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Short Communication

Chemical composition of essential oil from *Orthosiphon diffuses* Benth.

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In this study, the chemical composition of the essential oil of *Orthosiphon diffusus* was investigated. Extraction by hydrodistillation followed by gas chromatography and mass spectrometry (GC-MS) yielded 25 compounds representing 95.3% of the oil. The major volatile components of the oil were n-eicosane (19.5%), t-caryophyllene (18.6%), octocosane (12.2%), limonene (11.6%), β-ocimene (4.2), methyl palmitate (2.8%) and elemol (2.6%).

Key words: *Orthosiphon diffusus,* essential oil, n-eicosane, t-caryophyllene.

INTRODUCTION

Orthosiphon diffusus (Lamiaceae) is a small, perennial, aromatic herb (Singh et al., 1996). The genus *Orthosiphon* Benth, in tribe Ocimeae, comprises 40 species and was recorded from the Old World: in tropical and Southern Africa, Madagascar and tropical or subtropical Asia. The species usually occurs in grassland, woodland, or forest margins. Essential oil from members of Lamiaceae has been used for medicinal values (Harley et al, 2004). *O. diffusus* contain orthodiffenes A to D; these four constituents belong to the polychiral furanopyrans which are relatively rare in nature. The *in vitro* cytotoxic activity of these compounds was tested against Jurkat and HL-60 cells (Holla et al., 2011). Various species of this genus are known for their medicinal properties, but the chemical studies on these species are limited. For example, decoction of *Orthosiphon glabratus* is used to cure diarrhoea and piles, while leaves are applied to cuts and wounds (Singh et al., 1996). *Orthosiphon aristatus* possesses diuretic properties and is used in treating urinary lithiasis, oedema, eruptive fever, influenza, rheumatism, hepatitis and jaundice (Prajapati et al., 2003). The phytochemical analysis of essential oil of *O. aristatus* reveals that sesquiterpenes are the main components, including β-elemene, β–caryophyllene and its oxide, β-selenene, cadinene, humulene, and α-guayene (Schut and Zwaving, 1986; Fernandes et al, 2007). It has been demonstrated that the inflorescence of *O. aristatus* contains a benzopirane derivative, the metal-ripariocromeno (Guerin et al., 1989). Essential oils from members of the Lamiaceae have been widely used medicinally, although *O. diffusus* has aromatic properties, and there are no reports on the composition of essential oil from the leaves. As a part of a systematic study on the chemical composition of essential oil of *Orthosiphon* (Schut and Zwaving, 1986; Guerin et al., 1989), we report for the first time the chemical constituents of essential oil from the aerial parts of *O. diffuses.*

MATERIALS AND METHODS

Plant

Leaves of *O. diffusus* were collected from Department Campus, Hemagangothri, Hassan (latitude, longitude), Karnataka, India, during the flowering period in April 2007. The plants were identified and authenticated by comparison with Herbarium specimens.

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Table 1. Chemical composition of the essential oil of *O. diffusus*.

S/N	Name of the compound	Composition (%)	Retention index
1	α-pinene	1.4	928
2	β-myrcene	0.8	948
3	octanol	0.9	993
4	Limonene	11.6	1018
5	β-ocimene	4.2	1029
6	α-terpinolene	0.4	1052
7	Linalool	2.1	1082
8	Allyl acetate	2.4	-
9	t-caryophyllene	18.6	1430
10	β-seline	0.9	1485
11	Calarene	1.6	1417
12	β-bisebolene	0.7	1508
13	Cyclododecene	0.7	-
14	Elemol	2.6	1531
15	Nerolidol	0.8	1561
16	Kauran-18-al	1.9	-
17	Farnesol	1.7	1544
18	Methyl palmitate	2.8	-
19	Benzothiazoldisulfide	1.8	-
20	Hexadecane	0.7	1808
21	Stearaldehyde	0.7	-
22	Methylisostearate	1.8	1927
23	Heptadecane	2.2	-
24	n-eicosane	19.5	1954
25	Octocosane	12.2	1958

Voucher specimen (No. 15/2-5-07 PS) was been deposited in the Department of Bioscience, P.G. Centre, Hassan Karnataka, India.

