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SHORT COMMUNICATION

**Chemical investigation of the essential oil of *Laggera crispata*
(Vahl) Hepper & Wood from India**

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Abstract: Hydrodistilled essential oil of the aerial parts of *Laggera crispata* (Vahl) Hepper & Wood, collected from the Kumaon region of the western Himalayas was analysed by gas chromatography and gas chromatography–mass spectrometry. Eighty constituents, accounting for 83.9 % of the total oil composition, were identified. The oil was mainly dominated by sesquiterpenoids (45.3 %) and benzenoid compounds (33.9 %). Among them, 2,5-dimethoxy-*p*-cymene (32.2 %), 10-*epi*- γ -eudesmol (14.7 %), β -caryophyllene (6.9 %) and caryophyllene oxide (5.4 %) were major components of the oil.

Keywords: *Laggera crispata*; Asteraceae; essential oil; GC–MS; 2,5-dimethoxy-*p*-cymene; 10-*epi*- γ -eudesmol.

INTRODUCTION

The genus *Laggera* Sch.-Bip. ex Koch, belonging to the Asteraceae family, is represented by over 10 species prevalent in the tropical Asia and African continents. In India, it is represented by 3 species commonly found in tropical regions in both plains and altitudes up to 1500 m. The three species representing the genus are *Laggera alata*, *Laggera aurita* and *Laggera crispata*, of which *L. aurita* is characterized with stems not having wings while the other 2 species possess winged stems. In *L. alata*, the wings are broad, entire and continuous, while in *L. crispata*, they are narrow, toothed and interrupted. However, all three species are aromatic in nature.¹

Laggera crispata (Vahl) Hepper & Wood (Syn. *Laggera pterodonta* (DC.) Sch.-Bip. ex Oliver) is an annual, erect, highly branched, strongly aromatic and

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viscid pubescent plant. The leaves paste of *L. crispata* is used in the treatment of inflammation and swelling in north-east India and it is also said to possess ant-helminthic properties.² In traditional Chinese medicine, the aerial parts of this plant have been used as an anti-inflammatory, antibacterial and anti-leukaemia agent.³ Recently, great attention has been paid to *Laggera* species because of their diverse chemical components and biological activities.⁴ The plants have been noted to possess antiviral activity against Herpes simplex type I and II and anti-tuberculosis activity.^{5,6}

Many phytochemical investigations have been performed on the non-volatile and volatile constituents of different species of *Laggera* from various countries.^{7–17} In India, *L. aurita* has also been studied on a few occasions.^{18–20} However, a literature survey revealed that there are no reports available on the phytochemical aspects of *L. crispata* from India. Therefore, in present research, the essential oil derived from the aerial part of *L. crispata* was investigated by GC and GC–MS.

EXPERIMENTAL

Plant material

Fresh plant material (aerial parts) of *L. crispata* were collected at the vegetative stage from an experimental field of the Central Institute of Medicinal and Aromatic Plants, Research Centre, Purara, Uttarakhand in August, 2009. The plant material was authenticated at the Botany Department of the Centre (voucher specimens No. Cimpant-335). The site is located at an altitude of 1250 m in the Kattyur Valley, western Himalayas. Climatologically, it is categorized as a temperate zone. The monsoon usually breaks in June and continues to September.

Extraction of the essential oil

The essential oil was extracted from fresh aerial parts of *L. crispata* by hydrodistillation, for 3 h using a Clevenger apparatus.²¹ The percentage essential oil content (% v/w) was estimated on a fresh weight basis. The obtained oil sample was dehydrated over anhydrous sodium sulphate and kept in a cool and dark place before analyses.

