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Chemical composition of *Artemisia Vulgaris* L. from Kashan area isolated by nano scale injection

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Abstract: Essential oil from aerial parts of *Artemisia vulgaris* L., was obtained by hydro-distillation to produce oil in the yield of 0.25% (w/w). The oil was analyzed by capillary gas chromatography, using mass spectrometric detection. The amount of the samples injected by nano scale included were 1.0 nL (diluted 1.0 µL of sample in 1000 ml of *n*-pentane, v/v). Twenty three bioactive and flavour molecules were identified in the oil of *Artemisia vulgaris* with Trans-Caryophyllene (24.76%), 1,8-Cineol (18.64%), Trans-Salvene (14.87%), ß -Cubebene (11.82%), and α -Humulene (5.47%) as main constituents.

Keywords: *Artemisia vulgaris*, Essential oil composition, Nano scale injection, Trans-Caryophyllene, 1,8-Cineol, Trans-Salvene, ß-Cubebene, α-Humulene.

Introduction

Artemisia species belongs to the family Asteraceae, have received increasing interest because of their medicinal applications, especially with regards to flavonoids [1]. They are used in gastric diseases, in malaria, as antifungal, anthelmintic, sedative and emmenagogue agents [2]. *Artemisia vulgaris* L. (mugwort) is a tall aromatic perennial herb, which grows in the hilly districts of India in areas up to 2400 m elevation. In traditional medicine, this plant is widely used for the treatment of diabetes and extracts of the whole plant are used for epilepsy and in combination for psychoneurosis, depression, irritability, insomnia, anxiety, and stress [3]. Infusion of the leaves is given as a vermifuge. Mugwort is commonly used in traditional European medicine as a choleretic and for amenorrhoea and dysmenorrhoea [4]. In herbal medicine, aerial parts of *A. vulgaris* are being used as an anthelminthic, an antiseptic, an antispasmodic, and a tonic for vital organs and for various disorders including hepatosis [5]. In various studies, *A. vulgaris* showed antibacterial activity and showed efficacy in the correction of breech presentation [6]. Its crude extract has been used as an antimalarial agent for thousands of years, and it was found that artemisinin extracted from *A. vulgaris* had antitumor activity [7]. A paste or powder of the leaves is applied over skin diseases. It is used as an inferior substitute for cinchona for treating fever. The plant is used for leucorrhoea, threatened abortion, haemoptysis, vomiting, colic, rheumatism, and impetigo. The active components of *A. vulgaris* identified include flavonoids, coumarins, sesquiterpene lactones, volatile oils, inulin, and traces of alkaloids. The chief compounds of volatile oils include camphor, camphene, α-thujone, germacrene D, 1,8-cineole, and β-caryophyllene [4, 8, 9]. To the best of our knowledge, the essential oil of the aerial parts of this plant in Kashan area has 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. It is clear that, in some plants, the amount of essential oil is trace (less than 1 µL). Thus by this method, (dissolving in a solvent) we can inject the dissolved essential oil in G.C. or G.C./M.S. and find out the components.

Results and Discussion

Air-dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger-type apparatus to produce oil in the yield of 0.25% (w/w). The oil was analyzed by GC and GC/MS. Twenty three, flavour

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Table 1. Bioactive and fragrance components of the aerial parts of A. vulgaris

Compound[a]	A, %	RI [b]	Compound[a]	A, %	RI [b]
Trans-Salvene	14.87	869	Isogeraniol	0.95	1273
Artemisia triene	0.79	935	α -Copaene	2.46	1370
α-Pinene	0.33	937	ß -Cubebene	11.82	1382
ß - Pinene	0.89	970	(-)-ß -Elemene	2.51	1388
1-Octene-3-ol	2.06	977	Trans-Caryophyllene	24.76	1405
ß -Myrcene	2.12	991	α -Humulene	5.47	1447
1,8-Cineol	18.64	1035	Trans- ß -Franesene	1.01	1450
γ -Terpinene	0.43	1057	Bicyclogramcerne	1.69	1490
Trans-Sabinene hydrate	1.46	1096	δ -Cadinene	0.78	1511
Trans-Pinocanveol	0.82	1142	Spathulenol	1.75	1572
Pinocarvone	1.96	1159	Caryophyllene oxide	0.95	1578
Terpinene-4-ol	0.82	1172	**Total identified**	**99.34**	

