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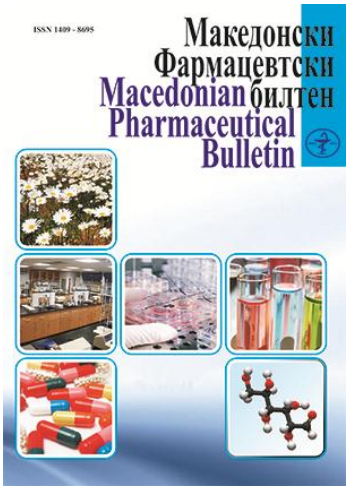

Title: **GC-MS analysis of the essential oil, aroma components and *n*-hexane extract of St. John Wort (*Hypericum perforatum* L., Hypericaceae)**

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GC-MS analysis of the essential oil, aroma components and *n*-hexane extract of St. John Wort (*Hypericum perforatum* L., Hypericaceae)

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Abstract

St. John Wort (*Hypercum perforatum* L., Hypericaceae) has been used as a medicinal plant for a long period of time as this plant is characterized by a diversity of bioactive constituents which possess well documented pharmacological activities including antiviral, antimicrobial, anti-inflammatory, antioxidant, hepatoprotective and anti-tumoral activity. Nowadays, special interest is put on its essential oil as some experimental studies showed great biological and pharmacological potential. According this, the main goal of this study was GC/MS analysis of the essential oil, aroma components as well as *n*-hexane extracts of *Hypericum perforatum* that grows in Western region in R. Macedonia. GC/FID/MS analyses of the isolated essential oils from leaf, flower and herb resulted in the identification of 84 compounds. The fraction of sesquiterpenes was dominated in all examined oils and the main constituents were germacrene D (17.77-39.03%), E-caryophyllene (11.37-25.71%) and *β*-selinene (0.69-4.77%). GC/HS/MS analyses of the aroma components resulted in the identification of 23 compounds. Among them, isononane was identified as main aroma component (up to 75%). GC/FID/MS analyses of the *n*-hexane extracts resulted in the identification of 60 compounds which were characterized by the presence of terpenoid (mono- and sesquiterpene) components and non-terpenoid constituents mainly consisted of hydrocarbons and their oxygenate derivatives and related components. The non-terpenoid fraction represented the largest part of the analysed extracts. The most abundant were nonacosane (15.45-49.28%), octacosane (1.33-40.05%) and pentacosane (1.68-9.04%).

The aerial parts of *H. perforatum* collected from Western part of R. Macedonia could be considered as a good source of essential oil with specific chemical profile as well as aroma

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components and high lipophilic compounds, but further investigation should be done in accordance to their possible commercial or medicinal use.

Keywords: *Hypericum perforatum*, essential oil, aroma components, *n*-hexane extract, GC-MS

Introduction

St. John Wort (*Hypercum perforatum* L., Hypericaceae) (HP) has been used as a medicinal plant and it is one of the best-known members of the genus *Hypercum* (Crockett and Robson, 2011). It's a perennial herbaceous aromatic plant characterized by an erect, cylindrical, 10-100 cm tall stem, branched in the upper part. It has opposite, sessile, narrow ovate to linear leaves, with transparent dots throughout the tissue. Flowers are organized in board cymes at the end of the upper branches, with a yellow colour. Blooming occurs from May to August (Mustafa et al., 2012; Tutin et al., 1972).

HP is characterized with a diversity of bioactive constituents, which include naphtodianthrones (pseudohypericin, hypericin), phloroglucinol derivates (mainly hyperforin and adhyperforin), flavonoids, catechin tannins, procyanidines and smaller amounts of essential oil (EO) (Bradley, 2006; Nahrstedt and Butterweck, 1997). The bioactive constituents of HP possess documented pharmacological activities including antiviral, antimicrobial, anti-inflammatory, antioxidant, hepatoprotective and anti-tumoral activity (Crocket and Robson, 2011; Linde et al., 1996; Muller, 2005; Saddiqe et al., 2010). Extracts and other preparations have been used for some medical treatment such as removing wounds and burns in skin, sciatica, eczema, menopausal disorders, premenstrual syndromes, central nervous system disorders, nerve damages, anxiety, diabetes mellitus, dyspepsia etc (Dulger, 2005a; Laakmann et al., 1998; Males et al., 2006; Milosevic et al., 2007; Rabanal et al., 2002; Radulovic et al., 2007). Naphtodiantrones, phenols, flavonoids, phloroglucinol derivates and EO of HP have been reported responsible for these activities (Bilia et al., 2002; Saroglou et al., 2007). Nowadays, special interest is put on the

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HP essential oil as some experimental studies showed their great antimicrobial, (Pirbalouti et al., 2013; Rabanal et al., 2002; Saddiqe et al., 2010; Sevim et al., 2010), antioxidant (Radulovic et al., 2007), antifungal (Sevim et al., 2010) and antidermatophyte potential (Laripour et al., 2009). There are many studies on HP EO composition and they show a significant variation in volatile profile of this species. Germacrene D, *α*-pinene, *β*-caryophyllene, 2-methyloctane and *n*-nonane are metioned as major components in HP essential oil reported by many authors (Cirak et al., 2010; Mockute et al., 2008; Radusiene et al., 2005; Schwob et al., 2004; Sevim et al., 2010). On the other hand, very little is known about HP aroma as well as HP highly unpolar components. For these resons, the main goal of this study was GC-MS analysis of essential oil, aroma components and *n*-hexane extract of *Hypericum perforatum* that grows in western region of the Republic of Macedonia.

Material and methods

Plant material

Plant material was collected from June till August from 2014, 2015 and 2017, in full blossom of the plant from three different locations in R. Macedonia: Tetovo, Mavrovo and Debar (Table 1). Plant identity was verified and voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje.

The plant material (aerial parts = Hb) was air dried, packed in paper bags and kept in a dark and cool place until analysis. For purpose of analysis, flowers (Fl) and leaves (Fol) of some samples were separated. For isolation of essential oil, fresh plant material was used.

Table 1

Essential oil isolation

The essential oil (EO) was isolated from fresh and minced plant material by hydrodistillation in all-glass Clevenger apparatus for 2 hours according to Ph. Eur. The obtained oil was dried with anhydrous Na_2SO_4 and dissolved in hexane for further analyses.

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Preparation of n-hexane extracts

The plant material was prepared by ultrasonic extraction at room temperature. Hexane was used as an extractive agent in ratio 1:20 (1 g of plant material was extracted 2 times with 10 ml of n-hexane). Extraction was performed on two occasions of 30 minutes (total extraction time was 60 minutes). The extract obtained after filtration was evaporated to dryness, and the dry residue then was reconstituted in *n*-hexane to obtain a solution with a concentration of 1 g/mL.

