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COMPOSITION OF *ARTEMISIA ANNUA* ESSENTIAL OIL OBTAINED FROM SPECIES GROWING WILD IN BULGARIA

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ABSTRACT

The Artemisia annua essential oil was obtained by hydro distillation of species growing wild in Bulgaria and was analyzed by GC/MS. Thirty six compounds were identified. The main ones were α-caryophillene (24.73%), α-cuvebene (13.53%), α-copaene (7.42%), α-selinene (8.21%) artemisia ketone (8.45%) and camphor (3.61%). The composition of the essential oil appeared to be different from those of species growing in other geographic locations.

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Introduction

The genus Artemisia, with up to 500 species, is one of the largest and most widely distributed genus of the tribe Anthemideae, family Asteraceae. In ancient Greece and in the Roman Empire the essential oil from the *Artemisia* species was used in infusion as poison antidote and for its abortive qualities indicated in gastric insufficiency. It also activates the blood circulation and has antimalaric and antihelmintic properties (3, 4). There are number of studies concerning the chemical composition of the *Artemisia annua* essential oil obtained from species growing in different geographic locations but there is no analysis concerning its composition from species growing in Bulgaria.

The aim of the present study is to find out how the geographic location influences the qualitative and quantitative composition of the *Artemisia annua* essential oil and to search for compounds which can serve as marker compounds for the identification of the *Artemisia annua* essential oil.

Materials and Methods

Plant material

Artemisia annua was collected in the area of Kazanluck (Bulgaria) in June 2009. Voucher specimens were determined by Dr Zornitsa Kamenarska and were deposited in the herbarium of the Joint Genomic Center.

Isolation and analysis of the essential oil

The aerial parts of the plant were immediately subjected to 2.5-hour hydro distillation in order to obtain the woodworm water. A sample of the wormwood water (20 ml) was consecutively extracted with 3x20 ml chloroform and another one with diethyl ether. The obtained essential oil (13.5 mg with diethyl ether and 16.5 mg with chlorophorm) was dried over anhydrous sodium sulfate and was subjected to GC/MS analysis.

GC/QQQ 7000A Agilent Technologies, with a capillary column HP-5MS (30 m\0.25 mm, 0.25 μm film thickness). Carrier gas was Helium and a flow was fixed to 1 $ml.min^{-1}$. Temperature programme was: 40°C with a 5 min hold to 250°C at $4°C \cdot min^{-1}$ and a 15-min hold at 250°C. The ion source was set at 260°C and the ionization voltage was 70 eV. The identification of the compounds was performed by using NIST05 chromatographic library.

Results and Discussion

The composition of the obtained essential oil from *Artemisia annua* is presented in **Table 1**. The components are listed in order of their elution on the column. Thirty-six compounds were identified representing 85.42% of the total oil. The oil consists of 19 monoterpenoids (18.00%) and 17 sesquiterpenoids (67.42%). The main sesquiterpenes are α-caryophillene (24.73%), α-cuvebene (13.53%), α-copaene (7.42%), α-selinene (8.21%) and the main monoterpenes are the irregular monoterpene artemisia ketone (8.45%) and the monoterpene aldehyde camphor (3.61%).

The composition of the *Artemisia annua* essential oil is pretty different from the composition of the essential oil obtained from species growing in Iran, in which the 7 sesquiterpenoids comprise 12.59% and the 24 monoterpenoids-83.72% of the total oil. The major components of the essential oil were camphor (48.00%), 1,8-cineole (9.39%), camphene (6.98%) spathulenol (4.69 %) and artemisia ketone (2.68%) (9).

