CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF THE *ARTEMISIA ARBORESCENS* L. GROWING WILD IN LEBANON

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ABSTRACT

The essential oil obtained by hydrodistillation from air dried aerial parts of Artemisia arborescens L. from Lebanon was analyzed by GC/MS. Forty three compounds representing 95.33% of the oil sample were identified. The major component was β-thujone (68.5%), followed by chamazulene (12.3%), and some lesser amounts of terpinen-4-ol (1.8%), myrcene (1.3%), α-thujone (1.2%), linalool (1%), cis-thuyanol-4-ol (1%), carvacrol (0.9%), β-cubebene (0.8%) and camphor (0.8%).

Keywords: *Artemisia arborescens* L., essential oil, *Asteraceae*, β-thujone, chamazulene

INTRODUCTION

The genus *Artemisia* (*Asteraceae*) is one of the largest and most widely distributed of the approximatively 60 genera in the tribe of the *Anthemideae*. This genus comprises a variable number of species, ranging from 200 to over 400. It is predominantly distributed in the northern temperate region of the world in the 0-50 cm precipitation area (Tan *et al.,* 1998), five of them are reported in Lebanon (Mouterde, 1983).

Many traditional uses from the genus were reported as antimalarial, antiviral, antitumor, antipyretic, antihemorrhagic, anticoagulant, antianginal, antioxidant, antihepatitis, antiulcerogenic, antispasmodic, anticomplementary and interferon inducing activity (Tan *et al.,* 1998), emmenagogue, diuretic, antispasmodic, abortive (Bnouham *et al.,* 2002) hypoglycemic, febrifuge (Arnold, 1985; Arnold *et al.,* 1993), antirheumatic and antiarthritis (El Beyrouthy *et al.,* 2008), antioxidant activity (Dessi *et al.,* 2001). Internally, infusion of

fresh leaves or flowering aerial parts: diuretic, anthelmintic, intestinal troubles, relieves gastro-intestinal cramps. Externally used as cataplasm of crushed leaves for snake and scorpion bites or decoction for wounds and sores applied locally as antiseptic and antifungal (Arnold *et al*., 1993).

The large genus of *Artemisia* has been the subject of numerous chemical studies (Marco & Barbera, 1990). *Artemisia arborescens* L. (syn: *Absinthium arborescens* Moench, *Artemisia elegans* Salisb., *Absinthium arborescens* (L.) Gaertn.) grows along the Mediterranean coast of European and African countries. In Lebanon, *A. arborescens* grows wild on rocks, old walls and especially in the coastal area at Ras al Biyyada. The flowering period is from May to August (Mouterde, 1983). *Artemisia arborescens*, which is commonly known in the Mediterranean area with the English denomination of "arboreous absinth" or "arborent mugwort" and in Lebanon "Daqn-al-sheikh, Shajarat Mariam, Shaybah and Rihān 'abyad" is a perennial and aromatic plant of about 60-100 cm in height with woody, erect and branched stems. The leaves have a whitish silvery color in the youngest part of the plants (Mouterde, 1983). In Lebanon this shrubby mugwort is valued as medicinal for its aerial parts (flowers and leaves) used in folk medicine for its antidiabetic, antipyretic and anti-inflammatory properties used by rural inhabitants of the areas where it grows wild.

Numerous reports on essential oils composition of different *Artemisia* species have been published, especially on those used for various purposes as flavorings, fragrances, rodent and mites repellent and as folk medicine for antispasmodic, antipyretic, anti-inflammatory and abortifacient activities (Gildemeister & Hoffman, 1961; Abu Zarga *et al.,* 1995). In previous investigations, aerial parts of this species have been shown to contain acetylenes flavonoids, sesquiterpenes lactones, homoditerpene endoperoxide and sesamine-type lignans, azulene derivative (Grandolini *et al.,* 1988; Betto *et al.,* 1988; Marco & Barbera, 1990; Abu Zarga *et al.,* 1995; Alberto Marco *et al.,* 1997).

In previous research the composition of *A. arborescens* has been reported in the literature by several authors for oils from different geographical origin: Liguria and Sardinia (Sacco *et al.,* 1983), Albany, Oregon (Tucker *et al.,* 1993), Mediterranean origin (Cotroneao *et al.,* 1993), Karpathos (Arnold *et al.,* 1993), Messina, Sicily (Cotroneao *et al.,* 2001), Morocco (Bnouham *et al.,* 2002), Calabria, Sicily and Island of Lipari (Lo Presti *et al.,* 2007). However, nothing is known about the composition of *A. arborescens* essential oil growing wild in Lebanon. The aim of the present study was to explore the composition of *A. arborescens* essential oil native to this country.

MATERIALS AND METHODS

Plant collection

The aerial parts (leaves, stems and flowers) of *A. arborescens* growing wild in Lebanon were collected in Ras al Bayyada (Fig.1). All samples were collected at the early flowering stage from May till July 2007, a voucher specimen number MNV425 has been deposited in the herbarium of H.S.U.K. (Holy Spirit University of Kaslik, Faculty of Agriculture). The taxa were identified by Prof. Nelly Arnold-Apostolides according to the analytical keys prescribed in the Flora of Lebanon (Mouterde, 1983).

