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Original Article

ESSENTIAL OIL COMPOSITION OF *ARTEMISIA VULGARIS* GROWN IN EGYPT

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ABSTRACT

Objective: The objective of this research was to evaluate the significance of the plant's origin and to assess the essential oil composition of *Artemisia vulgaris* grown in Egypt simultaneously evaluating the effect of environmental conditions on essential oil composition.

Methods: Seeds were planted and the essential oils extracted, using hydrodistillation, from the plants that grew. The resulting essential oils were examined, using gas chromatography linked to mass spectrometry (GC-MS). Thus also evaluating the essential oil chemotype "fingerprint" in *A. vulgaris*

Results: The study identified: the most abundant compounds being camphor, 3, 5-dimethylcyclohexane, germacrene D, cubebene, yomogi alcohol, artemisia alcohol, caryophyllene, while is lower concentrations thujopsene, muurolene, borneol, terpinen-4-ol, valencene, elemene and humulene. Despite the origins of the seeds, the chemical profile was very similar to those of plants grown in Egypt, thus suggesting essential oil composition was significantly influenced by the environmental conditions.

Conclusion: Based on the present study, It is suggested that seed origin may play a less significant part if the seed is planted in an environment different to that of its origin, this study proved that and favors the plant-environment interaction to influence the secondary metabolite composition. This supports that plant metabolite profiles are greatly affected by the environment they are grown in.

Keywords: Gas chromatography, Chemotype, Essential oils, Medicinal plant

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INTRODUCTION

The genus *Artemisia* L. is among the largest and most widely distributed genera of the Asteraceae family, consisting of 522 small herb and shrub species native to the northern hemisphere, South America, southern Africa, and the Pacific Islands [1, 2]. A large number of the Asteraceae family genera are important as cut flowers and ornamental crops, as well as medicinal and aromatic plants, many of which produce essential oils used in folk and modern medicine, including the cosmetics and pharmaceutical industry [3, 4]. Well reported for use as tonics, antimalarial, anthelmintic and antidiabetic aids, in treating wounds, bronchitis, ulcers, and tuberculosis in traditional Anatolian medicine [5-7]. There are also several reports concerning the antimalarial, antioxidant, cytotoxic, antipyretic, analgesic, antidiabetic, antimicrobial and antifungal activities of different *Artemisia* species [1, 8,-10].

Artemisia vulgaris L., commonly known as mugwort or common wormwood, is a perennial weed growing wild, native to Asia, Europe and North America, and abundantly in temperate and cold-temperature zones [11]. The plant is widely utilized in the Philippines for its antihypertensive properties. It has also been suggested that the plant possesses other medicinal qualities, such as anti-inflammatory, antispasmodic, carminative and anthelmintic properties, and that it has been used in the treatment of painful menstruation (dysmenorrhoea) and in the induction of labour or miscarriage [12]. Different parts of *A. vulgaris* have been reported to have antibacterial and antiviral activities [13]. Wang *et al.* [14] and Pugazhvendan *et al.* [15] reported on the insecticidal and insect repellent properties of *A. vulgaris* and that it showed great potential in insect control. This was further investigated by Chantraine and others [16] and by Sinha [17] of note is that the essential oils exhibited insecticidal activity.

Phytochemical studies on *A. vulgaris* indicate that a vast myriad of compound classes may be present in the genus, importantly, terpenoids and flavonoids. The rich accumulation of essential oils and other terpenoids in the genus is responsible for the use of the various species for culinary purposes, such as flavoring or liqueurs [1]. Williams *et al.* [18] identified 22 different components in the essential oil of *A. vulgaris* L. Major Components of the oil such as caryophyllene, alpha-zingiberene, borneol and ar-curcumene have all been reported to induce apoptosis [19-21].

Artemisia vulgaris L. has been the subject of numerous phytochemical studies. These studies attempted to explain the chemotypic variation brought about by: geographic origin, harvesting time and environmental edaphic effects on specifically the essential oils [22, 23]. Metabolite profiles are significantly influenced by plant-environment interactions [24, 25]. Coupled with geographic origin influencing genotypic variation, a significantly different chemotype may be observed and possibly with novel compounds when foreign *A. vulgaris* is grown in Egypt.

