Digest Journal of Nanomaterials and Biostructures Vol. 4, No. 4, December 2009, p. 819 – 822

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM LEAVES AND FLOWERING AERIAL PARTS OF *HAPLOPHYLLUM ROBUSTUM* BGE. (*RUTACEAE*)

M. RAHIMI-NASRABADI[a,b*], M. B. GHOLIVAND, H. BATOOLI[c]
[a]*Department of Chemistry, Imam Hossein University, Tehran, Iran*
[b]*Department of Chemistry, Razi University, Kermanshah, Iran*
[c]*Isfahan Research Center of Natural Sources and Agriculture, Kashan Station, Kashan, Iran*

The chemical composition of the hydrodistilled essential oil of the air-dried aerial parts of *Haplophyllum robustum* growing wild in Iran was obtained by hydrodistillation and was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The species is poor in essential oil (yield = 0.5%). Thirty constituents representing 99.23% of total oil have been identified. The main constituents of the oil were found to be 1,8-cineole (38.1%), Myrcene (10.69 %), α-Pinene (8.46%), 4-Terpineol (6.96%) and Sabinene (6.15%). Other representative compounds were identified as Methyl geranat (4.69%), γ-terpinen (4.3%) and α-terpinene (3.43%).

(Received August 9, 2009; accepted October 8, 2009)

Keywords: Essential oil, Haplofillum Robustum, Chemical composition

1. Introduction

The genus *Haplophyllum* A. Juss. which belongs to the *Rutaceae* family, is found in central and eastern areas of Asia. This genus consists of 22 species widespread in the Asia to N. Africa. Among the 18 species present in Iran, 9 species is endemic. The *Haplophyllum robustum* Bge., is distributed in regions of central to south-eastern of Iran. The Persian name of this plant is "Sodaby" (Mozaffarian, 1996).

The formation of essential oil in the plant, and consequently the yield and composition of the oil produced, depends on many factors. Genetic differences in plants of the same species that are otherwise indistinguishable (chemotypes) can result in widely different essential oil content. Geographic location and agricultural factors also influence oil production. This implies the possibility of different medicinal uses of a plant species grown in different regions. In the literature, there are many studies about the chemical composition and various activities of essential oil of haplophyllum (Al-Burtamani et al., 2005; Mohsen et al., 1989; Bessonova et al., 1990; Yuldashev et al., 2001) but to the best of our knowledge there are a little study on the essential oil composition of *Haplophyllum robustum* (Masoudi et al., 2004; Bamonieri et al., 2006). In this study, the chemical constituents of the essential oil of Arial part of *Haplophyllum robustum* were studied and its components were compared with previous reports.

*Corresponding author: rahimi1356@gmail.com

2. Experimental (materials and methods)

2.1. Plant material

The aerial parts of wild-growing *Haplophyllum robustum* were collected during the flaworing period in May 2008 from the sandy dunes of Aran and Bidgol deserts (Isfahan province, Iran). The aerial parts (leaves and flowers/inflorescences) were dried in the shade (at room temperature). A voucher specimen of the plant was deposited at the Herbarium of Kashan botanical garden.

2.2. Extraction of the essential oil

The essential oils were extracted by hydrodistillation of dried plant material for 8 h (50 g of sample in 500 mL of distilled water) using a Clevenger-type apparatus as recommended by British Pharmacopeia (Brithish Pharmacopeia). The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4-5°C prior to analysis. Yield based on dry weight of the sample was calculated.

2.4. Analysis of the essential oils

The analytical GC was carried out on Varian (Walnut Creek, CA, USA) Saturn 3400 GC system equipped with Flame Ionization Detectors (FID) and a DB-5 capillary fused silica column (30 m × 0.25 mm ID, film thickness of 0.25 μm). The oven temperature was held at 40 °C for 1 min then programmed at rate of 3 °C/min to 250 °C and held isothermal for 10 min. The carrier gas was nitrogen at a flow rate of 1.1 mL/min; injector temperature: 260 °C, detector: 280 °C. GC-MS analysis of the essential oils were performed using an HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column (60 m, 0.25mm I.D.; 0.25μm film thickness). Essential oil solution (1 μL) in hexane (HPLC grade) was injected and analyzed with the column held initially at 40 ℃ for 1 min and then increased to 250 ℃ with a 3 ℃/min heating ramp and subsequently kept at 250 ℃ for 20 min. Other operating conditions were as follows: carrier gas, He (99.999%); with a flow rate of 1 ml/min; injector temperature, 250 °C; split ratio, 1:50. Mass spectra were taken at 70 eV. Mass range was from *m*/*z* 20–500 amu. Oil constituents were identified by comparing linear retention indices based on a homologous series of even numbered n-alkanes (C8–C24) (Niles, Illinois, USA) with those of standard compounds and by comparison with literature data and MS data with those of reference compounds (Sigma–Aldrich and Acros Organics) and by MS data obtained from Wiley and NIST libraries (Sandra & Bicchi, 1987; Adams, 2001). Relative percentage amount were calculated from TIC by the computer.

