane Derivative	8-α, 15-epoxy-14-oxa-16 Norlabdane	$C_{18}H_{32}O_2$	280	30.782	8
6.	ß-citronellol	3,7- dimethyl oct-en-ol	$C_{10}H_{20}O$	156	36.320	5

Table: 3. Biological properties and uses of root hexane extracts of *C.forskohli*

Chemical constituents	Structure	Activity	Plant Source	Uses
α- Cedrene		Antifungal, Antimicrobial	*Helichrysum italicum*, Mastelic *et al*. 2005 *Coleus forskohlii*, Phytochemistry lab, IFGTB. 2007.	Used for aromatic preparations and Insecticidal preparations.
γ- Cadinene		Antifungal, Antimicrobial	*Cryptomeria japonica*, Sen *et al.2004* *Coleus forskohlii*, Phytochemistry lab, IFGTB. 2007.	Used in fungicidal preparations.
Citronellal		Antimicrobial, Insect Repellent	*Cymbopogon citratus*, Betty *et al*.2004 *Coleus forskohlii*, Phytochemistry lab, IFGTB. 2007.	Used for insecticidal preparations.
β- Citronellol		Antimicrobial, Insecticidal	*Cymbopogon citratus*, *Betty et al.2004* *Coleus forskohlii*, Phytochemistry lab, IFGTB. 2007.	Used in the antimicrobial and pesticidal formulations.

RESULTS AND DISCUSSION

GC-MS-MS analysis:

The root hexane extracts of *C. forskohlii* was analyzed using Varian 4000 GC-MS with Mass spectrophotometer operating in EI mode with VF 5MS column and the chromatogram was recorded and depicted in the Fig.1 and chromatogram was interpreted with available library in the GC-MS-MS. The derivatives of root hexane extracts of *C. forskohlii* were tabulated with chemical profile of the extracts, the percentage content, and retention indices of the constituents are summarized in Table 2. A total of six components were characterized on the basis of a typical library search and literature data from *C.forskohlii*. The main constituents of the root hexane extracts of *C.forskohlii* were found to be α-Cedrene (92.4%) a tricyclic sesquiterpenes, γ-Cadinene (67.6%) a bicyclic sesquiterpenes, Citronellal (6.25%) a monoterpenoid, Labdane Derivative (86.2%) and (8.35%) bicyclic diterpenes, ß-citronellol (5.49%) a monoterpene alcohol. The bioefficacy of these molecules were tested for antifungal, antibacterial as well as insect repellent in the bioassay studies conducted at Phytochemistry Laboratory, Division of Bioprospecting, Institute of Forest Genetics and Tree Breeding, Coimbatore in Table.3.

A supercritical extraction process with carbon dioxide was employed to the root powder of *C.forskohlii* and the extract obtained was analyzed using GC-MS-MS (Fig.1). The principle constituents identified were bornyl acetate (15.0%), 3-decanone (7.0%), an azulene derivative (sesquiterpene) (7.5%), alpha-pinene (2.0%) and beta-pinene (1.5%) [27]. From the aforementioned results it is learnt that the root extract of *C.forskohlii* yields terpenes as found in the present study. Yuenyongsawad and Tewtrakul, [28] reported that the essential oil from the leaves of *Coleus parvifolius* Benth. (Labiatae) analyzed using GC-MS possessed (*E*)-phytol (42.77%), followed by eicosatrienoate (16.39%), *n*-tetradecanoic acid (14.42%), octoil (6.54%), 2-methyl-7- octadecyne (5.97%), nonadecane (3.25%), germacrene-D (2.19%) and -humulene (1.42%). In a similar study by Giorgio *et al*., [29] the oil composition of *Stachys glutinosa* L. from different areas of Sardinia were analyzed by GC/MS and α-cedrene (19.2%), α-terpineol

