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STUDY OF ESSENTIAL OIL COMPOSITION OF SIDERITIS POPULATIONS FROM SOUTH OF ALBANIA

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ABSTRACT

Sideritis, known as mountain tea is a genus of flowering plants well known for their use as herbal medicine, commonly as a tisane. The Sideritis herbs were sampling in June, 2016 in different stations of mountains of South of Albania. The air dried plant samples were subjected to European Pharmacopoeia apparatus (Clevenger type) for 6 hours to obtain Sideritis oil in 1 ml Toluene. The chemical composition of the essential oils was analyzed using GC/FID technique. The oil of each Sideritis sample was injected in a Varian 450 GC. VF-1ms capillary column (30m x 0.33mm x 0.25 um) were used for separation of compounds. Alfa-Pinene (27.2 - 32.7 %), beta-Pinene (37.9– 45.4 %), beta-Phellandrene (2.2 - 4.4%), alfa-Copaene (1.6 – 3.5 %) and Bicyclogermacene (1 – 1.6 %) have been identified as the main components of the essential oils of Sideritis samples from South of Albania. Other compounds were found to be lower than 1%. The composition of the essential oil extracted from the Sideritis plant is associated with type, time of sampling and geographic position. The reported levels of the main compounds were the same with reported levels from other Balkan areas.

Keywords: Sideritis, Medicinal Plant, Essential Oils, alfa-Pinene, beta-Pinene, GC/FID;

Introduction

Mountain Tea is very popular in Albania and other Balkan countries used often in winter when levels of physical activity decrease and colds, aches, and pains increase. It is used for respiratory problems, digestion, the immune system, mild anxiety, and as an anti-oxidant. It is also used as an anti-inflammatory and to reduce fever. This plant is also the most popular between medicinal plant used in popular medicine. About 250 different plant species are wild harvested for medicinal and aromatic use in Albania. Medicinal and aromatic plant (MAP) species traditionally harvest in Albania include *Sideritis* (Mountain tea), sage *Salvia spp.*,*Origanum vulgare* and *Thymusspp.* Medicinal and Aromatic Plants is a major agro-forestry business in

Albania, especially in terms of international trade. Balkan countries are a major source of raw material or half finished products for many EU and US industries in different fields (food and beverage industry, healthcare, cosmetics & perfumes, additives etc.) and Albania, together with Bulgaria, is the main supplier. Since 2000 exports keeps increasing with destinations to Germany and USA. MAPs are widely used by individuals for cooking, preparation of herbal tea or traditional medicine; the industrial use of MAPs is extremely small and limited to the packaging of some herbal tea and to the small scale extraction of essential oils. No production of detergents, cosmetics or industrial medicines based on MAPs is recorded until now in Albania. MAPs are found and collected all over the country. The main areas are: Malesia e Madhe, Shkoder, Skrapar, Elbasan, Korce, Berat,Permet, Ersekeand Durres. In these districts the harvesting and consolidation system is also more organized. Collecting wild MAPs is an important activity and source of revenue for a large number of rural families. Collection of main MAPs is made mostly between July and August (Wolfgang et al., 2003; Ibraliu et al., 2011).Its flavor value and properties are due to the aromatic and other volatile compounds generally referred to as essential oils of the aromatic plant. Essential oils derived from MAPs have valuable pharmacological properties that have been investigated by many scientists around the world (Bozin et al., 2006).

Essential oils in everyday language called essences of plants used for medicinal purposes and as flavoring substances for cosmetics and culinary. Essential oils are chemical mixtures of different classes (terpenes, polyphenols, alcohols, aldehydes, ketones, acides, etc), obtained from plants through distillation and extraction. Their study included a variety of methods of analysis ranging from classics to the most modern methods of instrumental analysis methods such as chromatography combined with mass spectrometry. One of the key aspects in the analysis of essential oils is to identify its composition. Identification of compounds based on retention times with gas chromatography method is always uncertain because of the great number of components with similarities in their physical-chemical properties. One of the most typical interference in identifying components of an essential oil with gas chromatographic is the simultaneous determination of sesquiterpenes together with polar components (alcohols, esters, phenols, etc.). Processing industry essential oils applies various techniques to achieve their isolation. Stream distillation of dried plant is the main techniques used for extracting essentials oils from plants. Other extraction techniques such as Clevengar, Soxhlet,Tilepapeapparatus, etc. often used in laboratory tests of essentials oils.

MATERIALS AND METHODS

2.1. Sampling of *Sideritis* samples from South of Albania

Sideritis samples were taken in June 2016 in different stations of Nemërçka, Gramozi, and Dhëmbeli mountains from South of Albania. The sampling site was shown in Figure 1 and the geographical position in Table 1.