Head space method (GC/HS/MS analysis)

The analysis of aroma components was made on small amounts (0.3 g) of plant samples which were directly put in sealed vials and incubated for 5 min on 80 °C. For that purpose, agitator speed was adjusted at 500 rpm while syringe temperature was 85 °C. Only the gas phase (1 ml of highly volatile compounds) was injected and then investigated on an Agilent GC/FID/MS system.

GC/FID/MS analysis

The chemical composition of essential oils, aroma components and *n*-hexane extract were analyzed on Agilent 7890A Gas Chromatography system equipped with FID detector as well as Agilent 5975C mass quadripole detector. For that purpose, HP-5 ms capillary column (30 m × 0.25 mm, film thick-ness 0.25 μm) was used. Analytical conditions were as follows: initial oven temperature 60 °C (0 min) increased at a rate of 3 °C/min to 240 °C (1 min) and to 280 °C at a rate of 10 °C/min (1 min); helium as carrier gas at a flow rate of 1 mL/min; injector temperature 220 °C and that of the FID detector 270 °C. Each sample was injected at a split ratio of 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50-550 Da. The MS was operated in scan mode.

Identification of the components

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The compounds were identified on the basis of literature (Adams, 2007) and estimated Kovat′s (retention) indices that were determined using a mixture of homologous series of normal alkanes (C_9-C_{25}) analyzed under Automated Mass Spectral Deconvolution and Identification System (AMDIS) conditions. Also, identification of the components was made by comparing mass spectra of each constituent with those stored in the Wiley and NIST database and with mass spectra from the literature.

Results and discussion

Essential oils

GC/FID/MS analyses of the HP EOs (leaf - Fol, flower - Fl and herb - Hb) resulted in the identification of 84 compounds representing 84.98-97.5% of the total oil (Table 2). The isolated EOs were complex mixtures of hydrocarbons, monoterpenes and sesquiterpenes (67 mono and sesquiterpenes and 19 hydrocarbons and their oxygen-containing derivatives). The sesquiterpene fraction (ST) was the most abounded, in all examined oils reprersenting from 64.38-84.37% of the EOs chemical composition (Fig. 1).

Fig. 1.

The main constituents of the oils obtained from herb, flower and leaf were germacrene D (17.77-39.03% in HP/17 Fl-D and HP/17 Fol-T, respectively), E-caryophyllene (11.37-25.71% in HP/17 Hb-T and HP/17 Fl-T, respectively) and *β*-selinene (0.69-4.77% in HP/17 Fol-M and HP/17 Fol-T, respectively). Germacrene D was present in higher quantities (26.55-39.03%) in the EOs obtained from leaf and herb of HP in comparison to the oil obtained from flower of HP (11.98-17.77%). On the other hand, caryophyllene E was represented with higher percent in flower oil (22.23-25.71%) of HP than in the oil of leaf or herb (12.93-19.92%). Generaly, the content of germacrene D, E-caryophyllene and *β*-selinene was much higher in the samples collected from Tetovo in comparison to the samples from Mavrovo and Debar (Table 2).

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Monoterpene fraction (MT) was represented with *α*-pinene (identified in all samples, up to 8.75% in the sample of flower from Mavrovo) and ocimene (identified in all samples, up to 8.87% in the sample of flower from Tetovo) (Table 2). Other monoterpene components were presented in much smaller amounts but usually higher in flower oil than in oils obtained from herb or leaf of HP. Samples of oils obtained from HP from Tetovo contained smaller amounts of borneol and *p*-cymene, which were not identified in other investigated samples.

Hydrocarbons were presented in a small percentage in almost all tested samples and major compounds were hexacosan and tricosan (Table 2). The oxygen derivatives of alkanes such as some alcohols were present in all samples and dominated were dodecanol (5.15% in HP/17 Hb-M) and tetradecanol (3.84% in HP/17 Fol-D).

Great variations in the qualitative and quantitative composition were observed, depending on the part of HP from which the oils were isolated as well as the growing locations of the plant and year of collection.

Table 2.

Obtained results are in good accordance with literature data. According to Crocket (2010) essential oil and volatile constituents that have been the most frequently reported from *Hypericum* include the aliphatic hydrocarbons *n*-nonane and *n*-undecane, the monoterpenes *α*- and *β*-pinene and the sesquiterpenes *β*-caryophyllene and caryophyllene oxide. A number of major components have been identified from different *Hypericum* species that have a relatively limited occurrence among higher plants. Because some of these compounds may have a potential as food and/or beverage additives as well as aroma chemicals and could be utilize in cosmetics, an interest in further research on targeted breeding programs for some *Hypericum* species exists (Crocket, 2010). Besides *α*-pinene and E-caryophyllene, germacrene D was previously identified as the most abundant constituent of HP essential oil from Kosovo followed by higher percentages of 2-methyloctane, nonane, caryphyllene oxide and *n*-tetradecanol (Hajdari et al., 2014). Germacrene D was determined as an important constituent of the essential oil of HP that grows in Lithuania (Mockute et al., 2008). Previosusly, three

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different chemotypes of HP essential oil were identified: *β*-caryophyllene, caryophyllene oxide and germacrene D chemotypes. The oils of the first two chemotypes contained 17.30-46.90% of constituents with a caryophyllene skeleton while the germacrene D chemotype had not been detected earlier in *H. perforatum* species. The sesquiterpene hydrocarbons and oxygenated sesquiterpenes made up 62.00-81.80% of the oils. Additionaly, the identified aliphatic compounds varied from 1.70 to 19.60% (Mockute et al., 2003). The composition of essential oils obtained from flowers and leaves of HP from Lithuania showed that oxygenated sesquiterpene fraction was dominated in all investigated samples. Differences were attributed to the main components: caryophyllene oxide, spathulenol and viridiflorol. The data indicated some differences in sesquiterpene and aliphatic hydrocarbons, as well as in oxygenated aliphatics biosynthesis in flowers and leaves. The concentrations of *β*-caryophyllene and caryophyllene oxide in essential oils from leaves were higher than those from flowers, whereas dodecanol, spathulenol, viridiflorol, carotol and tetradecanol were present in higher quantities in flowers (Radusiene et al., 2005). Our results also showed differences as we found that *β*-caryophyllene was much higher presented in the oil of flower than in oil of leaves (22.23-25.71% and 12.93-19.92%, respectively), while the content of other mentioned components (spathulenol, caryophyllene oxide and viridiflorol) was almost the same in both oils from flower and leaf as well (Table 2).

