Digest Journal of Nanomaterials and Biostructures Vol. 5, No 1, March 2010, p. 169 – 172

STUDY OF THE BIOACTIVE AND FRAGRANT CONSTITUENTS EXTRACTED FROM LEAVES AND AERIAL PARTS OF *Haplophyllum glabrrimum* Bge ex Bioss FROM CENTRAL IRAN BY NANO SCALE INJECTION

A. BAMONIRI [a,*], BI BI FATEMEH MIRJALILI[b], A. MAZOOCHI [a], H. NAEIMI[a], H. GOLCHIN [a], H. BATOOLI[c]
[a]*Essential Oils Research Institute, University of Kashan, 51167 Kashan, I. R. Iran*
[b]*Department of Chemistry, College of Science, Yazd University, Yazd, I. R. Iran*
[c]*Isfahan Research Center of Natural Sources, Kashan Station, Kashan, I. R. Iran*

The dried leaves and aerial parts of *Haplophyllum glabrrimum* which belongs to Rutaceae family and grows in central Iran, were hydrodistilled to produce oils in the yields of 0.08% and 0.14% (w/w), respectively. The oils were analyzed by GC and GC/MS**.** The amount of the samples injected were 1.0 nL (diluted 1.0 μL of sample in 1000 ml of *n*-pentane, v/v). Ten and sixteen bioactive, flavour and fragrance molecules were identified, representing 93.86% and 96.89% of the leaves and aerial parts oil, one by one. The main component was Myrcene (52.89%, 65.08%) in leaves and aerial parts oils, respectively.

(Received February 17, 2010; February 26, 2010)

Keywords: Haplophyllum glabrrimum, Bioactive molecules, Nano scale injection, Essential oil, Hydrodistillation, Myrcene

1. Introduction

Plant volatile and fixed (nonvolatile) have wide applications in dietary regimens, food flavoring and preservation, folk medicine and fragrance industry [1,2]. Essential oils are secondary plant metabolites found in leaves, stems, flowers and fruits. They frequently have different chemical composition depending on the nature of the plant and the season.

The genus *Haplophyllum*, which belongs to the Rutaceae family, numbers ca. 70 species, is represented in the flora of Iran by 18 species including nine endemics [3].

The plants are perennial herbs with a pervasive smell, distributed from the Mediterranean to East Siberia. Fourteen species are found in Uzbekistan [4].

This genus is widespread in Central Asia and so has been commonly used for a long time in folk medicine by the local population. In the earliest scientific sources, such as the *Canon Medicinae* by *Avicenna*, it is indicated that "sadab-ruta" (the name given to different *Haplophyllum* species) could be applied for treating different diseases. It could be used alone or sometimes in combination with other plants, generally using some topical forms to treat, among other diseases, warts, herpes, lichens, tumour of the testes, and erysipelas. These folk uses could indicate biocidal properties (especially cytotoxic properties) [5].

To the best of our knowledge, the essential oils of the leaves and aerial parts of this plant in central Iran have not been considered before. The maters on hand of this study were the determination of the percentage bioactive and fragrant molecules by nano scale injection.

* Corresponding author: bamoniri@kashanu.ac.ir

2. Material and methods

Plant Material

Leaves and aerial parts (leaves and flowers/inflorescences) of *H. glabrrimum* were collected in May 2008 in around Kashan (province of Isfahan, Iran). Leaves and aerial parts were dried in the shade (at room temperature). The voucher specimens of the plant were deposited in the herbarium of Research Institute of Forests and Rangelands, Kashan, Iran.

Isolation of the Essential Oils:

The air-dried and ground leaves (50g) and aerial parts (70g) of *H. glabrrimum* were subjected to hydrodistillation for 3.5 h using a Clevenger-type apparatus [6]. After decanting and drying over anhydrous sodium sulfate, the corresponding yellowish oils were recovered from the leaves and aerial parts in a yields of 0.08%, 0.149% (w/w), respectively and stored at low temperature (4ºC) further analysis.

