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Essential oils composition of flower of *Leucas aspera in Tamilnadu*

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Abstract

Leucas aspera (Willd.) Link (Synonym Leucas plukenetii (Roth) Spreng.) is a species of annual branched herb belonging to the Lamiaceae, and distributed throughout in India, Bangladesh, Nepal, Malaysia, and Mauritius. L. aspera has been traditionally medicinally used as an antipyretic, insecticide, and as a remedy for toothache; its flowers are used as an expectorant, stimulant, diaphoretic, antirheumatic, and antipsoriatic, while its leaves are useful in treating snake bites. The present study was aim to identification of essential oils constituent and antimicrobial activity of Leucas aspera flowers. The essential oil was obtained from the flower of *L. aspera* in hydro-distilled method. The isolation of essential oils was analyzed by GC-MS method. Antimicrobial activity of essential oils of *L. aspera* were determine by the disk diffusion methods. The total yield of the essential oils was 2.34%. The results of the present study were forty-eight active compounds identified, representing 96.29% of the total oil. The main constituents were identified as isoamyl propionate (16.67) and isoamyl propionate (11.01%), hexadecane (6.9%). The maximum activity of essential oil of *L. aspera* flower active against *Staphylococcus aureus*. The conclusion of the present study is essential oils obtained from *L.aspera* flowers, which is shows in vitro anti-microbial potential effect may be act as the presence of isoamyl propionate (16.67) and isoamyl propionate (11.01%), hexadecane (6.9%).

Key words: Essential oils; flower; *Leucas aspera* (Willd.) Linn. ; Lamiaceae, antimicrobial activity.

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Original Research Paper

1. INTRODUCTION

Essential oils are mainly composed of aromatic and volatile compounds and can be obtained from different parts of plants, especially the leaves and flowers (Sharifi-Rad,2017). They are derived from plants and plant parts have demonstrated promising activity of antimicrobial potentials, insecticidal, anti-parasitic, antioxidant and cytotoxic properties (Kordali *et al.* 2005; Meshkibaf et al.,2011; Defera et al.,2000).

Leucas aspera (Willd.) Linn. is belonging to the family Lamiaceae. It is an herbaceous species and common weeds. it is distributed throughout India, Ceylon and Philippines. It is a locally tamil name known as Thumbai. It is found throughout India. The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperient, diaphoretic, insecticide, and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis, and other chronic skin eruptions. Bruised leaves are applied locally in conditions of snake bites (Kirtikar and Basu,1975).

The literature review of this plant was preliminary chemical examination of *L. aspera* revealed presence of triterpenoids in entire plant (Kamat and Singh,1994). Whole plant is reported to contain oleanolic acid, ursolic acid and 3-sitosterol (Chaudhury and Ghosh,1969). Aerial parts are reported to contain nicotine (Mangathayaru et al.,2006), sterols (Khaleque et al.,1970) two new alkaloids of α-sitosterol and β-sitosterol, reducing sugars (galactose), glucoside, (Chatterjee and Majumdar et al.,1969), diterpenes (leucasperones A and B, leucasperols A and B, isopimarane glycosides (leucasperosides A, B and C), together with other compounds like asperphenamate, maslinic acid, (-)-isololiolide, linifolioside, (Sadhu et al.,2006), nectandrin B, meso-dihydroguaiaretic acid, macelignan, acacetin, apigenin 7-O-[6'-O-(p-coumaroyl)-3-D-glucoside], chrysoeriol, apigenin, erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)propan-1-ol, myristargenol B, and machilin C, (-)-chicanine, (7R,8R)- and licarin A. (Sadhu et al.,2003). Among the 25 compounds identified from the leaf volatiles, μ-farnesene, α-thujene and menthol were the major constituents. The flower is reported to contain 10 compounds; among them amyl propionate (15.2%) and isoamyl propionate (14.4%) were dominant (Kalachaveedu et al.,2005). Seed is reported to contain palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (0.65%). The unsaponifiable fraction contained 3-sitosterol and ceryl alcohol (Jam et al.,1968; Badami and Patil ,1975). Shoot contained novel phenolic compounds (4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol) (Misra et al., 1975), aliphatic ketols (28-hydroxypentatriacontan-7-one, 7-hydroxydotriacontan-2-one),[Misra et al.,1992] long-chain compounds (1-hydroxytetratriacontan-4-one, 32-methyltetratriacontan-8-ol),(Misra et al.,1992), nonatriacontane,(Misra et al.,1995), 5-acetoxytriacontane, β-sitosterol (Misra et al.,1992) and dotriacontanol (Misra et al.,1992), Leucolactone (I), isolated from the root of *L. aspera* have been characterized as 3,3,16c-dihydroxyoleanan-28-1,3-olide (Pradhan et al.,1990). The essential oils from *L. aspera* possessed bacterial activity was reported in the earlier (Rao and Narasimha,1971). The methanol extract of *L. aspera* flowers, its fractions, the alkaloidal residue and the expressed flower juice showed good antibacterial activity for methanol extract and methanol fraction with maximum activity for the alkaloidal residue (Mangathayaru et al.,2005).