The essential oil of HP from Serbia contained *β*-caryophyllene (14.20%) and 2-methyl-octane (13.10%) as the most abundant constituents (Gudzic et al., 2001). According Saroglou et al., *α*-pinene, *β*-farnesene and germacrene D were dominated components and the oil generally contained higher content of sesquiterpene hydrocarbons (Saroglou et al., 2007). Other authors find that HP and several other species of *Hypericum* from southeastern Serbia contained essential oils characterized by a high content of non-terpene compounds and a low content of monoterpenes. A comparison that was carried out of the chemical composition of the essential oils from flower, leaf and stem of HP revealed that the highest concentration of non-terpene compounds was found in the flower and stem oil, while a high concentration of sesquiterpenes was characteristic for leaf oil. There were also a significant differences in the concentrations of the same compounds in the essential oils of HP, collected in different years

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from the same location which could be explained by seasonal differences of the plant. The main conclusion was that genetic and environmental factors both play a role in determining the composition of essential oils of the *Hypericum* species that were studied (Smelcerovic et al., 2007).

The essential oil isolated from fresh aerial parts of HP from Serbia revield 134 identified compounds accounted for 98.70% of the total oil. The main components of the oil were: germacrene D (18.60%), (E)-caryophyllene (11.20%), 2-methyloctane (9.50%), *α*-pinene (6.50%), bicyclogermacrene (5.00%) and (E)-*β*-ocimene (4.60%). The volatile profile of *H. perforatum* was characterized by a large content of sesqiuterpenoids (57.70%), especially sesquiterepene hydrocarbons (48.70%). Monoterpenoids (22.40%) also consisted mostly of hydrocarbons (21.40%). Nonterpenoid compounds amounted to 18.10% of the total oil (Djordjevic, 2015).

The essential oil from inflorescences of *H. perforatum* (var. *angustifolium*) growing wild and harvested in Sardinia (Italy) was characterized by higher content of 2-methyloctane (21.10%), germacrene D (17.60%) and *α*-pinene (15.80%) (Pintore et al., 2005). The main constituents of the essential oil of HP from Turkey were hydrocarbon and oxygenated sesquiterpenes such as *β*-caryophyllene (4.08-5.93%), *γ*-muurolene (5.00-9.56%), *β*-selinene (5.08-19.63%), *α*-selinene (4.12-10.42%), *δ*-cadinene (3.02-4.94%), spathulenol (2.34-5.14%), and caryophyllene oxide (6.01-12.18%). Principal component analysis was also carried out and, according to the results, these nine principal components were found to represent 100% of the observed variation in the oil composition. Monoterpenes, both hydrocarbon and oxygenated, were represented by scarce amounts of *α*- and *β*-pinene, myrcene, linalool, *cis*- and *trans*-linalool oxide, and *α*-terpineol. It was noted that the chemical variation among the populations is possible result of different genetic and environmental factors (Cirak et al., 2010).

In the samples collected in Greece, the main components were germacren D (22.80%) followed by 2-methyl-octane (10.80–17.80%), *trans*-(*E*)-caryophyllene (6.60–10.30%), *α*-pinene (5.20–10.10%) and bicyclogermacrene (4.10–4.80%) (Petrakis et al., 2005).

Aroma components

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GC/HS/MS analyses of the HP aroma components (ACs) (leaf and flower) resulted in the identification of 23 compounds representing 93.89-99.96% of the total ACs (Table 3). The main aroma component was isononane presented in very high percentage, up to 75% in flowers of HP collected in Tetovo in 2016. In other samples this component was presented in amounts from 34.68 to 74.62%. Only one sample contained much smaller amounts of isononane (7.85%, samples of flower from Tetovo collected in 2014). Important aroma components that were identified were *α*-pinene (8.06-35.08%), nonane (2.06-8.12%), 3-methylnonane (1.62-8.06%), 2-methyldecane (1.77-14.55%) and E-caryophyllene (0.11-7.94%).

Isononane (2-methyloctane) is found in alcoholic beverages and was identified as important constituent of *Hypericum perforatum* (St. John's Wort). Usually this compound was reported as constituent of HP essential oil (Mockute et al., 2003; Pirbalouti et al., 2013; Hajdari et al., 2014), probably important for the specific scent of the plant together with other hydrocarbons such as nonane, 3-methylnonane, 2-methyldecane and specific mono- and sesquiterpene components.

Table 3.

n-Hexane extracts

GC/FID/MS analyses of the HP *n*-hexane extracts (HEs) of 12 samples of HP (leaf and flower) resulted in the identification of 60 compounds representing 85.93-96.96% of the HEs (Table 4). The analysis showed that the HP HE is characterized by the presence of terpenoid (33 mono - MT and sesquiterpene - ST) components and non-terpenoid constituents (27 components). This fraction of other components mainly consisted of hydrocarbons and their oxygenate derivatives and related components and represented the largest part of HP HE (65.06-85.39%) followed by much smaller fractions of ST (10.31-19.27%) and MT components (3.64-11.09%) (Fig. 2).

Fig. 2.

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The content of identified components differed a lot depending on the location and the year of collection, but also from the part of the plant from which the extract was prepared. Important components from MT fraction of HP HE were eugenol (0.51- 4.04%) and menthol (0.44-2.24%). Both of these components were not identified in adequate samples of HP EO. Sesquiterepene fraction was characterized by higer quantities of E-caryophyllene (0.36-2.59%), dauca-5,8-diene (0,00-3.45%), selina-3,7(11)-diene (0.00-4.82%), caryophyllen oxide (0.32-2.88%) and 10-*epi*-γ-eudesmol (0.84-3.26%) (Table 4).

In relation to other compounds (OC), qualitative and quantitative variations were identified in the presence and the concentration of all identified aliphatic hydrocarbons. The most abundant were nonacosane (15.45-49.28%), octacosane (1.33-40.05%) and pentacosane (1.68-9.04%). Two samples contained higher amounts of tetratetraacontane (9.13% in HP/15 Fl-T and 15.61% in HP/14 Fl-T), both originated from the same location (Tetovo). The oxygenated derivatives of alkanes (mainly alcohols) were with C_{12}-C_{18} chain (dodecanol, tetradecanol, pentadecanol, hexadecanol, heptadecanol and octadecanol), presented in different but usually small amounts. Additionally, some fatty acids and their esters were also identified and other types of compounds such as octadecenamide as well (Table 4).

Table 4.

A comparison of the chemical composition of HE prepared from flowers and leafs has shown that there were not significant differences in the chemical composition between extracts. Some aliphatic hydrocarbons were presented in higher amounts in HP HE from flowers such as nanocosane and tetratetracontane. From terpenoid components caryophyllene E was more presented in HP HE of flowers, while all other components varied a lot and in some cases were presented more in HP HE of floweres and in other in HP HE of leafs (Table 4). Differences in the content of some compounds were identified in relation to the year of collection. It could be noticed that MT fractions was smaller in all samples of HP HE originated from Debar (average value 5.21%, 7.00% and 7.48% for MT fractions for samples from Debar, Tetovo and

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Mavrovo, respectively). The same ratio was noticed for OC (70.83%, 71.85% and 74.94% for samples originated from Debar, Mavrovo and Tetovo, respectively), whille the content of ST was higher in samples from Debar (16.31%, 15.17% and 13.57% for Debar, Tetovo and Mavrovo, respectively).

