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THE BIOACTIVE AND VOLATILE CONSTITUENTS OF *PRANGOS ACAULIS (DC) BORNM* EXTRACTED USING HYDRODISTILLATION AND NANO SCALE INJECTION TECHNIQUES

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This paper is devoted to an investigation carried out on hydrodistillation (HD) and nano scale injection techniques to extract bioactive volatile compounds from aerial parts of aromatic herb *Prangos acaulis (DC) Bornm* from Iran. This plant is a highly advanced and homogeneous species of Umbelliferae family, largely used in food industries, perfumery and medicine. Chemical composition of the volatile oil obtained by hydro-distillation of the *prangos acaulis* an endemic species of Iran at flowering stage was investigated by GC/MS. The amount of the samples injected by nano scale included were 0.1 nL (diluted 1.0 μL of sample in 1000 ml of *n*-hexane, v/v). Forty three bioactive and volatile compounds were identified. The major compounds of the oil were α -pinene (13.6%), limonene (12.94%) and myrcene (8.1%) β -pinene(5.4%) δ-3-carene(25.54%) α -terpinolene(14.76%) caryophylene(2.98%) γ –curcumene (2.65%)

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1. Introduction

Essential oils represent a small fraction of the composition of plants but confer the characteristics for which aromatic plants are used in the pharmaceutical, food and fragrance industries [1]. Essential oils have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons (terpenes and sesquiterpenes). Both hydrocarbons and oxygenated compounds are responsible for the characteristic odours and flavours [2-3].

The essential oils of plants have usually been isolated by either hydro-distillation or solvent extraction. The distillation method has traditionally been applied for the recovery of essential oils from plant materials. One of the disadvantages of the distillation method is that essential oils undergo chemical alterations and the heat sensitive compounds can easily be destroyed [1]. The other method applied for oil recovery from plant materials uses organic solvent extraction, which has limitations with regard to the loss of valuable volatiles during vacuum evaporation of solvent,

The genus *prangos acaulis* belongs to the Umbelliferae family which consists of about 30 species [4]. It is widespread in Iran to India. Some *Prangos* species have been used in the folk medicine as emulient, carminative [5], tonic, Anti flatulent, anathematic, antifungal andante bacterial agents [6]. It is herbaceous and perennial and grows up to 1 m high. The flowers are hermaphrodite (have both male and female organs) and are pollinated by Insects.

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Fifteen species of the genus *Prangos* are found in Iran, among which five are endemic*: P. gaubae, P. crossoptera, P. tuberculata, P. cheilanthifolia* and *P. cattigonoides*[7]. Hydro-distilled oil obtained from the aerial parts of *P. latiloba* has been the subject of a previous study. The major components of the oil were α -pinene (25.1%), limonene (16.1%) and myrcene (9.5%) [8]. A survey of the literature revealed that the oil composition of *P. uloptera* [9], P. *hissarica,P. seraivschanica, P. fedtschenkoi* [10], *P. ferulacea* [11-12], *P. latiloba* [8], *P. uechtritzzi* [13-14], *P. bornmuelleri* [14] and *P. heyniae* [6] have been reported.
To the best of our knowledge, the essential oil of the aerial parts at flowering stage of this plant in Lorestan area in south west of Iran have not been considered before. This study also was the determination of the percentage bioactive and volatile compositions by nano scale injection.

2. Material and methods

Plant material

The fresh plant of *prangos acaulis* (Family Umbelliferae) were collected during ful-flowering stage from of altitude 1600 m Zagros Mountain in the Lorestan state, west of Iran, in May 2009. The plant was identified and authenticated by Dr. Nasser Akbari at the faculty of agriculture Lorestan University.

Isolation of bioactive volatile components

The essential oil of fresh plant (100g) of was obtained by hydro-distillation in a Clevenger-type apparatus for 1.5 h. The essential oil was dried over anhydrous sodium sulphate. The yield of the oil obtained was found to be 1.35%.