[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.

and fragrance molecules, constituting 99.34% of the total components detected, were identified in this plant and listed in Table 1 with their percentage. Constituents are listed in order of their elution from HP-5MS column. The oil was characterized by a high content of Trans-Caryophyllene (24.76%), 1,8-Cineol (18.64%), Trans-Salvene (14.87%), ß -Cubebene (11.82%), and α -Humulene (5.47%).

In this paper, we illustrate biological properties and application of two important components from *A. vulgaris* essential oils:

Trans-Caryophyllene: Caryophyllene is one of the chemical compounds that contributes to the spiciness of black pepper, was shown to selectively bind to the cannabinoid receptor type-2 (CB_2) and to exert significant cannabimimetic anti-inflammatory effects in mice, used in food additive and ingested daily with food, it is the first dietary cannabinoid. Whether this compound is able to modulate inflammatory processes in humans via the endocannabinoid system is yet unknown. Beta-caryophyllene does not bind to the centrally expressed cannabinoid receptor type-1 (CB_1) and therefore does not exert psychomimetic effects.

1,8-Cineol (Eucalyptol): is used in flavorings, fragrances, and cosmetics. used as an insecticide and insect repellent, reduces inflammation and pain, kills leukaemia cells *in vitro,* It controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition, effective treatment for nonpurulent rhinosinusitis, treated subjects experienced fewer headaches on bending, frontal headache, and sensitivity of pressure points of trigeminal nerve, impairment of general condition, nasal obstruction, and rhinological secretion.

A number of earlier studies [10–13] generally showed large variation in chemical composition of officinal mugwort essential oils. The essential oils from French officinal mugwort was previously investigated by Carnat *et al.*, [14] who concluded that their representative main compounds are α- and β-thujone, as well as 1,8-cineole, camphor, terpinene-4-ol and borneol. At present it is known that geographic origin influences the chemical composition of officinal mugwort essential oil: those from India are rich in α- and β-thujone, *p*-cymene and camphor, while in those from Poland the major oil compound is 1,8-cineole and in those from England it is linalool [15]. Mucciarelli *et al.* [16] showed that camphor and camphene are the main compounds from Italian officinal mugwort, and Pino *et al.* [17] emphasized caryophyllene oxide as the main compound of this plant from Cuba. Meanwhile, it has been demonstrated that *A. vulgaris* grown in different countries possessed different compositions of essential oils. The oils from Italy are rich in camphor (47.7%), camphene (9.1%) and verbenone (8.6%) [16]. The oils from the Republic of Bashkortostan were found to contain large amounts of α-pinene (53.7%), trans-chrysanthenol (13.1%), β-myrcene (8.8%) and β-pinene (7.4%) [18]. 1,8-Cineole (28.9%), sabinene (13.7%), β-thujone (13.5%), and caryophyllene oxide (6.5%) were reported as the principal components in leaf essential oils of Egyptian *A. vulgaris* plants [19],