This study was therefore conducted to investigate the essential oil composition of *Artemisia vulgaris* grown in Egypt, and to identify the presence of a 'fingerprint' tentatively if any, simultaneously evaluating the effect of the environment on the essential oil composition.

MATERIALS AND METHODS

Plant material

Seeds of *Artemisia vulgaris* were obtained from the Komarov Botanical Institute, Saint Petersburg, Russia. Seeds were sown in the nursery on 25 October 2014 on the experimental farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt (30.0224 ° N, 31.2068 °E). Average minimum temperatures range from 4.3 to 17.1 °C, while average maximum temperature range between 29.5 to 43.5 °C annually. Relative humidity averages between 67.9 to 78.8 % with an annual rainfall average in the rainy seasons from 2 to 30 mm. The nursery plot had loamy clay soil. The aerial parts were harvested/collected at the end of May 2015.

Isolation of essential oils

The fresh samples were subjected to hydrodistillation, using a Clevenger-type apparatus for 3 h, as described in the method according to Gunther [26]. These were then dried over anhydrous sodium sulfate, and stored in a desiccator at 4 °C in darkness.

Gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of five essential oil samples was carried out in the second season, using a gas chromatography-mass spectrometry instrument housed at the National Research Center. A TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA); coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer), apparatus was used. The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm I.D., 0.25 µm film thickness). An analysis was carried out, using helium as the carrier gas at a flow rate of 1,0 ml/min and a split ratio of 1:10, and using the following temperature programme: 40 °C for 1 min; rising at 4.0 °C/min to 160 °C and held for 6 min; rising at 6 °C/min to 210 °C and held for 1 min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified by using two different analytical methods: (a) KI, Kovats indices in reference to alkanes (C9-C22) (National Institute of Standards and Technology); and (b) mass spectra (authentic chemicals, Wiley spectral library collection and NIST library).

RESULTS AND DISCUSSION

This study identified several compounds that have already been reported in the literature to be found on *A. vulgaris* (table 1). These include germacrene D, yomogi alcohol, artemisia alcohol, caryophyllene, thujopsene, muurolene, borneol, terpinen-4-ol, camphor, cubebene, elemene and humulene.

Table 1: Compounds detected using GC-MS in *Artemisia vulgaris* essential oil

Retention time	Compound	Kovat index	Area %	Relative % abundance
9.35	alpha linolenic acid	2191	0.45	0.3702
9.35	2-Phenyl-1,3-dioxolane	1215	0.45	0.3702
11.48	yomogi alcohol	1021	0.89	0.7322
12.41	eucalyptol	1059	9.10	7.4866
13.65	1,5-heptadien-4-one 3,3,6-trimethyl	1061	2.91	2.3941
14.53	artemisia alcohol	1068	0.76	0.6253
14.99	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	1139	0.36	0.2962
14.99	cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	1162	0.36	0.2962
14.99	cis-sabinene hydrate	1078	0.36	0.2962
15.56	2,4-dodecadiene	1230	0.90	0.7404
16.61	camphor	1121	13.83	11.3780
16.61	3,5-Dimethylcyclohexene	824	13.83	11.3780
17.13	cyclohexanol, 5-methyl-2-(1-methyle thenyl)	1172	1.37	1.1271
17.13	nerol	1228	1.37	1.1271
17.49	(+)-borneol	1150	3.15	2.5915
17.86	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	1160	0.60	0.4936
17.86	terpinen-4-ol	1137	0.60	0.4936
18.41	camphenol, 6-	1110	2.54	2.0897
18.62	myrtenol	1191	1.13	0.9297
20.19	thymyl methyl ether	1231	0.37	0.3044
20.19	carvacrol	1244	0.37	0.3044
21.54	bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	1302	2.07	1.7030
21.54	endobornyl acetate	1289	2.07	1.7030
23.23	a-Ylangene	1370	0.42	0.3455
23.23	a-muurolene	1491	0.42	0.3455
24.48	alfa-copaene	1221	0.85	0.6993
24.76	bourbonene	1531	0.65	0.5348
25.03	elemene	1398	1.65	1.3575
25.03	germacrene-A	1503	1.65	1.3575
25.56	alpha gurjunene	1495	0.43	0.3538
25.91	caryophyllene	1444	6.28	5.1666
25.91	valencene	1471	6.28	5.1666
26.93	humulene	1579	2.22	1.8264
27.14	valencene	1496	0.38	0.3126
27.88	germacrene-D	1503	10.44	8.5891
27.88	a-cubebene	1353	10.44	8.5891
28.00	thujopsene	1429	2.58	2.1226
28.28	cyclohexane, 1-ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethylidene)-	1488	2.40	1.9745
28.28	bicyclogermacrene	1580	2.40	1.9745
30.03	6,8,8-Trimethyl-2-methylenetricyclo[5.2.2.01,6]undecan-3-o	1599	0.36	0.2962
30.03	longipinocarveol, trans	1634	0.36	0.2962
30.35	palustrol	1567	0.74	0.6088
30.74	spathulenol	1605	4.05	3.3320
31.62	dihydroartemisinin, 3-desoxy-	2009	4.63	3.8091
32.89	cardinol	1660	0.94	0.7733
32.89	muurolol	1652	0,94	0.7733
44.24	ethanol, 2 (9 octadecenyloxy), (Z)	2336	0.57	0.4689
44.24	phytol, acetate	2168	0.57	0.4689

