MAIN COMPONENTS OF HYPERICUM PERFORATUM ESSENTIAL OIL FROM SOUTH-EAST ALBANIA

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Abstract

Albania as a Mediterranean country has suitable conditions for the growth of about 3500 different species of medicinal and aromatic plants. These plants have been used since ancient times in traditional medicine, culinary and cosmetics. *Hypericum perforatum, (*St John's wort), also called in Albania as "*lule basani*", is a flowering plant known for its traditional uses and well-established effects in mild form of depression due to its components. The data of the chemical composition of the essential oil of *Hypericum perforatum* plant from South-East Albania, in Kolonja area, were studied. The plants were sampled in June for a three-year period (2017 - 2019). The air-dried plant samples were subjected to hydrodistillation on European Pharmacopoeia apparatus (Clevenger type) to obtain *Hypericum perforatum* essential oil. The chemical composition of the essential oils was analyzed using GC/FID technique. From the gas chromatographic analyzes of essential oil for *Hypericum perforatum* resulted 30-40 compounds. The interpretation considered 21 main compounds that constitute from 92.09 % (2017) to 95.41% (2018). The byciclic monoterpenes comprise the largest group of components, with 41.38% to 50.59% of the total amount. The main compounds identified in all samples were: alpha-Pinene, beta-Pinene, beta-cis-Ocimene and Caryophyllene.

Key words: Hypericum perforatum, Essential oil, alpha-Pinene, beta-Pinene, Caryophyllene GC/FID.

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1. Introduction

The medicinal plants have been used since ancient times for the treatment of various diseases in traditional medicine, culinary and cosmetics. Albania has a good tradition for the use of medicinal plants in the treatment of various diseases, such as superficial wounds, respiratory problems, digestive tract and diseases that affect the nervous system. Albania as part of the Mediterranean area, and due to its favorable geographical position, stands out for its rich vegetation, with about 3500 different types of plants. Of this diverse vegetation, over 300 species are aromatic and medicinal plants, which constitute a good, important natural economic resource, still not fully exploited. The medicinal plants of our country are distinguished by their active ingredients and essential oils [1, 2, 3].

The collection process of the aromatic-medicinal plants constitutes one of the main activities that generates incomes for the livelihood of families in rural areas. Farmers want to invest in lands with low fertility, so to be plant with medicinal, essential oil and tanniferous plants. This would be a very good solution especially in abandoned agricultural areas and impossible to be treated by agricultural machinery and irrigation water. The chemical study of the essential oils of the most important medicinal plants of our country is a very valuable data not only for the people who deal with the collection, cultivation and acumulation of these plants but also for the certification of these plants and the pharmaceutical industry that in recent years has turned the eyes to the the medicinal plants, their active compounds to use in preparation as a more appropriate way of treating many disorders.

Hypericum perforatum, St John's wort, also called in Albania "*lule basani",* is a perennial herbaceous plant, widespread in the Mediterranean region. Flowering occurs from May to July. The plant is known for its traditional uses as tea or remedy to treat wounds, lesions and alleviate pain, and to treat minor burns and inflammation of the skin [1-5]. It has well-established effects in mild form of depression due to components such as hyperforin (phloroglucinol) and hypericin (naphthodiantrone). It is also rich in flavonoids such as rutine and quercetin, tannins and phenolic compounds [4-6]. It has topical uses employed for inflammatory conditions of the skin [5] and is known to have other properties including antibacterial, antiviral, anti-inflammatory and indications from preclinical studies, of a potential use of the plant in medical pain management [6-8].

2. Materials and Methods

2.1. Plant material sampling

The aerial parts of *Hypericum perforatum*, such as stems, leaves and flowers were selected from the Kolonja area, over 1000 m high. The plants were collected in June for a three-year period (2017 - 2019), a total of 15 plant samples from this area. Voucher specimens of the plant were deposited at the herbarium of Faculty of Natural Sciences, University of Tirana, Albania. Careful air drying of the samples was carried out, so to best preserve the morphological characteristics. The plant material was cut into small pieces (0.5-2 cm) and kept in a dark and cool place until further analysis.

2.2. Essential oils extraction

The plant material of *Hypericum perforatum* (50g plant material) were subjected to hydrodistillaton for 4 hours with Clevenger apparatus (Pharmacopeia European, 2014) and toluene was used for the isolation of the essential oil. The essential oil extracted from each sample was collected in small bottles. The amount of water present in the essential oil samples was removed using anhydrous sodium sulfate. After removing the water, the essential oil samples were placed in dark-colored vials, stored at +4 °C so to be used as final samples to perform their chemical analysis. The chemical composition of the essential oils was analyzed using GC/FID technique. [8,9,12,13].