2. MATERIAL AND METHODS

2.1 Collection of Plants

The plant materials of *Leucas aspera* were collected from the Rajagopalapuram pond, Palayamkottai, Tamilnadu. The plant material was identified by the help of regional flora.

2.2 Extraction of Essential Oil

Fresh flowers of *Leucas aspera* were collected and washed thoroughly under running water. 500 gms of the flowers of *Leucas aspera* was isolated from the essential oils by hydro distillation method. The essential oil was dried by anhydrous sodium sulfate and after filtration was stored in dark bottles at 4 °C until use for further studies. Essential oil yield was calculated based on weight percent (w/w). This process was repeated three times for the oil from each plant part.

2.3 GC-MS Analysis and Identification of compounds

The essential oils hydro distillation from the flower of *Leucas aspera* were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS condition were: EI (Electron Impact) ionization, injection temperature 280°C, detector temperature 280°C, Rtx-MS column (30m x 0.32 mm x 0.25 μm), temperature of column 70°C–280°C, carrier gas was used as helium. The active constituents of essential

oils were identified by comparing the mass spectra with those recorded in the National Institute of Standards and Technology (NIST) library and individual components were identified by comparison of both mass spectra and GC retention data with those of authentic compounds previously analysed and stored in the data system. The linear retention indices were also calculated for all volatile constituents using a homologous series of n-alkanes (Farag et al.,1989; Daferera et al.,2000 Adams, 1995).

2.4 Antimicrobial activity assay

The bacterial strains used in this study were: *Escherchia coli*, Salmonella typhi, *Staphyllococcus aureus*, and *Candida albicans*. Bacterial strains were cultured overnight at 37°C in Nutrient agar. Yeast was cultured at 30°C overnight in yeast malt agar. Antimicrobial activities of the essential oil against some bacteria and yeast strains were determined by the paper disc assay and broth dilution method. The disc diffusion method was employed to determine the antimicrobial activities of the essential oils. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells per mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 100µ L of the oil and placed on the inoculated plates. These plates were incubated at 37°C for 24 h for bacteria and at 30°C for yeast. The diameters of the inhibition zones were measured in millimeters (mm). All tests were performed in triplicates.

3. RESULTS AND DISCUSSION

3.1 Botanical Description

Leucas aspera is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 7.9 cm long and 1.24cm broad, with entire or crenate margin; petiole 2.2-5.7 mm long; flowers white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle-tipped, ciliate with long slender hairs; calyx variable, tubular, 7-14 mm long; tube curved, contracted above the nutlets, the lower half usually glabrous and membranous, the upper half ribbed and hispid; mouth small, very oblique, not villous, the upper part produced forward; teeth small, triangular, bristle-tipped, ciliate, the upper tooth being the largest. Corolla 1.3 cm long; tube 5.4 mm long and pubescent above, annulate in the middle; upper lip 3.6 mm long, densely white-woolly; lower lip about twice as long, the middle lobe obviate, rounded, the lateral lobes small, subacute. Fruit nutlets, 2.7 mm long, oblong, brown, smooth, inner face angular and outer face rounded (Kirtikar and Basu,1975; Hooker and London,1984).

3.2 Identification of Essential Oil

The total yield of the essential oils was 2.34%. Forty-eight compounds were characterized and identified according to their mass spectra and their relative retention indices determined on a non-polar stationary phase capillary column, comprising 96.29% of the total oil constituents. The main constituents were identified as isoamyl propionate (16.67) and isoamyl propionate (11.01%), hexadecane (6.9%). Earlier studies, GC and GC/MS analysis of the leaf and flower oils of *Leucas aspera* showed the presence of a total of 30 compounds (Mangathayaru et al.,2006).

