ESSENTIAL OIL COMPOSITION OF ARTEMISIA VULGARIS GROWN IN EGYPT

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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 antibiotic agents, 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, antiinflammatory, 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 (dysmenorrhea) 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-sinigrin, borneol and 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) KL 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 | 7.4666 |
| 13.65          | 1,5-heptadien-4-one, 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-methylthyl)- | 1139 | 0.36 | 0.2962 |
| 14.99          | cyclohexanol, 1-methyl-4-(1-methylthyl)- | 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 | 12.83 | 11.3780 |
| 16.61          | 3,5-Dimethylcyclohexene | 824 | 13.83 | 11.3780 |
| 17.13          | cyclohexanol, 5-methyl-2-(1-methyl thyl) | 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-methylthyl)- | 1160 | 0.60 | 0.4936 |
| 17.86          | terpinen-4-ol | 1137 | 0.60 | 0.4936 |
| 18.41          | campholen, 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-endol)-endo-(endo-acetate | 1302 | 2.07 | 1.7030 |
| 21.54          | endo-(endo-acetate | 1289 | 2.07 | 1.7030 |
| 23.23          | a-Yangene | 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          | cyclohexene, 1-ethenyl-1-methyl-2-(1-methylthyl)- | 1488 | 2.40 | 1.9745 |
| 28.28          | bicyclogermacrene | 1580 | 2.40 | 1.9745 |
| 30.03          | 6,8,8-Trimethyl-2-methyl enetricyclo[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 |
| 32.99          | murolol | 1652 | 0.94 | 0.7733 |
| 44.24          | ethanol, 2 (9 octadecenyl), (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, which is lower concentrations thujopsene, muurolene, bornel, 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 (41.4%), and caryophyllene (11.9%). 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, and 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 monoterpene species such as linalool, camphor, bornesol, bornel, terpinen-4-ol, isobornyl, Nonanone-3, (+)-β-thujone, bornyl acetate, β-pinene, myrcene, α-terpinene, limonene, and cineole. These compounds were also identified in samples tested in this study, inferring the presence of the same property.

Other studies on A. vulgaris growing in different European countries have been dominated mostly by the monoterpene fraction. German mugwort oil is rich in sabine (16%), myrcene (14%) and 1,8-cineole (10%) [32]. The oils from Italy contained camphor (47%), alone (27%) or bornel (3-18%) as the major constituents [33]. The amounts of monoterpene 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 sabineh 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-terpinol) 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 (22-12.2%), α-thujone (0-11.4%), camphor (15.7-23.1%) and isoborneol (9.3-20.9%) as predominant components, while 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 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-terpinol) 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 (22-12.2%), α-thujone (0-11.4%), camphor (15.7-23.1%) and isoborneol (9.3-20.9%) as predominant components, while 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.

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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 8.6%, trans-pinoacarvyl acetate 7.65%, davanone 6.98%, trans-anethole 6.54%, carene 5.6%, β-caryophyllene 4.31%, 2-methyl-naphthalene 4.45%, germacrene D 4.15%, limonen-6-ol 3.58%, hexahydror farnesyl acetone 3.54%, and β-elemene 2.7%, 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, bornel, 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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