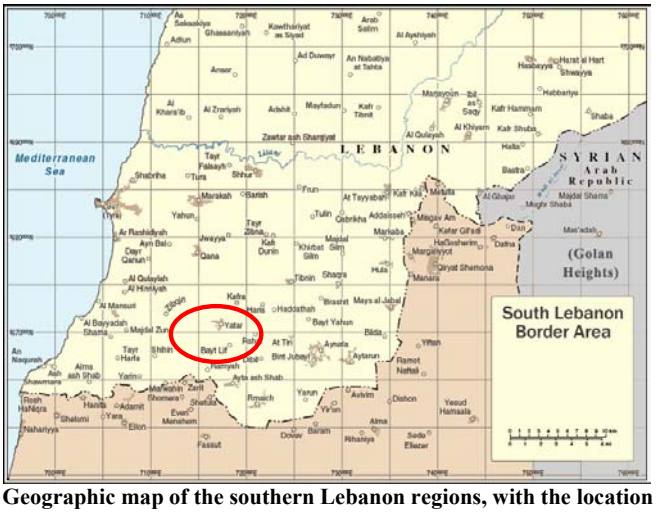

Figure 1. Geographic map of the southern Lebanon regions, with the location of Ras al Bayyada indicated.

Essential oil isolation

The aerial parts (100g of leaves, flowers and stems) were dried at room temperature (20-25°C), then ground and subjected to hydrodistillation using a Clevenger-type apparatus (for 4h) in accordance with European pharmacopoeia standards. The oil was dried over anhydrous sodium sulfate and stored under refrigeration at 4°C until used. The oil was dark blue green possessing a strong aromatic smell.

Experimental

Analysis of the essential oil was determined by GC and GC/MS. GC analysis was performed using a Varian gas chromatograph model 3300 equipped with a flame ionization detector 300°C. The parameters were:

- Capillary column: Carbowax 20M, 25m(length) x 0.31mm (diameter), 0.5µm film
- Detector temperature: 300°C
- Injector temperature: 250°C
- Oven temperature kept at 60°C for 3 minutes then programmed at 4°C/min to 200°C for 3 minutes

The carrier gas was nitrogen, at a working flow rate of 4ml/min and the injected quantity was 1µl of essential oil (diluted in ether to 40%).

GC/MS analysis was done on a Hewlett-Packard mass spectrometer model 5890Å coupled to electronic system HP 900/3000 and using a capillary column Carbowax 20M, 25m(length) x 0.32mm (internal diameter), 0.1µm film. The carrier gas was helium and the

working flow rate was 1ml/min. The oven temperature was kept at 60°C for 3 minutes then programmed at 4°C/min to 200°C for 10 minutes.

The identification of the peaks was assigned by comparing the GC relative retention time with those of authentic substances analyzed under the same conditions. The structure and stereochemistry of 43 constituents were identified by GC/MS analysis and by comparison of their mass spectra with those of authentic samples analyzed under the same conditions and with published ones (Stenhagen *et al.,* 1974; Masada, 1976; Swigar & Silverstein, 1981).

RESULTS AND DISCUSSIONS

TABLE 1

Chemical Composition of the Extract of Aerial Parts of *Artemisia arborescens* L. from Ras al Biyyada, Lebanon

Abbreviations: T= traces, TR= retention time, CPG=gas chromatograph, SM= mass spectrometer

The oil content was 1.7% (V/W) based on dry material of *A. arborescens* L. In Table 1, the list of 43 components identified in the essential oil of *A. arborescen*s is given. It was mainly composed of β-thujone (68.5%), followed by chamazulene (12.3%) and of some lesser amounts of terpinen-4-ol (1.5%), myrcene (1.1%), linalool and cis-thuyanol-4-ol (1%), α-thujone (0.9%), and sabinene (0.8%).

As can be seen, β-thujone, followed by chamazulene represent more than 80% of the essential oil. One notes that chamazulene is responsible for the dark blue green color of the volatile oil.

When these results are compared with those of previous research, some differences are found between them. This may be due to conditions of analysis as well as to climatic, seasonal, geographical and other extrinsic conditions, such as where and when the samples were collected. According to Tab. 2, the major components β-thujone and chamazulene reveal geographical variations in the Lebanese oil composition in comparison with published data from other origin, in Liguria and Sardinia (Sacco *et al.,* 1983), Albany, Oregon (Tucker *et al.,* 1993), Messina – Sicily (Cotroneao *et al.,* 2001), Morocco (Bnouham *et al.,* 2002), Calabria, Sicily and Island of Lipari (Lo Presti *et al.,* 2007), however the data obtained from Lebanon is quite similar to that described for the oil composition of *A. arborescens* from Karpathos island (Greece) (Arnold *et al.,* 1993).