Figure 1. The map of Sideritus sampling site from population of Gramozi, Dhëmbel and Nëmërçka Mountains, June 2016

Table 1. Geographic position of sampling station for Siderites samples from South of Albania

Station	**Geography position**		**Heigh**
	N	**E**	**m**
Nëmërçkë	40^0 04` 8.22``	20^0 31` 55.8``	980
Nëmërçkë/1	40^0 05` 8.15``	20^0 31` 8.74``	832

Nëmërçkë/2	40^0 05` 6.76``	20^0 31` 8.14``	920
Dhëmbel	40^0 12` 39.02``	20^0 19` 27.09``	1425
Gramoz/Greqi	40^0 23` 29.21``	20^0 50` 09.40``	1409
Gramoz/Albania	40^0 24` 24.8``	20^0 43` 9.55``	1310

2.2. Pretreatment of Sideritus plants

Sideritus samples (entire plant without their roots) were air dried in shadow for save the morphological and chemical characteristics. The samples plants were cut in small parts. 50 g of *Sideritis* samples were put in a 250 ml balloon. 150 ml distilled water and 2 ml Toluene were added to the balloon for isolation of *Sideritis* essential oils.

2.3. Isolation of Sideritus essential oil

Plant material (50 g of *Sideritis*) was subjected to hydro distillation for 3 h, using a Clevenger type apparatus recommended by European Pharmacopoeia for 6 hours to produce essential oil. The oil diluted to 2 ml Toluene was dried by anhydrous sodium-sulphate (Na_2SO_4) and kept sealed in dark glass vial at +4^{o}C until them were injected to GC/FID.

2.4. Apparatus and chromatography

Gas chromatographic analyses were realized with a Varian 450 GC instrument equipped with a flame ionization detector and PTV detector. The temperature of PTV injector was 260^{o}C. 1 ul of *Sideritis*essential oil diluted in Toluene was injected in splitless mode. A temperature for FID was held at 280°C. Nitrogen was used as carrier (1 ml/min) and make-up gas (25 ml/min). Hydrogen and air were flame detector gases with 30 ml/min and 300 ml/min, respectively. VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was used to isolate compounds of *Sideritis* essential oil. The oven temperature was programmed as follows: 40°C (held for 2 minutes) to 150^{o}C (with 4°C/min), after that to 280 °C with 10°C/min and held for 2 minutes. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and literature (Adams, 1995; David et al., 2010, Kong et al., 1999; Oladipupo and Adebola, 2009).

RESULTS AND DISCUSSION

The results of analysis of *Sideritis* essential oil samples from South of Albania were shown in Table 2. Representative *Sideritis* samples were analyzed from different stations of Nemërçka, Gramozi and Dhëmbeli Mountains from South of Albania. Samples were air dried in shadow for save physiological and chemical characteristics of *Sideritus* samples. The extraction of essential oil was made using Clevengar apparatus recommended from the literature. Chemical analyze of *Sideritis* essential oil samples were performed by capillary-GC/FID technique. The data presented a total of 16 the main compounds that were found for all *Sideritis* oil samples by the chromatographic method used. The data were in percent for the total of peaks except for the peak of Toluene that was the solvent use for dilution. The peaks lower than 0.01% was not present in this study. Chromatogram of the *Sideritis* essential oil for sample site in Dhëmbeli (Albania) was shown in Figure 2. Percent of analyzed compounds for all samples were shown in Figure 3. For all samples the main components was shown to be alfa-Pinene (27.3 - 32.6 %), beta-Pinene (38.9 – 45.3 %), beta-Phellandrene (2.3 - 4.5%), alfa-Copaene (1.7 – 3.4 %) and Bicyclogermacene (1 – 1.6 %). The other compounds were found to have the average lower than 1%. This composition of the essential oil from the *Sideritus* plant in Albanian South Mountains is associated with its type, time of harvesting the crop, its dry and geographic position and composition of thes oil where it grows. Distribution of analyzed components for *Sideritis* samples from South Albania was shown in Figure 4. Was the same distribution for all samples. This is due of the same *Sideritis* species analysed from the same area. The geographical position (particulary the height) is a important factor for the differences found between the analyzed *Sideritis* samples. Profle of analyzed components for *Sideritis* samples from South Albania mountains was shown in Figure 5. For all samples was found: beta-Pinene > alfa-Pinene >beta-Phellandrene>alfa-Copaene>Bicyclogermacene. The average levels of other studied compounds in Sideritis plants were found lower than 1%. Profile and leveles of *Sideritis* samples from South Albania mountains was the same with other reported studies from Mediterrean area (Trendafilova et al., 2013; Formisano et al., 2010)

