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Short Communication

Identification and Comparison of Effective Substances in Essential Oil of *Psammogeton Canescens* Plant Collected from Kerman Province / Iran

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ABSTRACT

Objective: The genus *Psammogeton*, belonging to the Umbelliferae family, has 4 annual species in Iran. In the present work, *Psammogeton canescens* (DC) **Methods:** Vatke was collected from Saheb-al-Zaman Mountain slope, northeast of Kerman province, Iran in May 2013. The essential oils of leaves, flowers and stems of the plant were separately extracted using hydrodistillation method and analyzed by GC and GC/MS. **Results:** In the leaf oil, 25 components were identified, representing 99.5% of the total oil, with β-pinene (40.3%), dill apiole (20.4%), limonene (15.1%) and 4aα,7β,7aα-nepetalactone (6.6%) as the main constituents. The flower oil was characterized by higher amount of dill apiole (44.3%), limonene (32.4%) and β-pinene (11.4%) among the 22 components comprising 99.8% of the total oil detected. Furthermore, 33 compounds were identified in the stem oil, representing 98.6% of the total oil. β-Pinene (33.6%), dill apiole (27.8%), limonene (16.3%) and γ-terpinene (5.3%) were found to be the major constituents. Consequently, β-pinene, dill apiole and limonene were the main components in all three oils. The dominant components of the leaf and stem oils were monoterpenes (76.0% and 64.5%, respectively), whereas phenylpropanoids (49.7%) and monoterpenes (49.2%) were the major groups of compounds in the flower oil.

1.INTRODUCTION

Umbelliferae family is one of the largest groups of flowering plants. This group consists of around 300 genus and more than 3000 species which are mostly distributed in northern hemisphere. Most of species of the group mainly grow in Mediterranean regions, Turkey, and Iran (Bamoniri et al., 2009; Ghahraman 1993). *Psammogeton* genus has 6 species distributed in central parts of Asia (Rahimi-Nasrabadi et al., 2009). The genus is called "Shen Ja'r" in Persian having 4 annual species which mostly grow in deserts (Mozafarian, 2006).

Psammogeton canescens (DC.) Vatke is an annual plant with 30 to 40 cm height. It is often split from the base or the lower half. Umbrellas have peduncle, umbelets are flowery, and bracteole are bayonet and ciliated. Leaves have widespread short shags, however; they are sometimes glabrous. Petals are firstly red and get gradually white. Flowers are small and white or pink. This plant flowers at the middle of spring. It grows in center, northeast and south east of Iran, however; it grows in other regions such as central Asia, Afghanistan, Pakistan, and Iraq (Mozafarian, 2007).

Some scientific reports are published about chemical compounds and biologic activities of *P. canescens*

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essential , but there is no significant research on other species of *Psammogeton*. Based on the studies, *P. canescens* essential showed significant antibacterial (Rahman & Gul, 2002) and antifungal (Rahman & Gul, 2000) activities. Antioxidant activities of the species essential oil and methanol extract have been also studied. Methanol extract and essential showed considerable antioxidant properties and are reported as natural antioxidant resources (Gholivand *et al.,* 2010). On the other hand, Iranian researchers have extracted essential oils from *P. canescens* leaves and shoots using hydro distillation method and the constituents were identified and reported (Rahimi-Nasrabadi *et al.*, 2009; Bamoniri *et al.*, 2009; Gholoivand *et al.*, 2010).

In this study, chemical compounds in the leaf, flower, and stem essential oils of *P. canescens* were separately identified and compared. No report has been published on the identification and comparison of chemical compounds in essential of different parts of the plant till now.

2. MATERIALS AND METHODS

1.2. Collecting the plant and extracting essential

P. canescens was collected from Saheb – al- zaman mountain slope at the height of 1700 to 1800 m in northeast of Kerman province. It was identified and kept with registration number of 8329 at Kerman Agricultural and Natural Resources Research Center using Iranian Flora (Mozafarian, 2007). At the first, leaves, flower, and stems were dried in the shade and at room temperature. After comminuting dried samples, 100 gr of each sample was separately transferred to Clevenger apparatus and the oil was extracted using hydrodistillation method for 3 hours. Then essential oils were separated and dehumidified by sodium sulfate and essential oils were determined based on the weight of each dried sample.

2.2. Characteristics of GC apparatus

Gas chromatograph (Shimadzu 15A) is consisted of a DB-5 shaft with the length of 50 m, internal diameter of 0.25 mm, and thickness of stationary phase layer of 0.25 µm. The heat plan is as follow: initial temperature of the shaft were set to 60° C for 3 minutes and then temperature was increased to 220° with the rate of 5 degree per minute, finally it was stopped at 220° C for 5 minutes. Injection chamber temperature was set to 250° C, FID (flame ionization detector) temperature was set to 270° C with flow of helium as carrier gas with speed of 1 mm/minute.