Regarding the data of usage of *n*-hexane as an extractive agent, they mainly goes in the direction of use of this solvent for isolation of the anthraquinone derivative, hypericin and the decomposition products of phloroglucinols (hyperforin and similar compouds) (Anand et al., 2005). Beside that, *n*-hexane is also used for the fractionation of primary ethanolic extracts from HP and for the production of hexane-soluble fraction that exhibits antimicrobial activity (Suntar et al., 2016). In terms of its nature, hexane is a high-lipophilic solvent that can be used for the extraction and isolation of lipophilic compounds such as fats, oils, resins and waxes, hydrocarbons, terpens and other non-polar components. For these reasons, it was use in our examination in order to define the chemical composition of HP in relation to the presence of the lipophilic compounds. The composition of the identified terpenes and hydrocarbon compounds in such prepared extracts can be compared with the qualitative composition of essential oil of the HP, since there are no data about the chemical composition of hexane extracts of HP.

Conclusion

A detailed investigation of the volatile constituents of *H. perforatum* essential oil resulted in the identification of 84 components. The oil was characterized by a sesquiterepene (64.38-84.37%) and monoterpene components (4.90-22.53%) followed by smaller amount of other compounds (4.79-12.13%). The main constituents of the oils obtained from herb, flower and leaf were germacrene D, caryophyllene E and *β*-selinene. In case of germacrene D, leaf and herb oil of HP contained higher quantities of this component (26.55-39.03%) in comparison to the oil obtained from flower (11.98-17.77%), while E-caryophyllene was much higher presented in the oil isolated from flowers (22.23-25.71%) than in other HP oils. The

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main aroma component was isononane presented in very high percentage, up to 75% in flowers of HP, followed by *α*-pinene (8.06-35.08%), nonane (2.06-8.12%), 3-methylnonane (1.62-8.06%), 2-methyldecane (1.77-14.55%) and E-caryophyllene (0.11-7.94%). HP *n*-hexane extracts contained 33 mono- and sesquiterpene components and 27 non-terpenoid constituents. The last fraction mainly consisted of hydrocarbons and their oxygenate derivatives and related components. This fraction represented the largest part of HP *n*-hexane extracts (65.06-85.39%) followed by much smaller fractions of sesquiterepene (10.31-19.27%) and lastly monoterpene components (3.64-11.09%).

The aerial parts of *H. perforatum* from western region of R. Macedonia can be considered as a good source of specific essential oil with authentic chemical composition and the oil can be used as a source of sesquiterpene compounds, in particular caryophyllene E and germacrene D. In addition, *H. perforatum* from western region of R. Macedonia contained also interesting aroma components as well as highly-lipophylic constituents. The possible utilization of these secondary metabolites shoud be further investigated.

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Резиме

GC-MS анализа на етерично масло, арома компоненти и *n*-хексански екстракт од жолт кантарион (*Hypericum perforatum* L., Hypericaceae)

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Клучни зборови: *Hypericum perforatum*, етерично масло, арома компоненти, *n*-хексански екстракт, GC-MS

Жолтиот кантарион (*Hypercum perforatum* L., Hypericaceae) долго време се користи како медицинско растение кое се карактеризира со присуство на различни биоактивни компоненти за кои се знае дека поседуваат добро документирани фармаколошки активности, вклучувајќи антивирусна, антимикробна, анти-инфламаторна, антиоксидативна, хепатопротективна и антитуморна активност. Денес постои голем интерес за испитување на етеричното масло, бидејќи некои експериментални студии укажуваат на голем биолошки и фармаколошки потенцијал на маслото. Според тоа, главната цел на оваа испитување била ГЦ/МС анализа на етеричните масла, арома компонентите, како и на *n*-хексанските екстракти од жолгиот кантарион кој расте во западниот регион на Р. Македонија. ГЦ/ФИД/МС анализите на изолираните етерични масла од лист, цвет и херба резултирале со идентификација на 84

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компоненти. Во сите испитувани масла, сесквитерпените биле доминантна фракција, а главните компоненти биле гермакрен Д (17,77-39,03%), Е-кариофилен (11,37-25,71%) и *β*-селинен (0,69-4,77%). ГЦ/ХС/МС анализа на арома компонентите резултирала со идентификација на 23 соединенија. Меѓу нив, изононанот бил идентификуван како главна арома компонента (до 75%). ГЦ/ФИД/МС анализата на *n*-хексанските екстракти резултирала со идентификација на 60 компоненти за кои било карактеристично присуство на терпеноидни (моно- и сесквитерпени) компоненти и нетерпеноидни конституенси кои главно се состоеле од јаглеводороди и нивни сродни деривати и сродни компоненти, кои го претставувале најголемиот дел од екстрактот. Меѓу нив, најзастапени биле нонакозан (15,45-49,28%), октакозан (1,33-40,05%) и пентакозан (1,68-9,04%). Надземните делови на жолтиот кантарион собрани од западниот дел на Р. Македонија можат да се сметаат за добри извори на специфични етерични масла, како и на ароматични компоненти и високо липофилни соединенија, но понатамошните испитувања треба да се направат во насока на нивната можна комерцијална или медицинска употреба.

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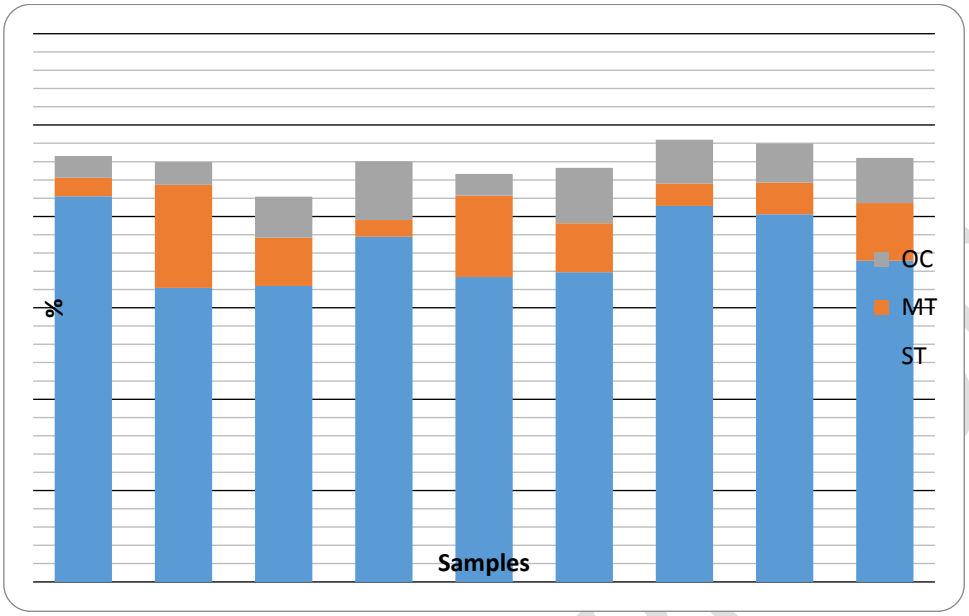

Fig 1. The amount of different fractions in the essential oils of 9 samples of HP EOs obtained from fresh herb, flower and leaf of *H. peforatum* from three different locations from Western part of R. Macedonia.