Gas Chromatography (GC):

GC analyses of the oils were performed on an Agilent HP-6890 gas chromatograph equipped with flame ionization detector (FID) and an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The oven temperature was programmed as follows: 50ºC (2 min), 50-130ºC (3ºC min^{-1}), 130ºC (2 min), 130-270ºC (5ºC min^{-1}). Injector and detector temperatures were maintained at 220ºC and 290ºC, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 μL of sample in 1000 ml of n-pentane, v/v) in the splitless mode. Helium was used as carrier gas with a flow rate of 1 mL/min.

Gas Chromatography-Mass Spectrometry (GC/MS):

GC-MS analyses of these oils were performed on a Agilent HP-5973 mass selective detector coupled with a Agilent HP-6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m × 0.25 mm i.d, film thickness, 0.25 μm) and operating under the same conditions as above was described. The flow rate of helium as carrier gas was 1 mL/min. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200ºC; resolution, 1000.

Identification of bioactive and fragrant components:

Essential oils were analyzed by GC and GC/MS systems using a non-polar column and identification of components in oils were based on retention indices (RI) relative to *n*-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature [7, 8]. The percentage composition of the samples was computed from the GC-FID peak areas without the use of correction factors.

3. Results and discussion

Air-dried leaves and aerial parts of the plant were subjected to hydrodistillation using a Clevenger-type apparatus and yellowish oils were obtained in the yields of 0.08%, 0.14% (w/w), respectively. Ten and sixteen bioactive, flavour and fragrance molecules, constituting 93.86% and 96.89% of the total components detected, were identified in this plant and listed in Table I with their percentage. Constituents are listed in order of their elution from HP-5MS column. myrcene (52.89%), Elemol (10.565), and *β*-caryophyllene (8.94%) were the major components of leaves oil. Meanwhile myrcene (65.08%), *α*-thujene (5.36%), and Trans- *β*-ocimene (4.69%) were the

major components of aerial parts oil.

Table 1. Bioactive and fragrance components of leaves (A) and aerial parts (B) of H. glabrrimum from Central Iran

Compound[a]	**A, %**	**B, %**	**RI** [b]	**Compound**[a]	**A, %**	**B, %**	**RI** [b]
α -thujene	-	5.36	920	β -Elemene	2.54	1.70	1383
Myrcene	52.89	65.08	992	β -caryophyllene	8.94	2.28	1408
α-terpinene	-	0.57	1017	α-Humulene	2.62	-	1438
δ-3-carene	-	4.35	1027	Germacrene D	3.84	0.66	1468
Trans- β-ocimene	3.39	4.69	1044	δ -cadinene	3.11	2.79	1515
γ -terpinene	-	2.69	1054	Elemol	10.56	-	1541
Terpinolene	-	1.50	1083	T-muurolol	3.87	0.73	1647
Linalool	-	1.97	1099	Neophytadiene	2.10	-	1828
Allo-Ocimene	-	1.34	1125	N-Nonadecane	-	0.58	1889
Terpinene-4-ol	-	0.60	1170	**Total**	**93.86**	**96.89**	

[a]Compounds listed in order of their RI.
[b]RI (retention index) measured relative to n-alkanes (c_8-c_{32}) on the non-polar HP-5MS column.
%, Relative percentage obtained from peak area.
[M] Monoterpenes, are natural compounds with ten carbon atoms in their skeleton.
[S] Sesquiterpenes, are natural compounds with fifteen carbon atoms in their skeleton.

Literature survey indicated that there is no report on the Chemical composition of *H. glabrrimum,* but previous studies on essential oils chemical composition a number of *Haplophyllum* genus showed various compositions [9-14]. It would also be noteworthy to point out that the constituents of any plant essential oil studied is influenced by the presence of several factors, such as local, climatic, seasonal and experimental conditions.

Acknowladgement

Financial support made by Research Chancellor of the University of Kashan, Kashan, Iran is gratefully acknowledged.

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