3.3 Antimicrobial activity

The results of the antimicrobial activity showed that the essential oil of *L. aspera* exhibited antimicrobial activities against the tested bacteria (Table-2). The observed results were maximum activity of essential oil of *L. aspera* flower active against *Staphylococcus aureus*. Previously, the methanolic extract of *L. aspera* flowers, its fractions, the alkaloidal residue and the expressed flower juice showed good antibacterial activity and methanol fraction with maximum activity for the alkaloidal residue (Mangathayaru et al.,2005). Similar results have been reported on other Gram-positive and -negative bacteria using the disc diffusion method (Fisher and Phillips, 2006; Fisher *et al.,* 2007). Plant essential oils and their components are known to exhibit antimicrobial activities (Cavaleiro *et al.,* 2006; Cha *et al.,* 2007; Craske *et al.,* 2005; Flamini *et al.,* 1999; Jo *et al.,* 2004). The high percentages of a-pinene, β-pinene, and linalool in the essential oil are known to be associated with the antibacterial activity (Couladis *et al.,* 2003; Fisher and Philips, 2006; Ulubelen *et al.,* 1994). Earlier report, essential oil composition of *L. aspera* collected from the different localities of India (Mangathayaru et al.,2006; Gerige et al., 2007). The conclusion of the present study is Leucas aspera contain rich essential oil which is very importance in pharmaceuticals industries, food, cosmetics etc. This work was suggested that possible use of the essential oils of *L. aspera* in the food, cosmetics, and pharmaceutical industries as natural components for use as antimicrobial agents.

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Table 1. Chemical Composition of the Essential Oil of L. aspera flowers

Sl.No.	Essential Oils	RI	Percentage	Identification Methods
1.	α-Thujene	9235	0.23	RI, GC-MS
2.	α-Pinene	9291	1.45	RI, GC-MS
3.	Camphene	9298	0.34	RI, GC-MS
4.	1-Octen-3-ol	1023	2.67	RI, GC-MS
5.	β-Pinene	1031	1.56	RI, GC-MS
6.	3-Octanol	1089	2.10	RI, GC-MS
7.	α-Phellandrene	1098	0.67	RI, GC-MS
8.	o-Cymene	1101	1.89	RI, GC-MS
9.	Limonene	1129	4.21	RI, GC-MS
10.	(Z)-β-Ocimene	1178	1.23	RI, GC-MS
11.	(E)-β-Ocimene	1199	2.10	RI, GC-MS
12.	γ-Terpinene	1201	2.34	RI, GC-MS
13.	Terpinolene	1209	3.10	RI, GC-MS
14.	Terpin-4-ol	1211	0.12	RI, GC-MS
15.	α-Terpineol	1234	0.34	RI, GC-MS
16.	Methylsalicylate	1256	0.89	RI, GC-MS
17.	Caprinaldehyde	1267	1.45	RI, GC-MS
18.	Pulegone	1298	0.10	RI, GC-MS
19.	Bornylacetate	1319	1.01	RI, GC-MS
20.	n-Tridecane	1321	0.30	RI, GC-MS
21.	Eugenol	1345	1.43	RI, GC-MS
22.	(Z)-Jasmone	1389	1.34	RI, GC-MS
23.	Methyleugenol	1391	4.67	RI, GC-MS
24.	β-Bourbonene	1398	1.90	RI, GC-MS
25.	β-Elemene	1403	1.11	RI, GC-MS
26.	1,7-di-epi-α-Cedrene	1409	0.61	RI, GC-MS
27.	β-Caryophyllene	1411	1.15	RI, GC-MS
28.	β-Gurjunene	1423	0.34	RI, GC-MS
29.	α-trans-Bergamotene	1445	1.32	RI, GC-MS
30.	Unkown	1451	0.29	RI, GC-MS
31.	dodecane	1461	1.61	RI, GC-MS
32.	α-Humulene	1478	1.34	RI, GC-MS
33.	(E)-β-Farnesene	1489	3.10	RI, GC-MS
34.	β-Chamigrene	1491	1.03	RI, GC-MS
35.	β-Selinene	1497	0.01	RI, GC-MS
36.	α-Selinene	1501	1.20	RI, GC-MS
37.	trans-α-Guaiene	1509	0.11	RI, GC-MS
38.	Caryophylleneoxide	1511	1.09	RI, GC-MS
39.	Selin-11-en-4-α-ol	1534	3.19	RI, GC-MS
40.	isoamyl propionate	1545	16.67	RI, GC-MS
41.	Unknown	1551	0.67	RI, GC-MS
42.	Khusinol	1567	3.41	RI, GC-MS
43.	α-Bisabolol	1571	1.48	RI, GC-MS
44.	amyl propionate	1606	11.01	RI, GC-MS
45.	epi-α-Bisabolol	1687	0.23	RI, GC-MS
46.	Benzylbenzoate	1699	0.12	RI, GC-MS
47.	hexadecane	1671	6.09	RI, GC-MS
48.	Bicyclovetivenol	1690	1.67	RI, GC-MS
	Total		96.29	

Table 2. Antimicrobial activity of the *L. aspera* flower essential oils as determined by the diffusion method

Sl.No	Tested organism	Zone of Inhibition	Results
1	*Escherchia coli*	14	Moderately
2	Salmonella typhi	16	Good
3	*Staphyllococcus aureus*	19	Good
4	*Candida albicans*	16	Good

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