whereas in Croatia, the chief components reported are β-thujone (20.8%), α-pinene (15.1%), and 1,8-cineole (11.7%) [20]. The oils isolated from North Lithuanian *A. vulgaris* plants were high in amounts of sabinene, β-pinene, 1,8-cineole, artemisia ketone, cis- and trans-thujone, chrysanthenyl acetate, germacrene D, and β-caryophyllene [20]. The oils from the plants from New York, USA were rich in α-pinene, camphene, β-pinene, 1,8-cineole, santolina triene, β-myrcene, and camphor [21]. The oils isolated from Indian grown plants were characterized by large amounts of camphor (38.7%), isoborneol (8.2%) and artemisia alcohol (4.5%) [9]. α-Thujone was reported as the main constituent of *A. vulgaris* oils. Furthermore, previous papers also reported percentages of α-thujone higher than those of β-thujone [15]. Thus, the oils isolated from different countries differed in composition. This clearly shows that the different geographical locations, environmental conditions, and stress factors the plant faces during its survival and growth affect the accumulation of essential oils. Thus, these findings provide a foundation for future investigations into medicinally active biomolecules in *A. vulgaris*. Also, in India, GC-MS results from leaves of *A. vulgaris* revealed the presence of 88 components and the extracted oil was rich in camphor (16.8%), α-thujone (11.3%), germacrene D (7.2%), camphene (6.5%), 1,8-cineole (5.8%) and β-caryophyllene (5.4%) [22]. essential oils produced from a population of *A. vulgaris* of Vietnamese origin cultivated near Hanoi were subjected to analysis by GC, GC/MS and ^{13}C-NMR. The oils were found to contain oxygenated monoterpenes as major components (1,8-cineole, camphor and α-terpineol). No significant difference was observed between compositions of leaf and flower oils [23]. On the other hand, the leaf oil of *A. vulgaris* collected from India was found to be rich in 1,8-cineole, α-thujone, camphor and isoborneol. The fruit oil contained α-thujone and artemisia alcohol as major components, while camphor predominated in the flower oil [9]. Identification of volatile compounds from fresh leaf tissue of *A. vulgaris* collected from New York (Ithaca), revealed mainly monoterpenes, including Santolina triene, α-pinene, camphene, β-pinene, β-myrcene, limonene, eucalyptol (1,8-cineole), and camphor [22]. Study of the other species of this genus also carried out, for example: essential oil of *A. Selengensis,* mainly included eucalyptol, camphor, borneol, bornyl acetate, α-ylangene, α- copaene, β-gurjuene, β-cedrene, 5,7-dimethyl-1-naphthol, 1-benzophenone, and 1,4-dimethyl-7-(1-methylethyl)-azulene-2-ol [24]. In other study, essential oils were isolated from *A. abrotanum* L., *A. absinthium* L., *A. alba* Turra, *A. annua*, L., *A. campestris* L. ssp. *campestris, A. campestris* L. ssp. *borealis* (Pallas) H. M. Hall et Clements, *A. chamaemelifolia* Vill., *A. genipi* Weber, *A. glacialis* L., A. *petrosa* Baumg. ssp. *eriantha* Ten., *A. umbelliformis* Lam., *A. vallesiaca* All., *A. verlotiorum* Lamotte, *A. vulgaris* L., growing spontaneously in the north-west Italian Alps. GC-MS analyses were carried out in order to determine the percentage composition of the oils. The data obtained were statistically processed in order to partition the species according to their oil composition. The results showed the presence of two main groups of plants. The first group composed of *A. genipi, A. umbelliformis* and *A. petrosa* was characterized by the presence of α-thujone, while camphor and 1,8-cineole characterized the oil of the remaining plants [16].

Experimental

Plant Material:

Aerial parts of *A. vulgaris* were collected in May 2009 in the center region of iran (around the Kashan area). 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:

Dried aerial parts (80g) of *A. vulgaris* were subjected to separate hydrodistillation for 3.5 h using a Clevenger-type apparatus [25]. After decanting and drying over anhydrous sodium sulfate, the sample oil which was dark yellow in color, recovered from the aerial parts in yield of 0.25% (w/w).

Gas Chromatography (GC):

GC analysis of the oil was 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 held at 60˚c for 3 min and then programmed to 250˚c at a rate of 3˚c/min. 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^{-1}.

Gas Chromatography-Mass Spectrometry (GC/MS):

GC-MS analysis of the oil was 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^{-1}. 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 oil was analyzed by GC and GC/MS systems using a non-polar column and identification of components in the oil was 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 [26, 27]. The percentage composition of the sample was computed from the GC-FID peak areas without the use of correction factors.

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