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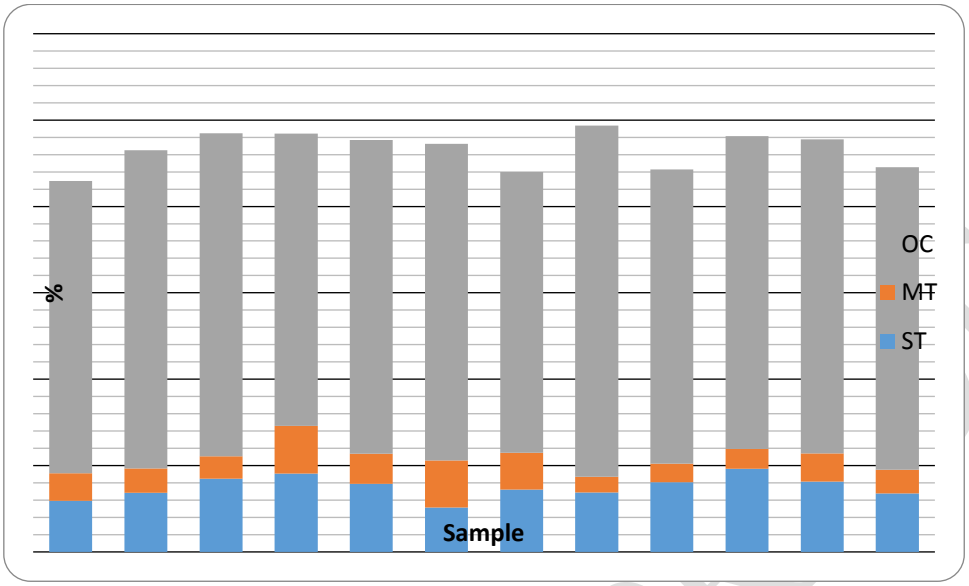

Fig 2. The amount of different fractions in the *n*-hexane extracts of 12 samples of HP HE obtained from dry flower and leaf of *H. perforatum* from three different locations from Western part of R. Macedonia.

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Table1. Plant samples of *H. perforatum*

	Sample	Part of the plant	Locality	Year
1	HP/14 FoL-T	leaf, dry	Tetovo	2014
2	HP/14 FL-T	flower, dry	Tetovo	2014
3	HP/15 FoL-T	leaf, dry	Tetovo	2015
4	HP/15 FL-T	flower, dry	Tetovo	2015
5	HP/14 FoL-M	leaf, dry	Mavrovo	2014
6	HP/14 FL-M	flower, dry	Mavrovo	2014
7	HP/15 FoL-M	leaf, dry	Mavrovo	2015
8	HP/15 FL-M	flower, dry	Mavrovo	2015
9	HP/14 FoL-D	leaf, dry	Debar	2014
10	HP/14 FL- D	flower, dry	Debar	2014
11	HP/15 FoL-D	leaf, dry	Debar	2015
12	HP/15 FL- D	flower, dry	Debar	2015
13	HP/17 FoL-T	leaf, fresh	Tetovo	2017
14	HP/17 FL-T	flower, fresh	Tetovo	2017
15	HP/17 Hb -T	herb, fresh	Tetovo	2017
16	HP/17 FoL – M	leaf, dry	Mavrovo	2017
17	HP/17 FL –M	flower, fresh	Mavrovo	2017
18	HP/17 Hb – M	herb, fresh	Mavrovo	2017
19	HP/17 FoL –D	leaf, dry	Debar	2017
20	HP/17 FL-D	flower, dry	Debar	2017
21	HP/17 Hb -D	herb, fresh	Debar	2017

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Table 2. The composition of essential oils extracted from leaf, flower and herb of *H. perforatum* from three different locations in Western part of R. Macedonia (%)

Components	RI	HP/17 Fol-T	HP/17 Fl-T	HP/17 Hb-T	HP/17 Fol-M	HP/17 Fl-M	HP/17 Hb-M	HP/17 Fol-D	HP/17 Fl-D	HP/17 Hb-D
Cumene	924	0.12	0.14	1.20	0.32	0.12	0.36	0.12	0.32	0.03
α -Pinene	939	0.22	4.84	3.43	0.34	8.75	3.82	0.38	2.86	4.74
3-Methylnonane	971	-	0.80	0.55	-	0.54	0.75	-	0.19	0.55
Sabinene	976	0.12	0.47	0.53	0.30	0.16	0.31	0.08	-	0.15
β-Pinene	980	0.25	1.72	0.88	0.28	1.39	0.84	0.13	0.42	0.80
Mycrene	988	0.14	0.91	0.28	0.21	0.75	0.61	-	0.18	0.66
n-Decane	1000	0.24	0.24	0.44	0.28	0.28	0.38	0.42	0.38	0.40
Δ^3-Carene	1001	0.05	0.31	0.16	0.03	0.07	0.19	0.33	-	0.19
p-Cymene	1020	-	0.06	0.08	-	-	-	0.06	-	-
Limonene	1024	0.06	0.15	0.09	0.07	0.14	0.06	0.06	0.21	0.08
β -Phellandrene	1025	0.11	0.29	0.16	0.08	0.10	0.16	0.16	0.14	0.15
β-(Z)-Ocimene	1032	0.41	1.43	0.54	0.24	0.80	0.49	0.36	0.28	0.43
β-(E)-Ocimene	1044	1.03	8.87	2.22	0.79	4.58	2.94	1.69	1.96	3.30
γ -Terpinene	1054	0.26	0.73	0.34	0.23	0.23	0.41	0.42	0.14	0.41
2-Methyldecane	1064	0.68	1.26	0.47	0.74	0.92	0.85	0.68	0.81	0.71
Terpinolene	1086	-	0.22	0.09	-	0.08	0.11	0.46	-	0.12
n-Undecane	1100	0.12	0.56	0.33	0.23	0.32	0.54	0.23	0.32	0.35
allo-Ocymene	1128	-	0.25	0.12	-	0.08	0.11	0.15	0.07	0.99
Borneol	1165	0.02	-	0.06	-	0.04	-	0.04	0.02	-
Terpinen 4-ol	1174	1.15	1.68	0.41	0.62	0.52	0.27	-	0.21	0.34
α -Terpineol	1186	0.15	0.46	-	0.10	-	-	0.46	0.14	0.22
2-Methyldodecane		0.65	0.55	0.28	0.24	0.35	0.19	0.20	0.66	0.24
Decanal	1201	0.12	-	0.03	0.16	-	-	0.18	-	-
Tridecane	1300	0.14	0.14	0.08	-	0.06	0.06	0.12	0.16	0.06
α -Cubebene	1345	0.15	0.14	0.10	0.08	0.12	0.19	0.24	0.22	0.20