2.3. Gas chromatographic examination

The analysis of *Hypericum perforatum* oil samples were performed using the gas chromatographic apparatus Varian 450 GC, equipped with a PTV injector and a flame ionization detector (FID). The injector temperature and detector temperature were maintained at 280ºC and 300ºC, respectively. An amount of 2µl of *Hypericum perforatum* essential oil (dissolved in toluene) was injected in split mode (1:50). Nitrogen was used as a carrier gas (1 ml/min) and as a make-up gas (25 ml/min). Hydrogen and air were the flame gases in the detector at 30 ml/min and 300 ml/min, respectively. The VF- 1ms capillary column (30 m x 0.33 mm x 0.25 mu) was used to isolate the essential oil compounds. The oven temperature was programmed as follows: first heated to 40°C (for 2 minutes), then the temperature was raised to 150°C at 4°C/min, then to 280°C at 10°C/min, and the final temperature of 280°C was maintained for 5 minutes. The identification of the compounds was based on the comparison of the exit time (RT) and with the literature data. Quantitative data of the analyzed compounds is given in % versus the total area of the curves. [10, 12, 14, 15]. Chromatogram of *Hypericum perforatum* essential oil for Kolonja (Borova) sample in year 2019 is demonstrate in Figure 1.

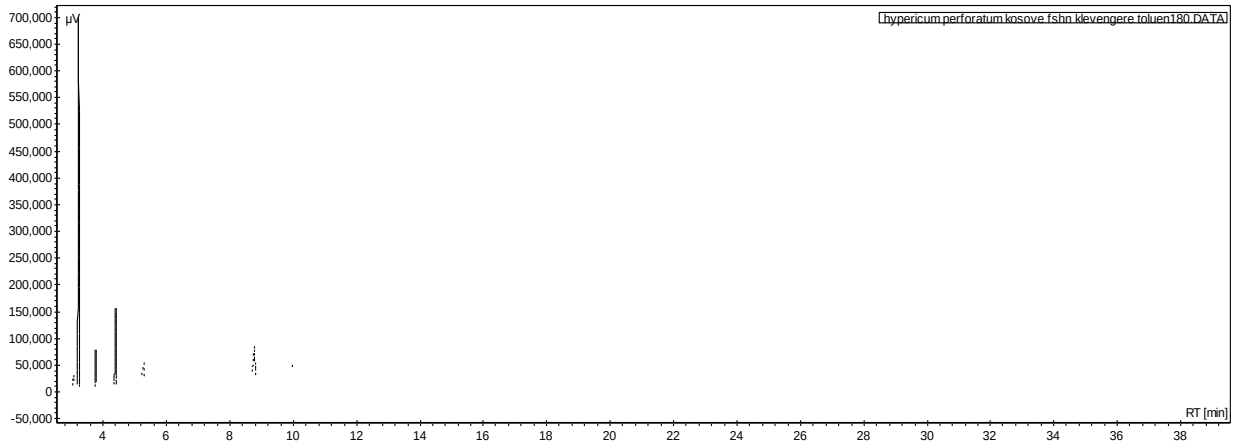

Figure 1: *Chromatograms obtain with GC/FD for Hypericum perforatum* essential oil for Kolonja (Borova) sample 2019.

3. Results and discussion

The results GC/FID analyses of essential oils are reported in Table 1. It gives the average percentages of the components analyzed for *Hypericum perforatum* from the area of Kolonja for the three-year period 2017-2019. Peaks with lower surface area than 0.1%, indicating the presence of many components in extremely small quantities, were not considered in this study.

Table 1: The Average percentages of the main components of *Hypericum perforatum* essential oils for the period 2017-2019.

Compounds	**2017**	**2018**	**2019**
alpha-Thujene	1.42	1.35	1.65
alpha-Pinene	**28.54**	**26.23**	**29.34**
Camphene	0.36	0.62	0.53
Sabinene	0.51	0.74	0.45
beta-Pinene	**16.38**	**11.43**	**18.25**
beta-Phellandrene	1.81	1.88	0.28
2-Methyl Decane	2.38	4.57	2.71
4-Carene	0.44	1.01	0.37
D-Limonene	0.72	0.56	0.54
p-Menth-3-ene	1.29	5.09	1.35
Undecane	0.61	1.11	1.28
beta-cis-Ocimene	**9.69**	**8.87**	**7.23**
gamma-Terpinene	1.15	0.24	0.68
2-Methyl dodecane	0.84	1.15	1.69
Caryophyllene	**12.57**	**8.71**	**10.42**
alpha-Muurolene	0.88	2.23	2.72
Germacene D	**6.38**	**9.62**	**6.32**
beta-Bisabolene	2.27	3.99	1.55
gamma-Elemene	1.68	3.61	2.37
gamma-Cadinene	1.49	1.32	2.94
gamma-Curcumene	0.68	1.08	0.78
Total	**92.09**	**95.41**	**93.45**
Monocyclic monoterpenes	4.97	7.77	2.85
Bicyclic monoterpenes	47.65	41.38	50.59
Aliphatic monoterpenes	13.52	15.7	12.91
Monocyclic sesquiterpenes	11.01	18.3	11.02