The most abundant compounds being camphor, 3, 5-dimethyl-cyclohexane, germacrene D, cubebene, yomogi alcohol, artemisia alcohol, caryophyllene, while is lower concentrations thujopsene, muurolene, borneol, terpinen-4-ol, valencene, elemene and humulene (Supplementary data fig. 1). Similar compounds have been reported by Williams [27] and Williams *et al.* [28]. Other studies by region include that by Burzo *et al.* [29] in Romania, who found that *A. vulgaris* oil was characterized by high quantities of germacrene D (41,46%) and caryophyllene (11,94%). Govindaraj and Ranjitha Kumari [30] reported that major components of *A. vulgaris* essential oils in India were camphor, camphene, α-thujone, 1,8-cineole, muurolene and caryophyllene, similar to the samples grown in Egypt from this study.

A study by Hwang *et al.* [31] isolated and identified mosquito repellent compounds in *A. vulgaris* essential oil against *Aedes aegypti*. The compounds isolated were mainly monoterpenoids such as linalool, camphor, isoborneol, borneol, terpinen-4-ol, isobornyl, Nonanone-3, (α+β)-thujone, bornyl acetate, β-pinene, myrcene, α-terpinene, limonene, and cineote. These compounds were also identified in samples tested in this study, inferring the presence of the same property.

Older studies on *A. vulgaris* growing in different European countries have been dominated mostly by the monoterpene fraction. German mugwort oil is rich in sabinene (16%), myrcene (14%) and 1,8-cineole (10%) [32]. The oils from Italy contained camphor (47%), alone (27%) or borneol (3–18%) as the major constituents [33]. The amounts of monoterpenes varied: camphor from 1 to 13%, 1,8-cineole 1–23% and terpinen-4-ol 1–19% in the leaves [34] or camphor (2–20%), together with myrcene (9–70%) and 1,8–cineole from oils investigated in France [35]. These suggest a slight "fingerprint", with those observed in this study mostly with the differences of the non-detection of 1,8 cineole, myrcene, limonene, beta pinene and sabinene that characterize the chemotypes reported in the European studies of *A. vulgaris*.

