**SUPPLEMENTARY MATERIAL**

**Profiling of the essential oil compositions from the flowers and leaves of *Tanacetum fisherae* Aitch. & Hemsl., an endemic plant in Kerman province, Iran**

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**Abstract**: *Tanacetum fisherae* Aitch. & Hemsl. is an endemic plant growing wild in some brackish regions of Iran. Since there are not enough reports concerning the quantitative and qualitative analyses of its essential oil, it was decided to characterize the respective water-distilled oils obtained from the flowers and leaves of this medicinal plant. Characterization of the corresponding essential oil profiles revealed that in both of the analysed oils, oxygenated monoterpenes constituted most of the chemical profiles. In this sense, the most prevailing natural compounds in the flower oils were *cis*-*p*-2-menthen-1-ol (11.2%), *trans-p*-2-menthen-1-ol (10.7%), *trans-*piperitol (7.8%), 1,8-cineole (6.1%), *cis*-piperitol (3.8%), α-terpineol (3.7%) and terpinene-4-ol (2.1%), whereas the main constituent components of the leaves oils were respectively 1,8-cineole (16.7%), *cis*-*p*-2-menthen-1-ol (14.6%), *trans-p*-2-menthen-1-ol (10.4%), *trans-*piperitol (12.8%), α-terpineol (5.4%), *cis*-piperitol (2.9%), borneol (2.7%), and terpinene-4-ol (2.1%). In addition, the second rank of natural compound constituting groups was due to oxygenated sesquiterpenes, as well.

Keywords: *Tanacetum fisherae* Aitch. & Hemsl., essential oil, oxygenated monoterpenes, *cis*-*p*-2-menthen-1-ol, *trans-p*-2-menthen-1-ol, α-bisabolol

**3. Experimental**

***Plant sampling, geographical coordinates and oil isolation***

The whole plant material (550 g) was gathered during the flowering stage on 12 June 2019 from the Kerman mountains (30.2839° N, 57.0834° E) (Figure 1) and identified by Dr. Gholami (taxonomist). Immediately after the sampling process, the plant was washed with water carefully in order to remove its unwanted contaminants. A voucher specimen was deposited at the herbarium of Agricultural Faculty of Shahrood University of Technology, No. TF280.901. The flowers and leaves of this plant were carefully separated and dried under the darkness at an ambient temperature. In the next step, these parts were separately pulverized into fine powder and subjected to water distillation using a modified Clevenger glassware. The hydrodistillation process was conducted three times and for each relevant separation and the average time dedicated was between 3-3.5 hours. Both of the obtained essential oils had a pale yellowish color and pungent odor and stored in browned and sealed sampling glass vials in the refrigerator (-4 °C) until further analysis. The average yields of the obtained oils from the flowers and leaves of *T. fisherae* Aitch. & Hemsl. where restrictively as 0.7 and 0.65 w/w% respect to the dry plant material.

***Gas chromatographic analyses***

The gas chromatographic determinations were performed using GC and GC-MS instrumentations with the general characteristics as follows. A Shimadzu C-R4A Chromatopac GC was used equipped with a capillary SE 30 column composed of 100% dimethylpolysiloxane (length: 30 m; internal diameter: 0.32 mm; film thickness: 0.25 μm) and flame ionization detector (FID). Nitrogen was used as the carrier gas with an average flow rate of 1 mL/min. The temperature programming for the employed column (SE 30) was according to the following steps. The final temperature of the injection port and the detector (FID) were subsequently adjusted at 230 and 280 °C. The initial temperature of the column was first kept at 60 °C for five minutes and programmed to reach a temperature of 200 °C with a mild ramp of 5 °C/min and finally raised to a total temperature of 280 °C with the aforementioned ramp. For each injection, exactly 1.0 µL of the diluted sample essential oil was injected onto the injection port of GC under the splitless mode. The surface area of each obtained peak was used for the quantitative determination of each constituent component of the essential oil in the relevant chromatogram (detector signal) regardless of any correlation factor.

The column used in GC-MS apparatus (Hewlett-Packard 6890/5973) was of HP-5 MS type composed of (5%-phenyl)-methylpolysiloxane phase (length: 30 m; internal diameter: 0.25 mm; film thickness: .25 μm) and programmed similar to that of SE30 in GC-based injections. The ultrapure He was used as the carrier gas with a flow rate similar to that of nitrogen in gas chromatographic analyses (1 mL/min) throughout the column. The operational characteristics of the GC-MS are as follows. The ionization potential of the instrument was adjusted at 70 eV, whereas the source temperature, multiplier voltage, emission current and the corresponding scan cycle were respectively set at 70 eV, 300 V, 200 µA and 1.5 s. The detailed characteristics of other involving variables have been extensively reported in our recent paper (Mohammadhosseini et al. 2017). To calculate the Kovats retention indices (RIs) of the oil constituents, a homologous series of normal alkanes (C9-C24) was injected separately exactly according to the oil analyses conditions and the relevant RIs were subsequently processed and considered as one of the main parameters for accurate characterization of each natural compound.