2.3. Characteristics of GC/ MS apparatus

5975C Agilent mass spectrometer was connected to 7890A Agilent gas chromatograph, with a HP- 5MS shaft with length of 30 m, internal diameter of 0.25 mm, and thickness of stationary phase layer of 0.25 µm. The heat plan follows as the initial temperature of the shaft was kept at 60°C for 3 minutes, then it was increased to 240°C with the rate of 5 degree per minute and it was kept at 240°C for 5 minutes. Temperature of injection chamber and FID was set at 250°C and 270°, respectively. The carrier gas was helium with the flow speed of 1 mm/ minute, scanning time of 1 second, ionization energy of 70 eV and mass region of 40 to 340.

2.4. Identification of compounds of essential oils

The essential oils were diluted by N-hexane and then were injected into GC apparatus through which the most appropriate heat plan for separating constituents was determined. Then essential oils were separately injected into GC/ MS apparatus and mass spectrums and chromatograms of each essential oil constituents were identified. Retention index (RI) of all constituents was calculated by injection of Normal alkanes (C_7 – C_{23}) into GC apparatus at same conditions using retention time.

The compounds of essentials were identified by comparing mass spectrums and RI, mass spectrums and RI of standard compounds (Davies, 1990; Adams, 2004), and using Wiley and NIST data banks in GC/ MS apparatus. The relative percentage of compounds of essential oils was determined considering their area under the chromatogram curve.

3. RESULTS

The results of the experiments included determining essential oil yield from different parts of the species as well as identification and recognition of percentage of constituents of the essential oils. Regarding the weight of each dried sample, the average yields of the essentials of *P. canescens* leaf, flower, and stem were 0.8%, 1.1% and 0.3%, respectively. Table 1 showed the identified compounds, retention index (RI), and the percentage of each compound in extracted essential oils. As it is seen in table 1, 25 compounds were identified in leaf oil which comprised 99.5% of the total essential oil. β-pinene (40.3%), dill apiole (20.4%), limonene (15.1%) and 4aα,7β,7aα-Nepetalactone (6.6%) were known as the main constituents. The flower essential was characterized by higher amount of dill apiole (44.3%), limonene (32.4%) and β-pinene (11.4%) among the 22 components comprising 99.8% of the total oil detected. Furthermore, 33 compounds were identified in the stem oil, representing 98.6% of the total oil. β-Pinene (33.6%), dill apiole (27.8%), limonene (16.3%) and γ-terpinene (5.3%) were found to be the major constituents.

4. DISCUSSION

As you can see in table 1, essential oils of *P. canescens* leaf, flower, and stem share considerable number of compounds. Consequently, β-Pinene, dill apiole, and limonene were found to be main constituents of all the three essential oils. On the other hand, there were more

compounds in stem oil rather than the two others, in a way that some compounds present in stem oil such as linalyl acetate, Thymol, Spathulenol, and (*E*)-β-Farnesene were absent in leaf and flower essential oils. There was a diterpene hydrocarbon called the Neophytadiene which is just present in stem essential. However there were also compounds existing in leaf and flower oils which were not identified in stem oil, of the most important compounds are Nepetalactone isomers.

Table 1.

Compounds indentified in leaf, folwer, and stem essential oils of *Psammmogeton canescens*

Compound Name	RI	Leaf (%)	Flower (%)	Stem (%)
α-pinene	934	2.1	0.6	1.6
camphene	950	0.1	–	0.2
β-pinene	978	40.3	11.4	33.6
Myrcene	990	1.9	0.8	2.0
α-phellandrene	1001	0.1	–	0.1
α-terpinene	1014	–	0.1	0.1
p-cymene	1023	0.7	0.2	0.8
Limonene	1029	15.1	32.4	16.3
(Z)-β-ocimene	1037	2.1	0.7	1.1
(*E*)-β-ocimene	1048	2.7	0.2	0.6
γ-terpinene	1058	2.9	1.2	5.3
terpinolene	1086	0.5	0.3	0.6
terpinen-4-ol	1174	0.2	0.5	0.4
-terpineolα	1187	0.3	0.1	0.4
linalyl acetate	1254	–	–	0.8
perilla aldehyde	1273	–	0.2	–
Thymol	1291	–	–	0.3
4aα,7α,7aα-nepetalactone	1359	0.4	–	–
neryl acetate	1361	–	–	0.1
α-copaene	1374	–	–	0.1
geranyl acetate	1380	–	–	0.2
β-bourbonene	1384	0.1	–	–
4aα,7α,7aβ-neprtalactone	1386	–	0.5	–
-elemeneβ	1389	–	–	0.2