(18.5%), terpinen-4-ol (12.6%) and α-terpinyl acetate (8.6%) were found to be the main compounds in the oil in accordance to the present study in which the essential oil of *C.forskohlii* also possessed α-cedrene (92.4%). The essential oil of *C.forskohlii* was mainly found to contain terpene and terpenoid fractions similar to the study reported by Mastelic *et al*., [30] where the essential oil of *Helichrysum italicum* (Roth) G. Don was fractionated into terpene and terpenoid fractions and analyzed using GC/MS, fifty-two compounds were identified and the terpenoid extracted from the bark of *Ziziphus jujuba* was a potential source to control the population of stored pest *Tribolium confusum* [31] similarly *C.forskohlii* possess various terpenoids componenets might be responsible for insecticidal properties. The main hydrocarbons of the oil were α -pinene (10.2%), α-cedrene (9.6%) aromadendrene (4.4%), β-caryophyllene (4.2%), and limonene (3.8%). Plant continue to be a major source of medicine, as they have throughout human history [32] as represented by the presence biologically active components in *C.forskohlii.*

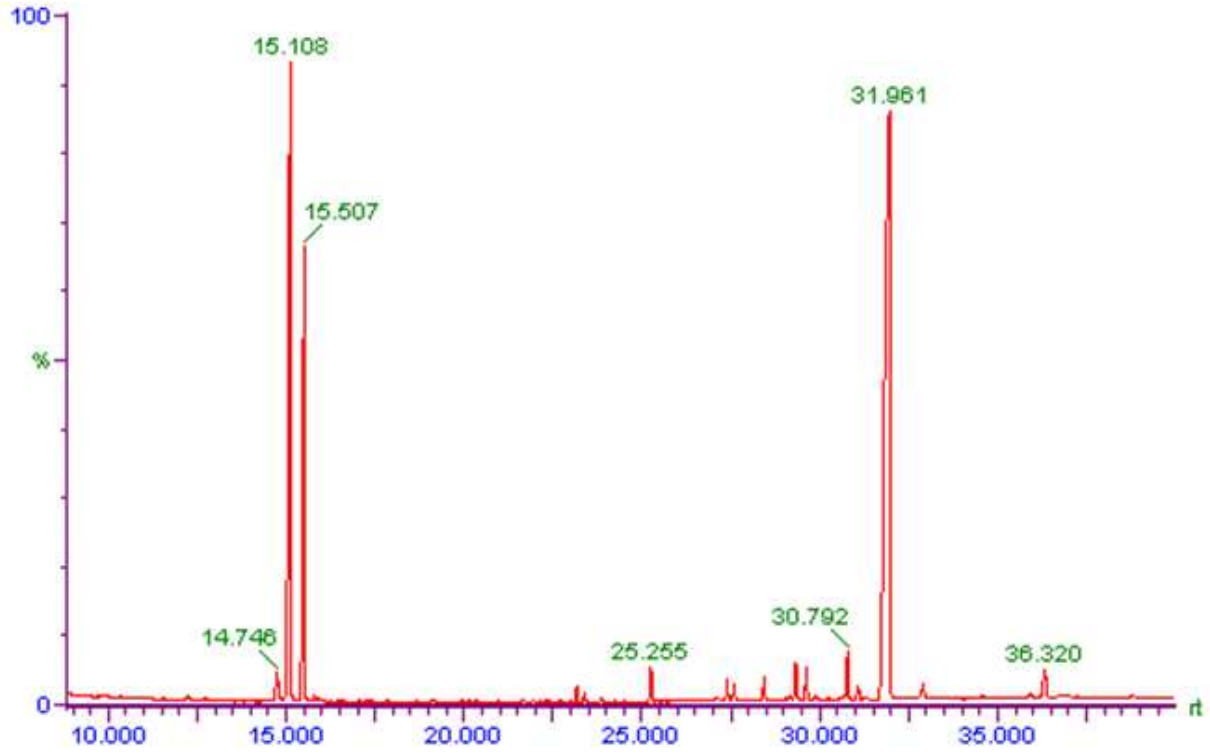


Fig.1 GC-MS-MS analysis of hexane extract of *C.forskohlii* roots

CONCLUSION

C.forskohlii roots showed the presence of bicyclic and tricyclic sesquiterpenes, bicyclic diterpenes, monoterpenoids and monoterpene alcohols. Among all the chemical constituents of *C.forskohlii* root hexane extracts contains six major components and α - Cedrene constituted 92.4% area followed by a Labdane Derivative which comprised 86.2%. In this study, the said constituents were comparatively high in coimbatore region, as mentioned by the earlier researchers in various species, and also it was found to be exhibiting antimicrobial / insecticidal activities in addition to their usefulness for the food, cosmetics and pharmaceutical industry.

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