***Identification of the relevant chemical profiles***

As is customary in the literature in such sort of studies, the main criteria to characterize a natural compound or the entire chemical profile are as follows.

a) Consistency between the calculated and literature available Kovats retention indices by co-injection of a series of normal paraffins (C9-C24) under the same optimized experimental conditions

b) Confirmation of mass spectral fragmentation patterns of the constituent components of the oil profile with those tabulated in the literature

c) Consideration of the mass library reports of advanced GC-MS instruments

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Table S1. Constituents of the essential oils from the flowers and leaves of*Tanacetum fisherae* Aitch. & Hemsl., an endemic plant in Kerman province, Iran.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sr. No. | Compound | Rt(Min.)a | RI(Cal.) b | RI(Lit.)c | Percentage |
| Flowers (%) | Leaves (%) |
| 1 | α-pinene | 5.86 | 935 | 936 | 0.3 | 0.5 |
| 2 | camphene | 6.29 | 945 | 946 | 0.1 | 0.9 |
| 3 | benzaldehyde | 6.45 | 953 | 952 | 0.3 | - |
| 4 | sabinene | 6.91 | 976 | 976 | 2.3 | 2.1 |
| 5 | β-pinene | 7.05 | 979 | 980 | 0.3 | 0.6 |
| 6 | myrcene | 7.45 | 991 | 991 | 0.3 | - |
| 7 | α-phellandrene | 7.87 | 1002 | 1003 | 3.7 | 0.1 |
| 8 | α-terpinene | 8.31 | 1014 | 1016 | 2.1 | 0.5 |
| 9 | *p*-cymene | 8.54 | 1024 | 1025 | 2.1 | 1.2 |
| 10 | 1,8-cineole | 8.78 | 1036 | 1033 | 6.1 | 16.7 |
| 11 | γ-terpinene | 9.80 | 1061 | 1059 | 0.9 | 0.6 |
| 12 | terpinolene | 10.99 | 1086 | 1086 | 0.2 | - |
| 13 | *cis*-sabinene hydrate | 11.01 | 1067 | 1068 | - | 0.3 |
| 14 | linalool | 11.34 | 1096 | 1095 | - | 0.2 |
| 15 | *trans-*sabinene hydrate | 11.45 | 1098 | 1098 | - | 0.3 |
| 16 | *cis*-*p*-2-menthen-1-ol | 11.74 | 1120 | 1121 | 11.2 | 14.6 |
| 17 | *trans-p*-2-menthen-1-ol | 11.78 | 1138 | 1137 | 10.7 | 10.4 |
| 18 | camphor | 13.30 | 1146 | 1145 | - | 0.4 |
| 19 | borneol | 14.30 | 1166 | 1167 | 0.8 | 2.7 |
| 20 | terpinene-4-ol | 14.70 | 1177 | 1178 | 2.1 | 2.1 |
| 21 | α-terpineol | 15.28 | 1190 | 1191 | 3.7 | 5.4 |
| 22 | *cis*-piperitol | 15.60 | 1195 | 1196 | 3.8 | 2.9 |
| 23 | *trans-*piperitol | 16.12 | 1207 | 1207 | 7.8 | 12.8 |
| 24 | piperitone | 17.98 | 1240 | 1240 | 0.9 | 1.6 |
| 25 | bornyl acetate | 19.42 | 1265 | 1266 | 0.4 | 0.2 |
| 26 | lavandulyl acetate | 19.63 | 1267 | 1268 | 0.8 | - |
| 27 | *trans*-jasmone | 24.20 | 1390 | 1390 | - | 0.8 |
| 28 | β-gurjunene | 25.94 | 1432 | 1431 | - | 0.2 |
| 29 | *cis-*β-farnesene | 26.84 | 1457 | 1457 | 0.2 | - |
| 30 | β-sesquiphellandrene | 29.70 | 1523 | 1523 | 0.2 | - |
| 31 | *trans*-γ-bisabolene | 30.05 | 1530 | 1529 | - | 0.1 |
| 32 | (*E*)-nerolidol | 31.31 | 1561 | 1562 | 2.6 | 0.9 |
| 33 | caryophyllene oxide | 32.15 | 1587 | 1586 | - | 2.1 |
| 34 | 1-*epi*-cubenol | 33.94 | 1630 | 1629 | 11.9 | - |
| 35 | bisabolol oxide | 35.09 | 1659 | 1658 | 3.1 | 2.7 |
| 36 | α-bisabolol | 36.18 | 1690 | 1689 | 15.8 | 14.3 |
|  |  | **Total** | **94.7** | **98.2** |

a Rt (Min.): Retention time (minute); b RI (Cal.): Calculated retention index; c RI (Lit.): Retention index in literature; Monoterpene hydrocarbons (MH): Sr. No. 1-2, 4-9; 11-12; Non-terpene hydrocarbons (NH): Sr. No. 3, 27; Oxygenated monoterpenes (OM): Sr. No.10, 13-26; Sesquiterpene hydrocarbons (SH): Sr. No. 28-31; Oxygenated sesquiterpenes (OS): Sr. No. 32-36



Figure S1. The geographical map of the sampling area (Kerman mountains (30.2839° N, 57.0834° E).



Figure S2. The gas-chromatogram of the essential oils from the flowers of *Tanacetum fisherae* Aitch. & Hemsl..



Figure S3. The gas-chromatogram of the essential oils from the leaves of *Tanacetum fisherae* Aitch. & Hemsl..

Figure S4. Comparison of the different distribution of the essential oils components from flowers and leaves of *T. fisherae* Aitch. & Hemsl. in the different classes of natural compound. Monoterpene hydrocarbons (MH); Non-terpene hydrocarbons (NH); Oxygenated monoterpenes (OM); Sesquiterpene hydrocarbons (SH); Oxygenated sesquiterpenes (OS).