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α-Longipinene	1350	-	0.15	0.13	-	-	0.13	-	0.08	0.07
α -Ylangene	1373	0.06	0.08	0.23	0.07	0.07	0.12	0.12	0.15	0.12
α-Copanene	1374	0.21	0.19	0.23	0.19	0.18	0.33	0.36	0.33	0.28
β-Bourbonene	1387	0.14	0.11	0.14	0.25	0.12	0.18	0.28	0.21	0.28
β-Elemene	1389	0.33	0.2	0.21	0.52	0.56	0.37	0.65	0.49	0.20
E-Jasmone	1390	0.04	0.02	-	-	0.08	-	0.02	-	-
α-Gurjenene	1409	0.20	0.08	0.08	0.21	0.04	0.04	0.15	0.09	0.14
β-Funebrene	1413	0.50	0.35	0.34	0.32	0.41	0.5	0.67	0.54	0.25
E-Caryophyllene	1417	15.75	25.71	11.37	19.92	25.13	12.94	12.93	22.23	12.99
β-Cedrene	1419	0.22	0.16	0.72	0.31	0.19	0.22	0.34	0.26	0.12
β-Copaene	1430	1.45	0.64	0.62	1.23	0.66	1.25	1.01	0.68	1.19
Aromadendrene	1439	0.15	0.21	0.34	0.16	0.19	0.36	0.28	0.67	0.30
β-(E)-Farnesene	1440	1.53	3.13	2.96	0.28	1.47	2.88	1.84	3.73	1.98
α-Hummulene	1452	1.00	1.07	0.83	1.37	0.17	0.91	1.11	1.12	0.82
9-*epi*-(E)-Caryophyllene	1464	0.91	0.39	0.54	0.92	0.41	0.31	0.92	0.40	0.39
cis-Muurola 4(14) 5 -diene	1465	-	0.24	0.15	-	0.27	0.62	0.64	0.26	0.52
Dodecanol	1469	0.11	0.47	2.54	4.74	0.71	5.15	1.62	1.91	2.82
γ-Muurolene	1478	0.75	1.15	2.13	-	1.23	-	2.12	1.91	1.13
Germacrene D	1484	39.03	14.78	21.45	26.55	11.97	26.9	26.74	17.77	26.21
β-Selinene	1489	4.77	0.82	6.85	0.69	2.93	0.75	0.87	3.37	2.32
γ-Amorphene	1495	-	-	-	-	0.16	-	0.18	-	-
Bicyclogermacrene	1500	1.2	2.42	2.74	3.38	4.11	4.2	2.47	3.96	4.07
α -Farnesene	1505	1.17	0.91	2.43	1.24	1.38	1.41	0.58	0.94	2.13
γ-Cadinene	1513	0.82	0.89	0.96	0.96	0.98	1.67	1.31	1.33	1.60
δ-Cadinene	1522	2.68	1.99	0.62	2.40	1.82	3.18	2.33	2.33	3.25
Zonarene	1528	0.14	0.11	0.21	0.15	0.12	0.19	0.19	0.17	0.16
trans-Cadina -1,4-diene	1533	0.12	0.15	0.22	0.14	0.16	0.26	0.22	0.22	0.25
α -Cadinene	1537	0.25	0.26	0.23	0.33	0.27	0.46	0.32	0.31	0.44
α-Calacorene	1544	0.72	-	-	0.07	0.06	-	0.15	0.13	0.06

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E-Nerolidol	1561	0.68	0.83	0.46	1.11	1.23	0.85	1.67	1.74	0.94
Dodecanoic acid	1565	-	0.45	0.5	0.24	0.28	-	0.36	0.44	-
Spathulenol	1577	0.36	0.25	0.14	1.14	0.29	0.20	6.80	2.65	0.20
Caryophyllen oxide	1582	0.44	0.97	0.13	2.67	2.26	0.25	7.16	5.67	0.34
Globulol	1590	0.48	0.32	0.23	0.36	0.29	-	0.36	0.45	0.49
Viridiflorol	1592	0.83	0.55	0.77	0.66	0.62	0.74	0.7	-	1.02
Ledol	1602	1.35	0.5	0.43	0.73	0.19	0.21	1.52	-	0.34
Humulene epoxy II	1608	0.24	-	-	0.14	0.12	0.18	0.44	0.64	-
1,10-di-*epi*-Cubenol	1618	-	0.23	0.43	0.3	0.18	0.17	-	-	0.19
Juneol	1618	-	0.47	0.51	0.6	0.44	-	0.46	0.64	0.65
1-*epi*-Cubenol	1627	0.28	0.22	0.35	0.21	0.24	0.88	0.13	0.23	0.29
Eremoligenol	1629	-	-	-	0.38	0.47	-	0.52	-	-
epi -α-Muurolol	1640	1.4	0.62	1.69	1.04	0.63	0.9	0.39	0.42	0.93
α -Muurolol (Torreyol)	1644	0.51	0.27	-	0.57	0.33	0.37	-	0.49	0.38
α-Cadinol	1652	3.38	2.2	2.5	2.95	2.27	2.49	1.95	2.19	2.65
Selin-11-en-4α-ol	1658	-	0.05	-	0.78	0.84	-	0.82	-	0.32
neo -Intermedeol	1665	-	0.38	0.14	0.08	0.84	0.07	-	1.06	-
Tetradecanol	1671	1.20	0.46	2.86	3.80	0.78	2.93	3.84	2.28	3.38
α-Bisabolol	1685	0.13	0.10	0.12	-	0.16	0.09	0.12	0.08	0.08
2E,6E -Farnesol	1742	-	0.07	0.05	-	0.10	0.04	0.08	0.17	0.04
Benzyl benzoate	1759	-	0.17	0.15	0.21	0.11	0.17	0.32	0.62	0.11
14-hidroxy- δ-Cadinene	1803	-	-	-	0.14	-	-	0.14	0.12	-
n-Hexadecanol	1874	0.62	0.12	0.3	0.55	0.11	0.29	0.67	0.42	0.46
Nonadecane	1900	-	0.10	0.01	-	0.08	-	-	0.12	0.05
Phytol	1942	-	-	0.54	1.35	0.20	0.53	0.91	0.49	0.57
Eicosane	2000	-	-	0.18	0.11	-	-	0.12	0.18	-
Heneicosane	2100	0.12	0.14	0.14	0.14	0.08	0.12	0.22	0.14	0.08
Tricosane	2300	0.15	0.08	0.05	-	0.09	0.06	0.09	0.09	0.05
Pentacosane	2500	-	-	-	0.22	0.09	-	0.17	0.15	-
Hexacosane	2600	0.64	0.07	0.16	0.24	0.14	0.28	0.15	0.29	0.17