Tabela 3. Sideritus samples data using GC/FID technique

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	Gramoz/Al	Gramoz/Gr	Dhëmbel	Nëmërçkë	Nëmërçkë/1	Nëmërçkë/2
alfa-Pinene	27.300	32.620	29.030	30.400	30.520	30.310
alfa-Thujene	1.230	0.783	0.980	0.780	0.400	0.450
Camfene	0.050	0.320	0.220	0.100	0.290	0.140
beta-Pinene	38.900	38.390	49.540	46.340	41.590	47.560
Sabinene	0.980	1.380	1.554	1.320	1.320	1.300
Myrcene	0.670	0.701	0.590	0.830	0.980	1.300
Limonene	0.730	1.540	1.230	1.400	1.200	1.480
beta-Phellandrene	4.300	4.600	3.000	3.400	2.300	3.870
gama-Terpinene	0.321	0.550	0.390	0.320	0.340	0.720
para-Cymene	0.470	0.100	0.087	0.110	0.090	0.780
1-Octen-3-ol	0.850	0.100	0.110	0.210	0.000	0.100
alfa-Copaene	2.100	1.980	3.400	2.400	1.670	2.760
beta-Burbonene	0.120	0.120	0.110	0.230	0.140	0.100
Linalool	0.350	0.250	0.320	0.350	0.200	0.430
Bornyl Acetate	0.430	0.120	0.540	0.530	0.360	0.220
Bicyclogermacene	1.540	1.020	1.200	1.340	1.320	1.320

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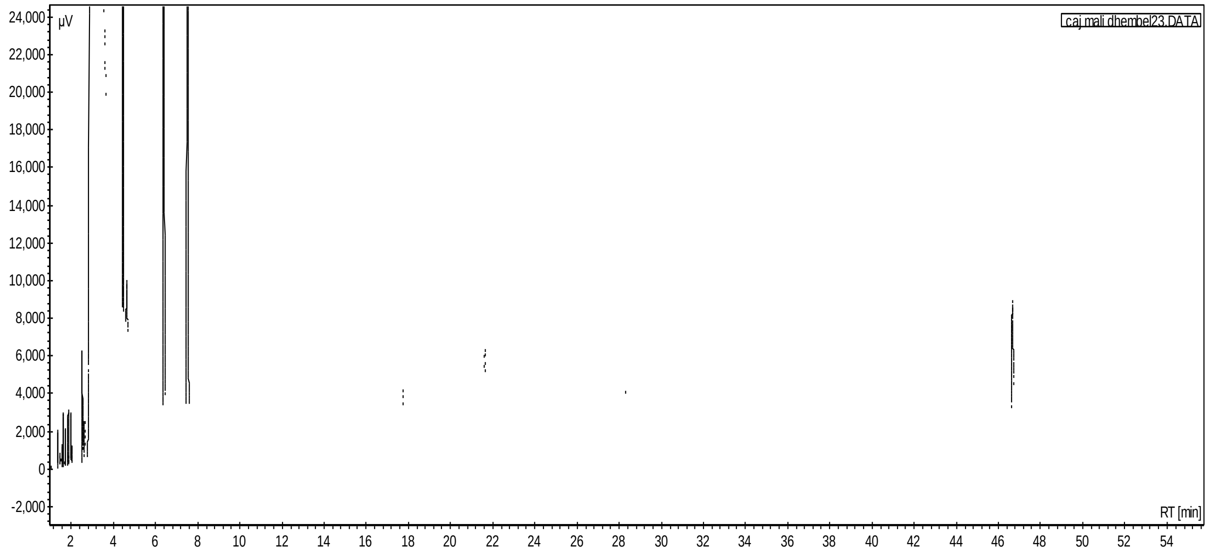