Isolation of the essential oil

Freshly harvested samples of leaves were hydrodistilled for 6 h using a Clevenger type apparatus to obtain the essential oils. The oils were stored at 0°C in air-tight containers after drying over anhydrous sodium sulphate for GC-MS analysis.

Gas chromatography–mass spectrometry (GC–MS)

GC-MS analysis was carried out in an Agilent gas chromatography N6890 fitted with a HP-5MS fused silica column (5% phenyl methyl polysiloxane 30 m × 0.25 mm, film thickness 0.25 µm), interfaced with an Agilent 5975C VLMSD with triple axis mass detector. Oven temperature program: 60 to 230°C at 5°C min^{-1}. Injector temperature was kept at 280°C. Helium was used as a carrier gas and was adjusted to column velocity flow of 1.0 ml/min. Split ratio was 1:10, whereas split flow was 10 ml/min, and mass range was 50 to 500. One microlitre of sample (dissolved in hexane 100% v/v) was injected into the system. Identification of oil components was achieved based on their retention indices, and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library [NIST database (G1036A, revision D.01.00)/Chem station data system (D.02.00.275, version 2.0d)].

RESULTS AND DISCUSSION

The yield of essential oils from fresh leaves of *O. diffusus was* 0.32%. The qualitative and quantitative composition of the oil, analyzed by GC-MS is presented in Table 1. In total, 25 compounds were identified, representing 94.3% in the oil.

The major compounds were n-eicosane (19.5%), t-caryophyllene (18.6%), octocosane (12.2%), limonene (11.6%), β-ocimene (4.2%), methyl palmitate (2.8%), elemol (2.6%), allyl acetate (2.4%), heptadecane (2.2%), linalool (2.1%) and kauran-18-al (2.1%). The minor compounds were methylisostearate (1.8%), benzothiazoldisulfide (1.8%), farnesol (1.7%), calarene (1.6%), α-pinene (1.4%), octanol (0.9%), β-seline (0.9%), β-myrcene (0.8%), nerolidol (0.8%), β-bisebolene (0.7%), hexadecane 0.7%), stearaldehyde (0.7%) and α-terpinolene (0.4). *n*-Icosane is part of the paraffin group, and is the shortest compound in the paraffin waxes and widely used to form candles and in the petrochemical industry. Caryophyllene is used in perfumery. Trans-caryophyllene was effective in reducing platelet activating factor, while bradykinin and ovoalbumin-induce mouse paw oedema (Schut and Zwaving, 1986). Limonene is active against a wide variety of tumour cell lines and possesses antiviral properties in low concentrations. Limonene is common in cosmetic products. D-limonene is used in food manufacturing and in some medicines, e.g. as a flavouring to mask the bitter taste of alkaloids, and as a fragrant in perfumery (EPA R.E.D 1994). It is also used as botanical insecticide (Singh et al., 1996). Methyl palmitate is a common botanical compound that occurs naturally in many plants.

Methyl palmitate seems safe for vertebrates, as reflected by its wide use in food, pharmaceutical, cosmetic, and industrial applications (Pearson, 2007). Methyl palmitate fed to rats proved to be nontoxic, and intraperitoneal injections in mice produced no significant changes in organ weight or phagocytic function of reticuoendothelial system (Pearson, 2007). Although, the safe and effective amount of methyl palmitate for humans has been reported to be in the range of 0.1 to 10 mg/kg body weight (Usha and Nazarine, 2003), this range is toxic to various phytophagous mites. Usha and Nazarine (2003) also indicate that methyl palmitate had a very mild effect on human skin and fatty acids could be used as a supplementary source of fat for animal feeds. The ocimenes are monoterpenes found within a variety of plants and fruits. β-ocimene (3,7-dimethyl-1,3,6-octatriene) exists in two stereoisomeric forms, *cis* and *trans,* with respect to the central double bond (Karl-Georg et al., 2002).

The ocimenes are often found naturally as mixtures of the various forms. They are used in perfumery, and like the related acyclic terpene, myrcene and ocimenes are unstable in air (Karl-Georg et al., 2002). The monoterpene and sesquiterpene alcohols have antibacterial properties (Deans and Waterman, 1993).

Conclusion

Conclusively, the study of the essential oil of *O. diffusus* showed the presence of t-caryophyllene, octocosane and limonene. Further research can be performed to determine methods of effectively administering extracts of *O. diffusus* for use medicinally.

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