TABLE 2

Comparative β-Thujone and Chamazulene Content in *Artemisia arborescens* L. Essential Oil from Different Origins

β-thujone		
%	**Origin**	**References**
74	Morocco	Codignola, 1984
68.9	Karpathos	Arnold *et al.,* 1993
68.5	Lebanon	Present work
60	Italy	Sacco *et al.,* 1983
23.97	Sardinia (Italy)	Lai *et al.,* 2005
6.6	Lipari (Italy)	Lo presti *et al.,* 2007
1.5	Sicily (Italy)	Lo presti *et al.,* 2007
0.17	U.S.A	Tucker *et al.,* 1993
0	Calabria (Italy)	Lo presti *et al.,* 2007

chamazulene		
%	**Origin**	**References**
37.6	Sicily (Italy)	Lo presti *et al.,* 2007
34.6	Lipari (Italy)	Lo presti *et al.,* 2007
27.1	Calabria (Italy)	Lo presti *et al.,* 2007
21.39	U.S.A	Tucker *et al.,* 1993
13.3	Karpathos	Arnold *et al.,* 1993
12.3	Lebanon	Present work
11.32	Italy	Sacco *et al.,* 1983
10	Spain	Hurabielle *et al.,* 1982
7.66	Sardinia (Italy)	Lai *et al.,* 2005
0.6	Morocco	Codignola, 1984

Previous studies have reported seasonal variations in the thujones content, relative to other Artemisia species (Boutekedjiret *et al.,* 1992). The percentage of β-thujone in this oil

is less than of the one from Morocco (74%) and higher than those of Karpathos Island, Greece (68.9%) and Italy (60%). The Lebanese *A. arborescens* belongs to the Moroccan high thujone type, characterized by deep blue to blue black oil with odor characteristic of plant and distinctly thujone-like; powerful, minty and woody, with a definite slight milk-like note presence. The essential oil of *A. arborescens* native to Lebanon, has had no formal safety testing, it is best avoided in pregnancy, and for babies and children especially because of the higher amounts of β-thujone. Thujone, which can be quite toxic, occurs in two isomeric forms, the α-form being considerably more toxic than the β-form. In Lebanon, α-form is quite negligible (0.9%) but the β-form is quite high (68.5).

In comparison with the essential oil of *A. arborescens* from other countries, one can conclude that the proportion of chamazulene in Lebanon (12.3%) was less than those of Sicily, Lipari and Calabria (Italy), Karpathos (Greece), with respectively 37.6%, 34.6%, 27.1% and 13.3% (Lo presti *et al.,* 2007), U.S.A (21.39 %) (Tucker *et al.,* 1993) more than those of Italy (11.32%) (Sacco *et al.,* 1983), Spain (10%) (Hurabielle *et al.,* 1982), Sardinia (Italy) (7.66%) (Lai *et al*., 2006) and Morocco (0.6%) (Codignola, 1984). Essential oils that contain chamazulene are important in therapeutic applications because of its apparent radical scavenging activity. Interesting research supporting this activity was carried out by Rekka *et al*. (1996), who investigated the role of chamazulene *in vitro* experiments using an iron (II)/ascorbate system to generate hydroxyl radicals inducing membrane lipid peroxidation in liver microsomes. It was found that chamazulene is a potent hydroxyl radical scavenger and effectively inhibits lipid peroxidation. The ability of chamazulene in radical scavenging is what is thought to be responsible for its effectiveness in treating inflamed skin conditions. *A. arborescens* was shown to have weak antibacterial action except towards *Clostridium sporogenes* when tested with twenty-five test bacteria. Skin care applications may include: burns, sensitive skin, acne, keratosis, sunburn, inflammations and infections of the skin (Ikan, *et al*., 1993). For respiratory problems, inhalation could be beneficial for bronchitis, (asthmatic & catarrhal), coughs, sinus and chest congestion.

Chamazulene is a component of the *Anthemideae* tribe which has also been isolated from the essential oil of other species of the genus *Artemisia*: *A. copa* (6.5%) (Collin *et al.,* 2004), *A. absinthium*, *A. caruthii* and *A. macrocephala* (Hurabielle *et al.,* 1982), *A. canariensis* (Bellomaria *et al.,* 1990), but also other genus of *Asteraceae* family which contain variable quantities of chamazulene: *Stevia serrata* (54.7%), *Achillea millefolium* subsp. *alpestris* (43.1%) (Carnat & Lamaison, 1990), *Achillea millefolium* (25.4%) (Haggag *et al.,* 1975), *A. collina* (15.1-18.4%) (Verzár-Petri & Cuong, 1977) and *Matricaria recutita* (10%) (Vuorela *et al.,* 1989). In *Achillea millefolium* ssp. *elbursensis,* the percentage of chamazulene in the oil obtained by hydrodistillation from the flower was 54%, and 35% in the leaf extract (Jaimand *et al.,* 2006).

CONCLUSION

The chemical composition of the essential oil of *A. arborescens* growing wild in Lebanon was investigated. The data obtained in this study showed a remarkable quantitative variation of constituents in the oil. To the major constituents belonged β-thujone (68.5%), chamazulene (12.3%), followed in some lesser amounts by terpinen-4-ol (1.5%), myrcene (1.1%), linalool and cis-thuyanol-4-ol (1%), α-thujone (0.9%), and sabinene (0.8%). Most of these constituents dominated in mugwort oils from other countries.

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