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Total	93.25	92.52	84.98	92.46	89.71	90.79	97.5	97.05	92.94

RI - Retention index - literature data (Adams, 2007), Fol – leaf, Fl – flower, Hb – herb, T – Tetovo, M – Mavrovo, D - Debar

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Table 3. The composition of aroma components of 18 samples of *H. perforatum* (leaf and flower) from three different locations in western part of R. Macedonia (%)

No.	Components	RI	HP/14 Fl-T	HP/14 Fol-T	HP/15 Fl-T	HP/15 Fol-T	HP/16 Fl-T	HP/16 Fol-T	HP/14 Fl-M	HP/14 Fol-M	HP/15 Fl-M	HP/15 Fol-M	HP/16 Fl-M	HP/16 Fol-M	HP/14 Fl-D	HP/14 Fol-D	HP/15 Fl-D	HP/15 Fol-D	HP/16 Fl-D	HP/16 Fol-D
1	Isononane	858	7.85	70.32	63.00	68.06	75.01	58.62	74.62	71.78	69.69	61.42	66.22	73.1	57.37	34.68	72.46	67.53	57.34	48.82
2	Nonane	900	2.97	2.46	3.10	6.00	3.60	7.65	3.28	-	2.06	2.26	5.77	4.70	8.12	2.92	3.08	3.21	3.43	2.38
3	*α* -Pinene	932	16.03	13.73	24.68	17.31	14.33	17.03	14.18	10.84	17.76	12.34	17.6	8.06	20.68	32.00	17.38	15.72	30.63	35.08
4	3-Methylnonane	971	1.95	2.56	4.14	3.15	2.28	3.02	3.43	-	2.58	2.96	5.23	8.06	2.25	5.05	1.62	2.28	2.25	2.31
5	*β*-Pinene	974	0.4	-	1.24	-	-	3.31	0.46	-	0.98	-	1.86	-	-	-	1.21	2.96	1.85	4.35
6	Mycrene	988	-	-	0.18	-	0.22	-	-	-	-	-	0.31	-	-	-	-	-	0.55	0.46
7	*n*-Decane	1000	-	-	-	-	0.12	-	-	-	-	-	-	-	-	-	-	-	0.10	-
8	Limonene	1024	-	-	-	-	0.11	-	-	-	-	-	0.18	-	-	-	-	-	0.27	-
9	*β*-(Z)-Ocimene	1032	-	-	-	-	0.08	-	-	-	-	-	0.17	-	-	-	-	-	-	-
10	*β*-(E)-Ocimene	1044	-	-	-	-	0.23	-	-	-	-	-	0.17	-	-	-	0.22	-	0.26	-
11	2-Methyldecane	1067	3.06	6.51	2.15	3.18	1.77	3.18	2.37	7.93	3.2	5.56	1.28	2.8	5.06	14.55	2.51	5.20	1.93	3.09
12	*n*-Undecane	1100	0.57	1.42	0.41	1.28	0.40	4.32	0.38	-	0.46	-	0.47	-	3.29	-	0.49	1.30	0.39	0.77
13	*allo*-Ocymene	1128	-	-	-	-	0.04	-	-	-	-	-	0.08	-	-	-	-	-	-	-
14	Tridecane	1300	0.06	-	0.03	-	0.03	-	0.19	-	0.08	-	0.14	-	0.56	-	0.06	-	-	-
15	*α*-Longipinene	1350	-	-	-	-	-	0.37	-	-	-	-	-	-	-	-	-	-	-	-
16	*α*-Copanene	1374	-	-	0.03	-	0.01	0.54	0.04	1.34	0.08	1.41	0.01	0.26	0.16	1.04	0.02	-	0.02	-

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17	E-Caryophyllee	1417	0.32	1.03	0.32	0.61	0.25	1.03	0.33	4.53	1.68	7.94	0.27	2.1	1.93	4.32	0.11	0.57	0.34	1.27
18	*β*-(E)-Farnesene	1454	-	-	0.04	-	0.01	0.16	-	-	0.06	-	0.05	-	-	-	-	-	-	-
19	*γ*-Muurolene	1478	-	-	-	-	-	-	0.04	-	1.10	-	0.01	-	0.2	1.37	-	0.37	0.02	0.17
20	*β*-Selinene	1489	-	-	-	-	-	-	0.03	-	0.09	-	-	-	0.2	-	-	-	0.07	0.11
21	*α*-Selinene	1498	-	-	-	-	-	-	0.03	-	0.08	-	-	-	-	-	-	-	0.06	0.10
22	*γ*-Cadinene	1513	0.03	0.59	0.03	-	-	-	-	1.59	-	-	0.01	0.34	-	-	0.02	-	-	-
23	*δ*-Cadinene	1522	-	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-
	Total		99.24	98.62	99.35	99.59	99.81	99.23	99.38	98.01	99.96	93.89	99.83	99.42	99.82	95.93	99.18	99.14	99.51	98.91

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Table 4. The composition of *n*-hexane extracts from 12 samples of *H. perforatum* (leaf and flower) from three different locations in Western part of R. Macedonia (%)