Gas chromatography (GC)

The GC analyses of the oil samples were realised on a Perkin Elmer Auto XL GC and a Nucon gas chromatograph model 5765 equipped with a FID using two different stationary phases, *i.e.*, DB-5 (30 m×0.32 mm; 0.25 µm film coating) and CP-Wax 52 CB (30 m×0.32 mm×0.25 µm film thickness) fused silica columns, respectively. Hydrogen was used as the carrier gas at 1.0 ml min⁻¹. The oven temperature was programmed from 70–250 °C at 3 °C min⁻¹ for the DB-5 column and from 70–230 °C at 4 °C min⁻¹ for the CP-Wax 52 CB column. The injector and detector temperatures were 210 and 230 °C, respectively. The injection volume was 0.02 µl neat (syringe: Hamilton 1.0 µl capacity, Alltech, USA) and the split ratio was 1: 30.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis of the essential oil sample was performed on a Perkin Elmer Auto-System XL GC interfaced with a turbomass quadrupole mass spectrometer fitted with an Equity-5 fused silica capillary column (60 m×0.32 mm i.d., film thickness 0.25 µm). The oven temperature was programmed from 60–210 °C at 3 °C min⁻¹ using helium as the carrier gas at

1.0 mL min⁻¹. The injector temperature was 210 °C, injection volume 0.1 µl prepared in *n*-hexane (dilution 10 %), split ratio 1: 40. The MS were taken at 70 eV with a mass scan range of 40–450 amu and scan rate 1.0 s with an interscan delay of 0.5 s.

Identification of the components

The constituents were identified based on their Retention Index (*RI*, determined with reference to a homologous series of *n*-alkanes, C₉–C₂₄, run under identical experimental conditions), co-injection with standards (Aldrich and Fluka) or known essential oil constituents, an MS Library search (NIST/EPA/NIH version 2.1 and Wiley registry of MS data 7th edition) and by comparing with MS literature data.^{22,23} The relative amounts of the individual components were calculated based on the GC peak area (FID response) without using correction factors.

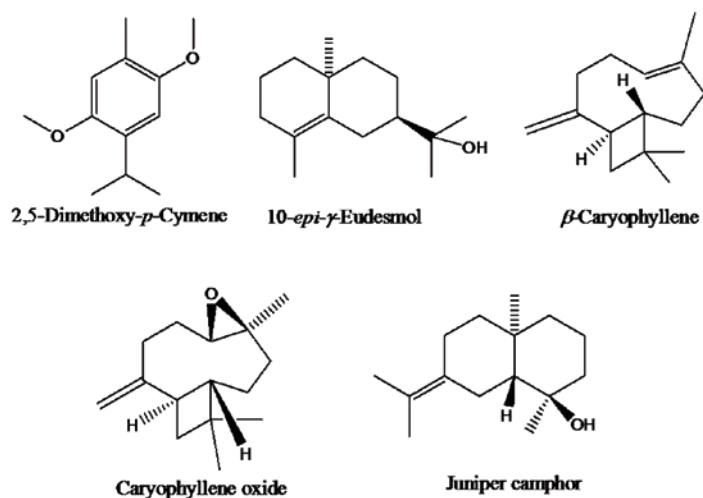
RESULTS AND DISCUSSION

The fresh plant material of *L. crispata* collected at vegetative stage yielded 0.11 % (v/w) essential oil. GC–FID and GC–MS analyses of the oil enabled the identification of eighty components, representing 83.9 % of the total oil content. The components with their relative percentage are summarized in Table I–S, given in the Supplementary material to this paper. The oil was mainly composed of benzenoid compounds (33.9 %), oxygenated sesquiterpenes (33.9 %), and sesquiterpene hydrocarbons (11.4 %). Monoterpene hydrocarbons and their oxygenated counterparts were detected in small amounts (2.2 and 2.5 %, respectively) in this oil. The benzenoid class of components was mainly represented by 2,5-dimethoxy-*p*-cymene (32.2 %), which was the major component of the oil. The oxygenated sesquiterpenes detected in significant amounts were 10-*epi*- γ -eudesmol (14.7 %), and caryophyllene oxide (5.4 %). Moreover, the major sesquiterpene hydrocarbons of the oil were β -caryophyllene (6.9 %), α -humulene (2.0 %) and bicyclogermacrene (1.8 %). Furthermore, among the oxygenated monoterpenes, only terpinen-4-ol was detected above 1 %. The structures of major constituents of the essential oil are given in Fig. 1.