Figure 2. Chromatogram of *Sideritus* sampling site from Nëmërçka Mountain

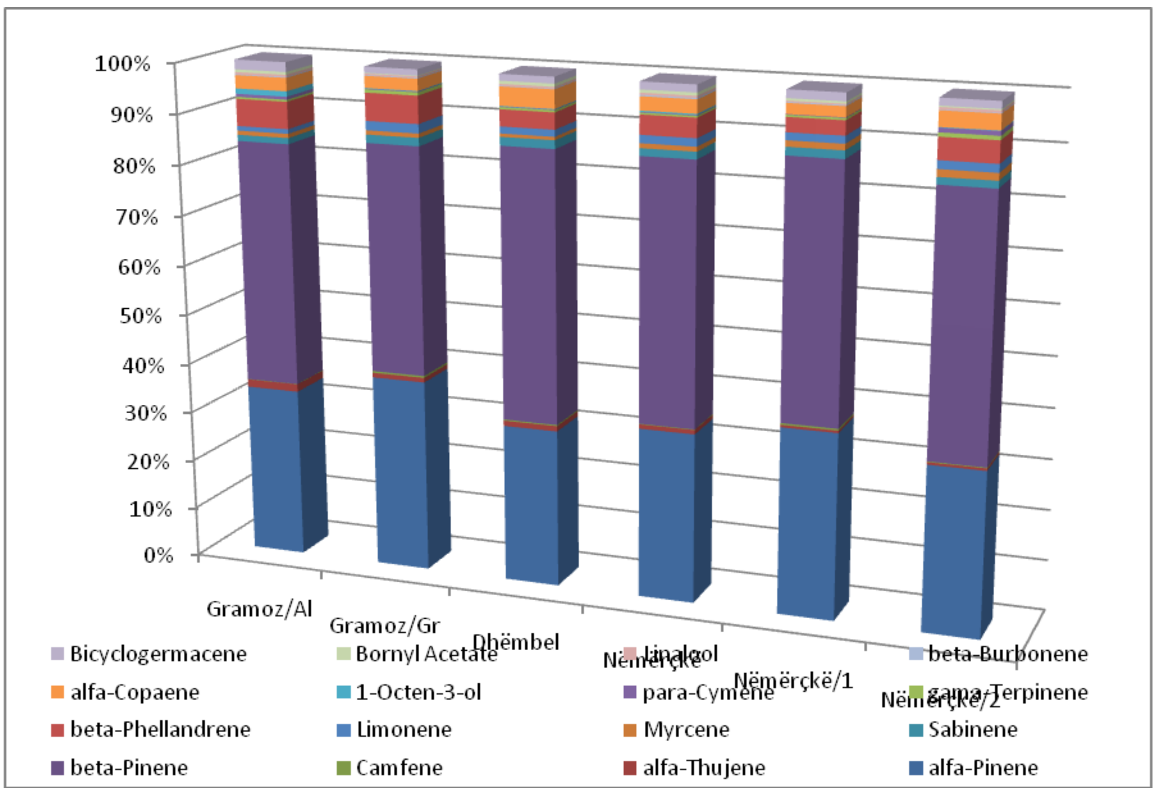

Figure2. Percent of compounds analysed in Sideritis samples from mountains of South Albania