GC and GC–MS analysis condition

The oil was analyzed by GC/MS using a Gas Chromatography Analysis GC analysis of the oil was conducted using a Varian CP-3800 instrument equipped with a DB-1 fused silica column (60 m × 0.25 mm id., film thickness 0.25 μm).Nitrogen was used as the carrier gas at the constant flow of 1.1 mL/min. The oven temperature was kept at 60°C for 1 min, then programmed to 250°C at a rate of 4°C/min, and kept constant at 250°C for 10 min. The injector and detector (FID) temperatures were kept at 250°C and 280°C, respectively.GC-MS analysis was carried out on a Thermoquest-FinniganTrace and DB-Wax columns under the same conditions.GC-MS instrument equipped with a DB-1 fused silica column (60 m× 0.25 mm i.d., film thickness 0.25 μm).The oven temperature was raised from 60^{o}C to 250^{o}C at a rate of 5^{o}C/min, and then held at 250^{o}C for 10 min.; transfer line temperature was 250^{o}C . In this case, the oven temperature was raised from 40^{o}C to 250^{o}C at a rate of 4^{o}C/min, then held at 250^{o}C for 10 min. with the transfer line temperature adjusted at 250^{o}C The flow rate of helium as carrier gas was 1.1 mL/min. split ratio was, 1/50.The quadrupole mass spectrometer was scanned over the 45-465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA. The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for Identification of individual n-alkanes (C_6–C_{24}) and the oil on DB-1 compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with authentic compounds or with those of reported in the literature [15].

Quantitative data was obtained from FID area percentages without the use of correction factors. compounds identified in the oil of *Prangos acilus* can be seen in Table 1.

3. Results and discussion

The volatile oil of the *prangos acaulis* at flowering was obtained by a conventional hydro-distillation method using a Clevenger-type apparatus and the yield of the oil was found to be in 1.35% (w/w) components. Identification of the constituents was accomplished by comparing their mass spectra and retention indices with those given in the literature and those authentic samples [15]. Relative percentage amount were calculated from TIC by computer. This study showed that prangose sp growing in this region contained a maximum amount of delta-3- carene (25. 54%). The results of the GC/MS analysis of the oil of aerial parts of (at flowering stage) are listed in Table I. Forty three compounds were identified representing 91.9 % of total oil The major components of the oil were 3-carene (25.54%), α -terpinolene(14.76%), α-pinene (13.6%), β-pinene(5.4%), limonene (12.94%), nerol(2.5%),caryophylene(2.98%), gama-curcumene(2.65%).Thus the oil consisted mainly of oxygenated monoterpenes (5.25%) ,hydro-carbonated monoterpenes (76.03%) and sesquiterpenes (7.89%). Comparing these results with previous studies on prangos species, it is worth mentioning here that there is variation in the chemical composition of *p.acilus* of this region with other species [9-12].

Table 1. Bioactive and volatile components of prangos acaulis.

No	COMPOUNDS	Tn	RI	Area%
1	α -thujene	5.3	925	0.1
2	α -pinene	5.5	932	13.6
3	Camphene	5.8	944	0.26
4	β -pinene	6.5	973	5.4
5	β -myrcene	7	992	8.1
6	α -terpinene	7.3	1011	0.15
7	δ-3-carene	7.7	1015	25.54
8	p-cymene	7.9	1024	1.09
9	Limonene	8.1	1030	12.94
10	Trans-beta-ocimene	8.3	1037	0.32
11	Trans-ocimene	8.7	1050	2.2
12	γ-terpinene	8.9	1057	1.07
13	α -terpinolene	9.8	1087	14.76
14	α -methylstyrene	10.1	1095	0.5
15	linalool	10.2	1100	0.87
16	Verbenol	11.4	1138	0.16
17	α -terpineol	12.4	1173	0.32
18	p-cymene-8-ol	12.6	1180	0.07
19	Linalyl propionate	12.8	1184	0.18
20	Myrtenol	13.02	1193	0.1
21	Nerol	14.9	1255	2.5
22	Bornyl acetate	15.7	1282	0.34
23	carvacrol	16.2	1300	0.08
24	Eugenol	17.7	1357	0.19
25	neryl acetate	18.2	1361	0.16
26	β -bourbonene	18.5	1382	0.16
27	α -copaene	18.6	1385	0.12
28	β -elemene	18.7	1389	0.14
29	Di-epi- α -cedrene	18.9	1399	0.1
30	β -Funebrene	19.2	1407	0.34
31	caryophyllene	19.4	1415	2.98
32	α -cedrene	19.6	1422	0.06
33	Aromadendrene	19.7	1426	0.01
34	α -caryophyllene	20.3	1449	0.88

35	β -farnesene	20.5	1456	0.17
36	Acoradiene	20.7	1464	0.11
37	γ-curcumene	21.1	1480	2.65
38	Bicyclogermacrene	21.5	1494	0.4
39	β -elemene	21.7	1501	0.18
40	(E,E)α -farnesene	21.8	1506	0.08
41	Germacrene B	23	1552	0.16
42	Caryophyllene oxide	23.7	1579	0.11
43	α -cadinol	25.5	1653	0.11

Tn: retention time
RI : retention index measured relative to n-alkanes (C6-C24) .
Area % : Relative percentage obtained from peak area.

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