According to literature surveys, 2,5-dimethoxy-*p*-cymene and 10-*epi*- γ -eudesmol have been previously detected in different species of the genus *Laggera* (Table I). The essential oil of *L. alata* grown in Nigeria¹² and Cameroon,¹⁴ *L. aurita* grown in India;¹⁸ *L. gracilis* grown in Cameroon;¹⁴ and *L. pterodonta* grown in Cameroon¹³ and West Africa¹⁶ were all dominated by thymol dimethyl ether and eudesmol isomers. However, *L. alata* grown on the Comoros Islands was dominated by sesquiterpene hydrocarbons;¹⁷ *L. oloptera* grown in Cameroon was found to possess mainly sesquiterpene and monoterpene hydrocarbons,¹⁴ while *L. tomentosa* grown in Ethiopia contained the oxygenated monoterpene, chrysanthenone.¹⁵

TABLE I. Major components in the essential oil of the genus *Laggera* growing in different countries

Species	Plant part	Major compounds	Country
<i>Laggera alata</i>	Leaf	Thymoquinol dimethyl ether (11.17–29.17 %), α -eudesmol (7.68–12.55 %)	Nigeria ¹²
<i>L. alata</i>	Leaf	Dimethoxy- <i>p</i> -cymene (34.1 %), γ -eudesmol (21.4 %)	Cameroon ¹⁴
<i>L. alata</i>	Leaf	β -Caryophyllene (30.5 %), α -muurolene (21.1 %)	Comoros Islands ¹⁷
<i>L. aurita</i>	Whole plant	2,3-Dimethoxy- <i>p</i> -cymene (? %), laggerol (? %)	India ¹⁸
<i>L. gracilis</i>	Leaf	Dimethoxy- <i>p</i> -cymene (33.4 %), γ -eudesmol (10.7 %)	Cameroon ¹⁴
<i>L. oloptera</i>	Leaf	β -Caryophyllene (15.2–20.4 %), sabinene (2.9–28.9 %), germacrene D (10.3–17.9 %)	Cameroon ¹⁴
<i>L. pterodonta</i>	Leaf	2,5-Dimethoxy- <i>p</i> -cymene (28.2 %), γ -eudesmol (26.2 %)	Cameroon ¹³
<i>L. pterodonta</i>	Leaf	2,5-Dimethoxy- <i>p</i> -cymene (30.5 %), 10- <i>epi</i> - γ -eudesmol (24.6 %)	West Africa ¹⁶
<i>L. tomentosa</i>	Leaf and inflorescence	Chrysanthenone (57.5 %), isochrysanthenone (6.8 %)	Ethiopia ¹⁵

Fig. 1. Chemical structures of marker constituents of the essential oil of *L. crispata*.

CONCLUSIONS

The *L. crispata* collected from the Kumaon region of the Himalayas was rich in thymol derivatives. The thymol derivatives are thought to be responsible for medicinal uses of *Laggera* species. Modern research also showed that phenolic compounds are known for a number of biological activities. Therefore, in light of

the presence of interesting molecules in the essential oil of the *L. crispata*, it can be said that this plant could potentially be used in various food and pharmaceutical preparations.

SUPPLEMENTARY MATERIAL

The components of essential oil with their relative percentage are available electronically at <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

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ИЗВОД

ИСПИТИВАЊЕ ХЕМИЈСКОГ САСТАВА ЕТАРСКОГ УЉА БИЉКЕ *Laggena crispata* (VAHL) HEPPER & WOOD ИЗ ИНДИЈЕ

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Етарско уље изоловано дестилацијом воденом паром из надземних делова биљке *Laggena crispata* (Vahl) Hepper & Wood, сакупљене у западним Хималајима, анализирано је методама гасне хроматографије и гасне хроматографије–масене спектрометрије. Идентификовано је осамдесет састојака, који су чинили 83,9 % уља. Доминантни су сесквитерпеноиди (45,3 %) и бензеноидна једињења (33,9 %). Главни састојци уља су 2,5-диметокси-*p*-цимен (32,2 %), 10-*epi*- γ -еудезмол (14,7 %), β -кариофилен (6,9 %) и кариофилен-оксид (5,4 %).

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