Components	RI	HP/14 Fl-T	HP/14 Fol-T	HP/15 Fl-T	HP/15 Fol-T	HP/14 Fl-M	HP/14 Fol-M	HP/15 Fl-M	HP/15 Fol-M	HP/14 Fl-D	HP/14 Fol-D	HP/15 Fl-D	HP/15 Fol-D
Monoterpene													
α-Pinene	932	0.02	-	-	0.85	-	0.08	-	0.65	0.42	-	1.24	-
α-Campholenal	1122	-	-	-	1.14	-	-	-	0.84	-	-	0.64	-
trans-Verbenol	1140	-	-	-	1.14	-	-	1.54	-	-	-	-	-
Isopulegol	1145	0.05	-	0.05	-	-	0.35	-	-	-	-	0.56	0.46
Menthone	1148	2.45	-	-	-	-	1.78	-	-	-	-	-	2.54
iso-Menthone	1158	-	-	-	-	-	2.11	-	-	-	-	-	-
neo-Menthol	1161	-	-	-	-	-	2.44	3.23	-	-	-	-	-
Menthol	1167	0.70	2.24	0.77	4.70	0.44	1.39	-	-	-	2.24	-	-
Verbenone	1204	-	-	-	0.73	-	-	0.75	-	-	-	-	-
Pulegone	1233	-	-	-	-	-	0.60	-	-	-	-	-	-
Piperitone	1249	-	-	-	-	-	0.32	-	-	-	-	-	-
Thymol	1289	1.41	-	-	-	2.80	-	0.63	-	-	-	-	-
Carvacrol	1298	0.04	-	0.84		0.32	-	-	0.82	1.42	1.24	-	-
iso-Menthyl acetate	1304	-	-	-	-	-	1.26	-	-	-	-	-	-
Eugenol	1356	1.72	3.41	3.48	2.53	3.41	0.51	2.33	1.33	2.47	1.09	4.04	2.54
Sesquiterpene													
β-Bourbonene	1387	0.06	0.02	-	0.05	0.05	0.09	0.04	0.04	0.04	0.02	-	0.05

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β-Elemene	1389	0.23	0.34	0.28	0.33	0.08	0.08	-	-	0.02	0.02	0.06	-
Vanillin	1393	0.02	0.05	-	0.02	-	0.24	0.24	0.37	0.57	-	0.08	-
E-Caryophyllene	1417	2.36	1.28	2.34	1.28	2.12	2.18	1.95	0.38	0.36	2.59	0.45	0.08
β-Copanene	1430	0.02	-	0.02	-	-	0.03	-	-	-	0.02	-	-
γ-Elemene	1434	-	1.20	1.32	0.08	0.06	0.04	-	0.02	-	1.15	1.24	1.08
α-Humulene	1452	-	-	-	-	-	-	-	-	-	1.58	-	-
9-*epi* (E) Caryophyllene	1464	0.08	0.08	1.21	0.82	0.28	0.64	0.02	0.04	0.05	-	0.08	0.19
Dauca-5,8-diene	1471	-	2.45	3.12	2.18	2.18	0.49	3.45	3.45	3.24	2.43	1.45	2.89
Germacrene D	1484	0.02	0.04	0.04	0.02	-	0.12	0.14	0.12	-	0.02	0.04	0.02
Eugenol acetate	1521	0.02	-	0.01	-	-	0.12	-	0.04	0.04	0.02	-	-
Selina-3,7 (11)-diene	1545	4.25	3.45	2.28	5.22	4.82	1.28	-	2.22	2.28	2.71	2.42	3.89
Spathulenol	1577	0.12	0.22	0.34	0.42	0.34	0.02	0.04	0.04	0.82	-	0.02	0.02
Caryophyllene oxide	1582	-	-	1.98	1.5	1.31	0.62	3.20	0.55	2.47	0.32	2.88	0.4
Guiol	1600	1.24	0.89	1.24	2.34	1.28	1.26	0.84	2.45	2.48	2.36	2.34	1.11
Humulene epoxide II	1608	0.02	0.05	0.02	-	-	0.02	0.85	-	0.46	0.62	0.24	0.46
10-*epi*-*γ*-Eudesmol	1622	2.14	2.12	1.89	1.84	1.42	0.84	1.24	2.27	1.84	3.26	2.84	1.48
Bulnesol	1670	1.25	1.48	0.89	2.02	1.84	2.24	2.45	1.74	1.46	2.15	2.18	1.84
Hidrocarbons and related components													
Tetradecane	1400	-	-	-	-	-	-	-	-	-	-	-	1.10
Dodecanol	1469	-	1.43	-	-	-	-	-	-	-	-	-	0.62
Tetradecanol	1671	-	-	-	0.70	-	-	-	-	-	-	0.39	-
N-Pentadecanol	1773	-	3.52	-	-	0.73	0.15	-	1.21	0.03	-	2.80	1.96
Octadecane	1800	-	-	-	-	-	-	-	-	-	-	-	0.51
Hexadecanol	1874	-	3.12	0.48	0.85	-	0.17	-	1.62	-	1.05	2.60	2.01

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Nonadecane	1900	-	-	-	-	0.28	-	-	-	0.02	-	5.65	-
Methyl hexadecanoate	1921	-	-	0.81	-	-	-	-	-	-	-	-	-
Hexadecanoic acid	1959	-	-	-	3.58	-	-	-	-	-	-	-	-
Eicosane	2000	-	-	-	-	0.32	-	-	-	-	-	2.87	-
Octaecanal	2022	-	2.61	3.1	-	-	-	-	-	-	-	-	-
Heneicosane	2100	1.62	-	-	-	2.82	-	1.82	-	0.16	-	-	-
Docosane	2200	-	-	-	-	0.41	-	-	-	0.02	-	-	-
Tricosane	2300	0.93	-	1.71	-	-	-	1.53	1.01	0.11	3.35	6.83	6.79
9(Z)Octadecenamide	2375	1.76	-	-	-	-	-	-	-	-	-	-	7.19
Tetracosane	2400	-	-	-	-	0.32	-	-	-	-	-	-	-
Pentacosane	2500	5.18	2.69	2.27	5.74	9.04	3.35	4.42	1.69	2.24	5.34	1.68	2.24
Hexacosane	2600	-	-	-	-	-	-	1.53	-	-	-	-	-
Heptacosanol	2609	-	-	-	-	-	-	-	-	-	-	-	12.05
Heptacosane	2700	-	-	-	4.24	-	0.35	-	7.52	-	-	-	-
Octacosane	2800	8.42	17.25	12.25	11.14	8.42	23.61	4.33	40.05	38.25	38.68	34.45	17.54
Nonacosane	2900	48.57	34.2	41.87	38.14	42.06	40.32	49.28	28.13	24.43	18.68	15.45	15.68
Octacosanol	3010	-	-	-	-	-	0.12	0.27	0.15	-	-	-	-
Dotriacontane	3200	-	-	-	1.78	-	-	-	-	-	-	-	-
Tritriacontane	3300	3.3	2.89	3.22	6.01	3.24	5.25	1.88	-	2.88	5.32	-	-
Tetratriacontane	3400	-	-	-	-	-	-	-	-	-	-	-	2.36
Tetratetracontane	4400	15.61	-	9.13	-	-	-	-	-	-	-	-	-
OC (total fraction)		85.39	67.71	74.84	72.18	67.64	73.32	65.06	81.38	68.14	72.42	72.72	70.05
Total of HE		85.93	93.03	96.96	96.85	95.39	94.47	88.00	98.75	88.58	96.26	95.52	89.1

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