4aα,7β,7aα-nepetalactone	1391	6.6	–	–
methyl eugenol	1400	–	–	0.1
β-caryophyllene	1417	1.1	0.4	0.6
α-humulene	1452	0.2	–	–
(*E*)-β-farnesene	1454	–	–	0.4
drima-7,9(11)-diene	1468	–	–	0.3
germacrene D	1481	0.3	0.1	1.0
-selineneβ	1487	0.3	0.4	1.0
(*E*)-methyl isoeugenol	1493	–	1.7	–
α-selinene	1495	0.3	–	–
myristicin	1518	0.4	1.2	0.9
elemicin	1553	–	2.2	0.2
spathulenol	1576	–	–	0.9
caryophyllene oxide	1581	0.2	–	0.2
dill apiole	1625	20.4	44.3	27.8
trans-isodillapiole	1713	0.2	0.3	0.3
neophytadiene	1836	–	–	0.1
Total	-	99.5	99.8	98.6

Leaf oil is consisted of eleven monoterpenes hydrocarbon (68.5%), four oxygenated monoterpenes (7.5%), six sesquiterpenes hydrocarbon (2.3%), one oxygenated sesquiterpene (0.2%), and three derivations of phenylpropanoid (21.0 %). There were also ten monoterpenes hydrocarbon (47.9%), four oxygenated monoterpenes (1.3%), three sesquiterpenes hydrocarbon (0.9%) and five derivations of phenylpropanoid (49.7%). Thus there was no oxygenated sesquiterpenes in flower essential. Finally stem essential included twelve monoterpenes hydrocarbon (62.3%), six oxygenated monoterpenes (2.2%), seven sesquiterpenes hydrocarbon (3.6%), two oxygenated sesquiterpenes (1.1%), five derivations of Phenylpropanoid (29.3 %), and one diterpene hydrocarbon (0.1 %). Therefore we can conclude that monoterpenes allocates the highest percentage of leaf and stem oils (76.0% and 64.5%, respectively), while the highest percentage of flower essential oil is for derivations of phenylpropanoid (49.7%) and monoterpenes (49.2%).

One of the most sensible characteristics of *P. canesscens* is the presence of significant percentage of derivations of phenylpropanoid in its different parts essential oils. Therefore dill apiole (a derivation of phenylpropanoid) allocate around half of flower essential and considerable percentage in leaf and stem essential.

Although monoterpenes and sesquiterpenes are generally the major constituents of herbal essential oils, Phenylpropanoid derivations even as the major constituents also exist in some essential oils. Phenylpropaniods forms a large group of secondary metabolites which are produced by plants using Phenylalanine amino acid and influenced by many factors such as pollutants, ultra-violet radiation, exposure to ozone, and other environmental factors. Recently there have been a large number of studies on the Pharmaceutical usage of natural and synthetic phenylpropaniods as wound healing, antioxidant, anticancer, antiviral and antibacterial (Davey et al., 2004; Delazar et al., 2006; Korkina, 2007).

Chemical compounds of *P. canescens* shoots, collected from Aran and Bidgol region, Isfahan province, was previously studied. Finally β-Bisabolen (33.35%), apiol (28.34%), α-Pinene (11.86%), and Dill apiol (8.17%) were reported as the main components of the essential oils (Gholivand et al., 2010; Rahimi-Nasrabadi et al., 2009). In another study, essential oils of leaf and shoots

of the mentioned species, collected from Kashan around, Isfahan province, were analyzed. Trans- methyl iso Eugenol (32.6%), Trans- caryophyllene (9.64%), Myrcene (6.94%), alo-Ocimene (6.39%), and α-Pinene (6.11%) were reported as the main compounds of leaf oil and β-Bisabolen (33.59%), α-pinene (32.24%), Trans- iso dill apiol (9.76%), and myrcene (8.98%) were the main compounds of the shoots essential oil (Bamoniri et al. 2009).

Despite all the similarities between results of the previous researches and the present research in terms of identified compounds, there are significant differences between them. As an example, most of identified phenylpropanoid derivations in this research were also reported in previous researches such as dill apiol, Trans-iso dill apiol, Myristicin, almacén, and (E)- methyl iso Eugenol; but β-Bisabolen which was reported in two previous as a major constituent of *P. canescens* shoots, was not identified in any studied parts in our research. Nepetalactone isomers which were also identified in essential oils of leaf and flower in this study were not reported in previous studies (Gholivand et al., 2010; Bamoniri *et al,* 2009; Rahimi- Nasrabadi *et al*., 2009). These differences can be due to different climatic conditions, time of harvest, and/or test conditions.

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