The majority of the compounds identified in the essential oil of *Artemisia annua* growing in Ethiopia were monoterpenes which account for 57.89% of the constituents. Sesquiterpenes and phenols comprised 36.84% and 5.55% of the identified compounds, respectively. In that analysis camphor was identified as the major component (43.84%). Among the identified monoterpenes 52.17% were monoterpene alcohols, 30.43% - monoterpene hydrocarbons, 13.04% - monoterpene aldehydes and 4.35 % were monotrpene oxides (8). Similarly

to our sample in which α-caryophyllene is the major compound a derivative of caryophyllene caryophyllene oxide was found in relatively high concentration (7.55%).

TABLE 1

Composition of the essential oil (% of the total volatile compounds*)

№	Compound	Sample	Compound type
1	Artemisia triene	0.25	Monoterpene hydrocarbon
2	α-Pinene	1.26	"
3	Camphene	0.4	"
4	Phellandrene	<0.1	"
5	Pseudopinene	0.32	"
6	Cymol	<0.1	"
7	Sylvestrene	<0.1	"
8	1,8-Cyneol (Eucaliptol)	2.55	Monoterpene oxygenated
9	trans-Ocimene	<0.1	Monoterpene hydrocarbon
10	γ-Terpinene	<0.1	"
11	Artemisia ketone	8.45	Monoterpene aldehyde
12	trans-Pinocarveol	0.43	Monoterpene alcohol
13	Camphor	3.61	Monoterpene aldehyde
14	Pinocarvone	0.73	"
15	Borneol	<0.1	Monoterpene alcohol
16	Terpinen-4-ol	<0.1	"
17	α-Terpineol	<0.1	"
18	Myrtenol	<0.1	Oxygenated monoterpene
19	Elixene	0.45	Sesquiterpene hydrocarbon
20	γ-Elemene	0.26	"
21	α-Copaene	7.42	"
22	α-Bourbonene	<0.1	"
23	α-Elemene	3.41	"
24	cis-Lanceol	0.26	Sesquiterpene alcohol
25	α-Cedrene	0.28	Sesquiterpene hydrocarbon
26	Caryophyllene	24.73	"
27	Muurolene	0.54	"
28	Farnesene	2.23	"
29	α-Humulene	3.68	"
30	α-Cuvebene	13.53	"
31	α-Selinene	8.21	"
32	Cadinene	1.03	"
33	Ledene oxide	1.39	Oxygenated Sesquiterpene
34	Isoaromadendrene epoxide	<0.1	"
35	Corymbolone	<0.1	Sesquiterpene hydrocarbon
36	Eugenol	<0.1	Monoterpene alcohol

*The ion current generated depends on the characteristics of the compound and is not a true quantification

The essential oil of *Artemisia annua* aerial parts, that were obtained from species growing in Marseilles (France) consisted of camphor (44%), germacrene D (16%), *trans*-pinocarveol (11%), β-selinene (9%), β-caryophyllene (8.9%) and artemisia ketone (3%) (5).

The essential oil, extracted from the aerial parts of plants grown at Lucknow (India) contained as main constituents artemisia ketone (58.8%), 1,8-cineole [eucalyptol] (10.2%) and camphor (15.8%) while the essential oil, extracted from aerial parts of plants grown in Kashmir contained artemisia ketone (52.3%), 1,8-cineole (13.1%) and camphor (15.5%) as major compounds (1).

Conclusions

It is evident that the geographic location greatly influences the type and concentration of the main constituents. Other factors which determine the chemical composition and thus the biological activity are the chemical and biological treatment. Malik et al. (2009) (7) have found different composition of the essential oil when the plant was treated with *Azospirillum*, basal N, P, K and S and *Glomus*. Bagchi et al. (2003) (2) have found out that the different growth periods at which the sample is collected also affect the essential oil composition. Another factor which affects the composition of the essential oil is the drying temperature (6). Despite the observed qualitative and quantitative differences due to the different geographic location, compounds such as camphor and artemisia keton are typical for *A. annua* essential oil and can serve as marker compounds for its identification. Further investigations concerning species grown in different locations in Bulgaria at different environmental conditions and collected at different periods would lead to further information concerning the composition of *A. annua* essential oil.

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