

STUDY OF THE BIOACTIVE AND FRAGRANT CONSTITUENTS EXTRACTED FROM LEAVES AND AERIAL PARTS OF *Psammogeton canescens* (DC.) Vatke FROM CENTRAL IRAN BY NANO SCALE INJECTION

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The dried leaves and aerial parts of *Psammogeton canescens* DC. which belongs to Umbelliferae family and grows in central Iran, were hydrodistilled to produce oils in the yields of 0.32% and 0.79% (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 μ L of sample in 1000 ml of *n*-pentane, v/v). Twenty three and fourteen bioactive, flavour and fragrance molecules were identified, representing 99.07% and 99.5% of the leaves and aerial parts oil, one by one. The main components were trans-methyl isoeugenol (32.60%) in leaves and β -bisabolene (33.59%) in aerial parts oils. The compositions of the oils were mostly quantitatively rather than qualitatively different.

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1. Introduction

Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro- or steam distillation and by expression. For centuries essential oils have been isolated from different parts of plants and also are used for fragrant and biological activities. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant and anticarcinogenic properties. Others are biocides against a broad range of organisms such as bacteria, fungi, viruses, protozoa, insects and plants [1].

Many factors can influence the amount of essential oil in aromatic herbs, e.g., climate and environmental conditions, season of collection, age of plants and etc [2]. The genus *psammogeton* is a plant belonging to the Umbelliferae family. Umbelliferae, a family of about 300 genera and 3000 species is cosmopolitan in distribution, chiefly in north temperate regions [3, 4]. Plants of this family are grassy, permanent, biennial or annual and mostly grow in north hemisphere; also more species of this family distributed in Mediterranean areas, Turkey and Iran [5, 6]. This family is of considerable economic importance for food, flavoring and ornamental plants [7]. These plants are known to accumulate flavonoids mainly in the form of flavones and flavonols [8]. Umbelliferae family includes some well known medicinal and toxic plants as well as common food plants such as carrot, celery and parsley and some of the most bioactive polyacetylenes are also found in them [9].

Psammogeton canescens is annual with 15-30 cm height, stems are tomentulose. Leaves are ternate and each part has pinnatisect with segments petiolulate, flowers are white or purple-pink in color and small, also umbels are longley pedunculate. Flowering period of this plant is

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May-June [10]. Studies indicated that the essential oil of this plant has antimicrobial activities such as bactericidal and antifungal [11-13].

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 matters on hand of this study were the determination of the percentage bioactive and fragrant molecules by nano scale injection.

2. Material and methods

Plant Material:

Leaves and aerial parts (leaves and flowers/inflorescences) of *P. canescens* were collected in April and May 2008, respectively, in khatab shekan area (North of Aran and Bidgol), around Kashan (province of Isfahan, Iran) at an altitude of 970m. Leaves and aerial parts were dried in the shade (at room temperature). The voucher specimens of the plant were deposited in the herbarium (Voucher NO.KBGH 1872) of Research Institute of Forests and Rangelands, Kashan, Iran.

Isolation of the Essential Oils:

The air-dried and ground leaves (62g) and aerial parts (144g) of *P. canescens* were subjected to hydrodistillation for 3.5 h using a Clevenger-type apparatus [14]. After decanting and drying over anhydrous sodium sulfate, the corresponding light yellow and colorless oils were recovered from the leaves and aerial parts in a yields of 0.32%, 0.79% (w/w), respectively and stored at low temperature (4°C) 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: 50°C (2 min), 50-130°C (5°C/min), 130°C (2 min), 130-200°C (3°C/min), 200°C (2 min) and 200-280°C (20°C/min). Injector and detector temperatures were maintained at 220°C and 290°C, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 µL of sample in 1000 µL 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, 200°C; 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 [15, 16]. 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 light yellow, colorless oils were obtained in the yields of 0.32%, 0.79% (w/w), respectively. Twenty three and fourteen bioactive, flavour and fragrance molecules, constituting 99.07% and 99.5% 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. Trans-methyl isoeugenol (32.60%), trans-caryophyllene (9.64%), myrcene (6.94%), allo-ocimene (6.39%) and α -Pinene (6.11%) were the major components of leaves oil. Meanwhile β -Bisabolene (33.59%), α -pinene (32.24%), trans-isodillapiole (9.76%) and myrcene (8.98%) were the major components of aerial parts oil.

Table 1. Bioactive and fragrance components of leaves (A) and aerial parts (B) of *P. canescens* from Central Iran

Compound ^a	A, %	B, %	RI ^b	Compound ^a	A, %	B, %	RI ^b
α -Pinene ^M	6.11	32.24	929	β -Copaene ^S	1.17	-	1422
β -Pinene ^M	-	4.71	969	α -Humulene ^S	1.85	-	1445
Myrcene ^M	6.94	8.98	985	Germacrene D ^S	3.39	-	1472
Limonene ^M	3.65	2.06	1022	Trans-Methyl isoeugenol ^M	32.60	0.52	1492
Cis-Beta-Ocimene ^M	2.18	0.51	1031	β -bisabolene ^S	3.50	33.59	1506
Trans-Beta-Ocimene ^M	5.07	0.65	1042	β -Sesquiphellandrene ^S	2.25	-	1515
γ -Terpinene ^M	2.61	-	1052	Trans-Cadinene ether ^S	1.26	-	1552
Allo-Ocimene ^M	6.39	1.23	1124	Spathulenol ^M	0.69	-	1569
Thymyl Methyl Ether ^M	1.05	-	1229	Cis-isomyristicin ^M	1.00	-	1608
Bicycloelemene ^S	-	0.31	1329	Dill apiole ^S	-	2.83	1618
α -Cedrene ^S	1.76	0.54	1407	Cis-Asarone ^M	2.35	-	1645
Trans-Caryophyllene ^S	9.64	1.57	1414	Trans-Asarone ^M	2.05	-	1674
β -longipinene ^S	0.60	-	1418	Trans-isodillapiole ^S	0.96	9.76	1706
				Total	99.07	99.5	

^aCompounds listed in order of their RI.

^bRI (retention index) measured relative to n-alkanes (C₈-C₃₂) 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.

In this paper, we illustrate biological properties and application of some important components from *P. canescens* essential oils:

α -Pinene: is employed as a fragrance substance to improve the odor of industrial products such as insecticides, antiseptics.

β -Pinene: is employed as antifungal, insecticides and polymer industries, and as a fragrance material in household perfumery.

Myrcene: is employed as a solvent or diluting agent for dyes and varnishes, and in the production of terpene polymers, terpene-phenol resins and terpenemaleate resins.

Limonene: is employed as component of artificial essential oils, and as fragrance material for perfuming household products.

Allo-Ocimene: is employed as a diluting agent for varnishes and dyes, as a component for terpene polymers, and is used to a small extent in the perfume industry.

Cis, Trans-Beta-Ocimene: is employed as a botanical insecticide, used as a solvent for cleaning purposes.

β -Longipinene: has antiinflammatory effects.

α -Humulene: has antimicrobial and insecticidal properties

Trans-Methyl isoeugenol: is employed as a attracting to an agricultural pest.

Trans-isodillapiole: is employed as a killing pests and bacteria.

Germacrene D: has antimicrobial and insecticidal properties.

β -Bisabolene: is employed as a perfume and fragrance industries.

Literature survey revealed that the chemical composition of the essential oil of *P. canescens* obtained by steam distillation was reported by Rahman *et al.*, the major components of this oil were limonene, myricitin, methyl eugenol, linalool and terpineol [17]. 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.

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