3. Results and discussion

The hydrodistillation of the flowering aerial parts of *Haplophyllum robustum* gave light yellowish oil with yield of 0.5% (w/w). The identified constituents from the aerial parts of *Haplophyllum robustum*, their retention indices and their percentage composition are summarised in Table 1. All the compounds are arranged in order of their elution from the HP5-MS column. A total of 30 compounds have been identified representing around 99.23% of the total oil. 1,8-cineole (38.1%), Myrcene (10.69 %), α-Pinene (8.46%), 4-Terpineol (6.96%) and Sabinene (6.15%) were major constituents in the volatile oil of *Haplophyllum robustum*. Other representative compounds were identified as Methyl geranat (4.69%), γ-terpinen (4.3%) and α-terpinene (3.43%). The oil showed a high content of Oxygenated monoterpenes (50.23%), with 1,8-cineole (38.1%) and 4-Terpineol (6.96%) as the major components. The sesquiterpene fraction was relatively small and representing only 1.26% of the total oil.The oil of *Haplophyllum robustum* consists of 21 monoterpenoids (90.4%) and 3 sesquiterpenoids (1.26%). The essential oil of *Haplophyllum robustum* is rich in monoterpenoids. In 2004, sabinene (30.5%), β-pinene (18.2%) and limonene (12.1%). were identified as main components in the essential oil of the

aerial parts of *Haplophyllum robustum* (Masoudi et al., 2004). Whereas the sample studied by us is different from this sample. According to Masoudi and Rustaiyan (Masoudi et al., 2004), sabinene (30.5%) β-pinene (18.2%) and limonene (12.1%) were among the main components of *Haplophyllum robustum*, whereas they were detected in the present study as sabinene (6.15%) β-pinene (2.82) and limonene was not detected. An earlier report shows the major components of C. copticum fruits essential oil as 1,8-cineole. But there is no any trace of sesquiterpenes in all samples of this work (Bamonieri et al., 2006). These differences might have been derived both from harvest time and local, climatic and seasonal factors or we may hypothesize that this sample belongs to a different chemotype. However, further investigations are needed to elucidate this hypothesis.

Table 1. Essential oil composition of Haplophyllum robustum.

Compound[a]	RI[b]	Area[c] (%)
α-thujene	926	2.23
α-Pinene	936	8.46
Sabinene	969	6.15
β-pinene	976	2.82
Myrcene	982	10.69
α-phellandrene	1001	0.41
α-terpinene	1013	3.43
1,8-cineole	1026	38.1
z-β-ocimene	1037	0.17
γ-terpinen	1052	4.3
Cis sabinene hydrate	1057	1.31
2-nonanone	1070	0.25
Verbenol	1079	0.14
p-mentha-1,4(8)-diene	1082	1.51
Trans-sabinnene hydrate	1087	0.5
δ-Terpineol	1152	0.91
1-Nonanol	1156	0.25
1,8-Menthadiene-4-ol	1163	0.26
4-Terpineol	1167	6.96
α-Terpineol	1176	1.39
Coahuilensol methyl ether	1196	0.97
Borneol formate	1217	0.14
Piperitenone	1245	0.16
Trimethylamine	1273	1.09
Geraniol formate	1281	0.19
Methyl geranat	1301	4.69
1-Undecanol	1356	0.49
β-caryophyllene	1429	0.73
γ-muurolene	1478	0.32
γ-cadinene	1521	0.21
Grouped components		
Monoterpene hydrocarbon		40.17
Oxygenated monoterpene		50.23
Sesquiterpene hydrocarbon		1.26
Oxygenated sesquiterpene		-
Other compound		6.46
total		99.23

[a]Order of elution on HP5-MS.
[b]Retention indices
[c]tr = trace, less than 0.05%.

Amounting to 99.7% of the total oil, the Monoterpene hydrocarbons and Oxygenated monoterpenes had the highest contribution (90.4%), these fractions dominated by 1,8-cineole (38.1%), followed by Myrcene (10.69%) and α-Pinene (8.46%).

References

[1] V. Mozaffarian, A Dictionary of Iranian Plant Names, p. 260, Farhang Moaser, Tehran (1996).
[2] S.K.S. Al-Burtamani, M. O. Fatope, R.G. Marwah, A.K. Onifade, S.H. Al-Saidi, Journal of Ethnopharmacology **96**, 107-112 (2005).
[3] Z.H. Mohsen; H.J. Jaffer; M. Alsaadi; Z.S. Ali, Pharmaceutical Biology **27**, 17 (1989).
[4] I.A. Bessonova, D. Kurbanov and S.Yu. Yunusov, Chemistry of Natural Compounds 26 (1990).
[5] M. P. Yuldashev, Chemistry of Natural Compounds **37**(3), (2001).
[6] S. Masoudi, A. Rustaiyan, PA. Azar, Journal of Essential Oil Research **16**, 548 (2004).
[7] A. Bamonieri, J. Safaei-Ghomi, H. Asadi, H. Batooli, Journal of Essential Oil Research **18**, 379 (2006).
[8] Brithish Pharmacopeia, vol. 2, Appendix XI F, HMSO, London, p. A138 (1988).
[9] P. Sandra, C. Bicchi, Capillary Gas Chromatography in Essential Oil Analysis, Alfred Huethig-Verlag: Heidelberg (1987).
[10] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Corporation, Carol Stream, IL, USA, pp. 63–344 (2001).