While α-Thujone or thujone isomer and camphor were determined as the main components in *A. vulgaris* from India [36] the oils from Morocco were also rich in isothujone and camphor [37]. Oxygenated monoterpenes (1,8-cineole, camphor, α-terpineol) dominated in the essential oils of *A. vulgaris* of Vietnamese origin [38]. The plants cultivated under Indo-gangetic plain conditions produced leaf essential oil with 1,8-cineole (2.2–12.2%), α-thujone (0–11.4%), camphor (15.7–23.1%) and isoborneol (9.3–20.9%) as predominant compounds, while flower oil was found to be rich in camphor (38.7%) [25]. The sesquiterpene fraction dominated in the mugwort oils from Cuba[38], where caryophyllene oxide (31%) was the predominant component and those from Vietnam [40] with β-caryophyllene (24%), β-cubebene (12%) and β-elemene (6%) as the major constituents. In this study, the oil composition was observed to be closely related to those from India and Morocco.

It is known that the composition of essential oils is characterized by significant variation, depending on the ecological niche occupied. Additionally, sample cytotype is vital in determining essential oil characteristics. Azimova and Glushenkova [41] detected the major essential oils to be camphor (30.0%) and thujone (35.0%), while other compounds included: *iso*-thujone, *α*-thujone, *β*-thujone, camphene, Δ^3-carene, myrcene, *α*-terpinene, 1,4-cineole, 1,8-cineole, artemisia alcohol, *iso*-borneol, *α*-terpineol, *α*-copaene, *β*-caryophyllene, humulene, *α*-guaiene, *δ*-guaiene, allo-aromadendrene, *δ*-cadinene, *iso*-artemisia ketone, borneol, terpineol-4, caryophyllene epoxide 1, caryophyllene epoxide 11, artemisiatrien, artemisia ketone, artemisia alcohol acetate, yomogi alcohol, santalinatriene, liratol, liratyl acetate, artemisia propionate, liratyl propionate, esters of liratol, (*Z*)-liratyl acetate and methyl-5-methylid-2-en–vinyl-3-hexenyl-4-acetate. These results are in full agreement with our own results, as well as with similar studies situated in the same geographic area, despite the seeds originating from a different geographic area (Russia).

Haider *et al.* [25] in India concluded that there were differences in the chemical composition of the essential oil produced from plants harvested at different growth periods; the leaf oil was found to be rich in 1,8 cineole 2.2–12.2%), α-thujone (0–11.4%), camphor (15.7–23.1%) and isoborneol (9,3–20,9%). The fruit oil contained α-thujone (15.5–16.0%) and artemisia alcohol (16.3–17.7%) as major components, while camphor (38.7%) predominated in the flower oil. Sadaka *et al.* [42] found that the major components of the essential oil of the aerial parts of *A. vulgaris* L. grown in Syria were camphor 8,65%, trans-pinocarvyl acetate 7.65%, davanone 6.98%, trans-anethole 6.54%, carene 5.69%, β-caryophyllene 4.31%, 2-methyl-naphthalene 4.45%, germacrene D 4.15%, limonen-6-ol 3.58%, hexahydro farnesyl acetone 3.54%, and β-elemene 2.70%, revealing a marked difference in the composition, caused by the different climatic and geographical conditions.

The study, therefore, identified several compounds that have been reported in earlier studies, showing marked similarities in the chemotypes by geographical area. Simultaneously, the study revealed in this case that seeds originating from a different area may still have to be studied in detail to confirm the effect of genotype-environment on the chemotypes.

CONCLUSION

Application of GC-MS in evaluating essential oil "fingerprint" chemotypes in *A. vulgaris* may be a valuable way of differentiating or identifying where the populations in question can be inferred to originate from. The study identified: germacrene D, yomogi alcohol, artemisia alcohol, caryophyllene, thujopsene, muurolene, borneol, terpinen-4-ol, camphor, cubebene, elemene and humulene, which have been reported in other studies, and also inferred that insecticidal and insect repellent properties may be present. Despite the origins of the seed, plants growing in a particular geographic region tend to produce similar chemotypes, as they are exposed to the same edaphic and geo-climatic conditions. However, there is a requirement to ascertain to what extent the genotypic variation has an effect on the essential oil composition of *A. vulgaris* grown in different geographic areas from that of the seed's origin

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CONFLICTS OF INTERESTS

Declared none

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