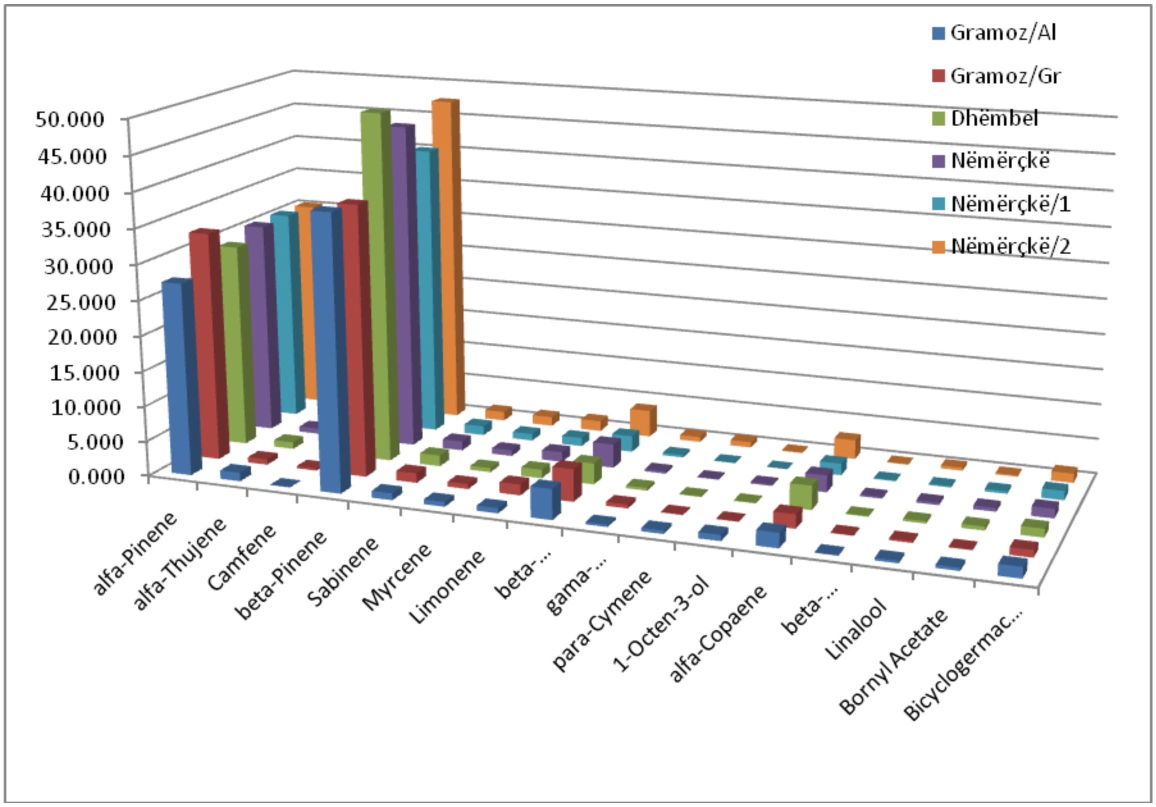

Figure3. Distribution of compounds analysed in Sideritis samples from South Albania

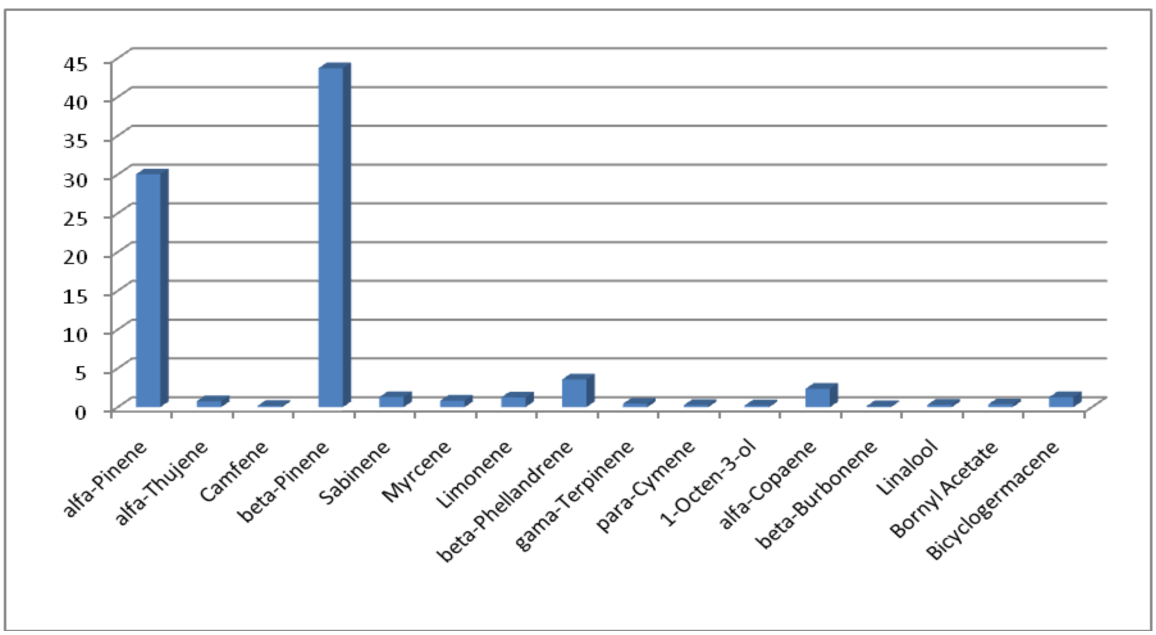

Figure4. Profile of compounds analysed in Sideritis samples (South Albania),

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CONCLUSION

Representative *Sideritis* samples were analyzed from different stations of Nemërçka, Gramozi and Dhëmbeli Mountains (South Albania mountains). Samples were air dried in shadow, essential oil was extracted using Clevengar apparatus recommended from the literature. Chemical analyze of *Sideritis* essential oil samples were performed by gas chromatographic technique with VF-1ms capillary column and flame ionization detector. The data presented 16 the main compounds found for all *Sideritis* samples. For all samples was found: beta-Pinene > alfa-Pinene >beta-Phellandrene>alfa-Copaene>Bicyclogermacene. The peaks lower than 0.01% was not present in this study. This composition of the essential oil from the Sideritus plant in Albanian South Mountains is associated with its type, time of harvesting the crop, its dry and geographic position and composition of the oil where it grows. Was the same distribution for all samples. This is due of the same *Sideritis* species analysed from the same area. The geographical position (particulary the height) is a important factor for the differences found between the analyzed *Sideritis* samples. Profile and leveles of *Sideritis* samples from South Albania mountains was the same with other reported studies from Mediterrean area.

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