Bicyclic sesquiterpenes	14.94	12.26	16.08

The analysis performed for *Hypericum perforatum* essential oil demostrated that the chromatograms contained 30-40 compounds. The interpretation considered 21 main compounds ranging from 92.09% in 2017 to 95.41% in 2018. The data examinated reveals that the main compounds identified in all samples were: alpha-Pinene, beta-Pinene, beta-cis Ocimene, Caryophyllene, Germacrene.

The following were the main components in major quantitative percentages compared to the total amount of essential oil: alpha-Pinene, from 26.23% (2018) to 29.34 % (2019), beta-Pinene from 11.43% (2018) to 18.25% (2019), Caryophyllene, from 8.71% (2018) to 12.57% (2017), beta-cis-Ocimene from 7.23% (2019) to 9.69 % (2017), Germacrene from 6.32% (2019) to 6.38 % (2018). Byciclic monoterpenes comprise the largest group of components, with percentage values ranging from 41.38% (2018) to 50.59% (2019) with the dominant compound α-pinene 29.34 % (2019), followed by beta-Pinene 18.29 % (2019). The quantity of all these compounds was higher in 2019 samples essential oils (Table 2). Bicyclic sesquiterpenes was the second major group of components (Table 1) the quantity of which varied from 12.26% in 2018 to 16.08% in 2019 with the main compound Caryophyllene (12.57% in 2017). Monocyclic sesquiterpenes was the third group of components which showed a higher quantity in 2019 (18.3%) with germacrene D as major component of the group (9.62%) and beta-Bisabolene (3.99%). While alifatic and monocyclic monoterpenes showed the smallest quantity, respectively 15.7% (2018) and 7.77% (2018).

Table 2. Profile of the major group of components (byciclic monoterpenes) for *Hypericum perforatum* samples (2017-2019)

Bicyclic monoterpenes	**Year 2017**	**Year 2018**	**Year 2019**
alpha-Thujene	1.42	1.35	1.65
alpha-Pinene	28.54	26.23	29.34
Camphene	0.36	0.62	0.53
Sabinene	0.51	0.74	0.45
beta-Pinene	16.38	11.43	18.25
4-Carene	0.44	1.01	0.37
Total	47.65	41.38	50.59

The data obtained from the analyzes of *Hypericum perforatum* from South-East Albania (Kolonja area) demonstraded that the chemical composition for the period of three years, showed similarity with those reported in other studies from the Balkan and Mediterranean areas [10-16].

According to the literature, the main compounds of the Italian *Hypericum perforatum* essential oils, were β-caryophyllene and 2,6-dimethylheptan, in the meantime in our oils 2,6-dimethylheptan was not a main component. Similar was the main group of components, sesquiterpenes, which was found as main group also in Italian oils [15]. In Greek essential oils the main compound α-pinene (21.0%) was found as main component, but differently from our oils, 2-methyl-octane (12,6%) was the other abundant component of *Hypericum Perforatum* essential oil [16]. The profile of the main components of the Serbian oils were similar to our oils, but the quantity of these compounds was different, such as α-pinene (6.5%) was lower than in our oils, and germacrene D (18.6%) was in higher quantity [12].

4. Conclusions

Hypericum perforatum, St John's wort, also called in Albania "*lule basan*i", is a medicinal plant, widespread in the Mediterranean region and in many areas of our country. It is well known for its traditional uses as tea or remedy to treat wounds, and minor burns.

In this study are presented the chemical data for *Hypericum perforatum* essential oils from the South-East area of our country (Kolonja), known for the variety of medicinal plants grown and collected by its residents. For all analyzed samples, the number of identified compounds was between 30 and 40 compounds, but the main 21 compounds identified in all samples were taken into analysis.

The main compounds identified in all samples were: alpha-Pinene, beta-Pinene and Caryophyllene.

Bicyclic monoterpenes comprise the largest group of terpenes, with the dominant compound α-pinene (29.34%) followed by beta-Pinene (18.29%). The quantity of all these compounds was higher in essential oils from plant samples collected in 2019. The chemical changes observed for the plants analyzed from the same area for three different years should be mostly related to the different atmospheric conditions (temperature, humidity, etc.), in this area. The profile and levels of the main compounds of *Hypericum perforatum* essential oil samples, showed minor variations with those reported from other studies the Balkan and Mediterranean areas.

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