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# *Chemical composition and antioxidant activity of the essentials oil of hyssop (Hyssopus officinalis L. ssp. officinalis)*

Skład chemiczny oraz aktywność antyoksydacyjna olejku eterycznego z hyzopu lekarskiego *(Hyssopus officinalis* L. ssp. *officinalis)*

## INTRODUCTION

Hyssop (*Hyssopus officinalis* L*,* F. *Lamiaceae*) probably originates from south-west Asia and south Europe. It can be found on lowlands and foothills, seldom appearing in mountain areas. Hyssop grows on dry and insolated slopes and meadows with calcareous soil, and sometimes in gardens commonly in old monastic gardens [22]. This herb is cultivated in eastern and central Europe, in France, Italy, the Balkans, Ukraine (Crimea) and in Asia [8]. Essential oil obtained form hyssop is used in food, cosmetics and within the pharmaceutical industry. It also possesses antibacterial, antiviral, antifungal and expectorant properties [4, 5, 16]. Recent research suggests that essential oil present in hyssop shows some antiplatelet activity [19]. What is more, spasmolytic action of this oil was described [10].

Essential oil is the main physiologically active constituent of hyssop. In leaves the content of the oil oscillates between 0.3% and 1.5%, in flowers between 0.9% and 2.0% whilst in stems, it is only present in trace amounts. It is obtained during steam distillation of dried or fresh herb (efficiency is about 0.15–0.3% and 0.3–0.8, respectively). On a large scale, it is produced in Mediterranean countries – in France and Italy and also in former Yugoslavia and the Ukraine [6]. Hyssop oil is a light green or light yellow liquid with a sweet camphoric scent. The amount and composition of essential oil from hyssop depends on many external factors (e.g. climatic conditions, type of soil), on the origin of the plant and harvesting time [22,23]. In previous studies, 31 chemical compounds were described in oils from different subspecies of hyssop. The main compounds usually found were *cis*-pinocamphone and *trans*-pinocamphone. Furthermore *β*-pinene, pinocarvone, limonene, linalool, *β*-caryophyllene, germacrene D, thujones and myrtenol were also often present [6, 7, 11, 12, 14].

The purpose of this work was to examine the composition of essential oil obtained from aerial parts of *Hyssopus officinalis L*. ssp. *officinalis* grown in Lublin (Eastern Poland). The full GC/MS and GC/FID analysis of essential oil,obtained from hyssop grown in Poland was performed for the first time. Due to the fact that numerous studies conducted have described that toxic compounds like methyl eugenol, a compound with confirmed carcinogenic activity, or monoterpenic ketones with strong epileptogenic properties can be present in essential oil isolated from hyssop it was necessary to perform the analysis of this oil [3, 18, 21].

## MATERIAL AND METHODS

Plant material. Hyssop (*Hyssopus officinalis* L. ssp. *officinalis*) was grown in the herb garden at the Faculty of Pharmacy, Medical University of Lublin, Poland (N 51°16' E 22°34'). Aerial parts of hyssop were harvested during the flowering stage in August 2005. The taxonomic identification was confirmed by the plant taxonomist, Stanisław Kwiatkowski, in the Dept. of Pharmacognosy within the Medicinal Plant Laboratory of the Faculty of Pharmacy (Medical University of Lublin, Poland). After identification the plant material was dried at 35ºC and then ground. The voucher specimen was deposited at the Herbarium of the Department of Pharmacy, Medical University of Lublin, Poland (No. 0501).

Isolation of essential oil. Essential oil was isolated using steam distillation in the Deryng-type apparatus – 50 g of dried hyssop was distillated with 400 ml of water for three hours. This method of obtaining essential oil is recommended by Polish Pharmacopoeia [15]. The obtained oil was dried with anhydrous sodium sulphate and stored at 4°C until tested and analysed.

GC/MS and GC/FID analysis conditions. The qualitative and quantitative analysis of particular components of the essential oil was made by means of gas chromatography techniques: GC/MS and GC/FID. For GC/MS analysis ITMS Varian 4000 GC/MS/MS (Varian, USA) apparatus equipped with a CP-8410 autoinjector and a VF-5ms column (column size: 30 m x 0.25 mm, film thickness: 0.25 μm Varian, USA) was used. Operating conditions were as follows: injector temperature – 220°C, detector temperature – 200°C, carrier gas: Helium at the flow rate of 1 ml/min, split injection with split ratio 1:20 and inject volume 1 μl. The temperature gradient was applied – initially 60°C for 5 minutes, then raised to 246°C at the rate of 3°C/min and finally held at that temperature for 10 minutes. For GC/FID analysis Varian 3800 with DB-5 column (J&W, USA) was used. Operating conditions were similar to GC/MS. The FID detector's temperature was 256°C.

The qualitative analysis was carried out on the basis of MS spectra which were compared with the spectra of the NIST library [13], and data available in literature- Kováts' retention [1, 9]. Identity of the compounds was confirmed by their retention indices taken from literature and own data [1, 9]. The composition of the essential oil was determined by GC/FID, by assuming the totality of all the particular oil to be 100% pure.

## RESULTS AND DISCUSSION

The amount of the essential oil distillated from hyssop was 0.18% (v/w) on dry weight basis of herbage. The GC chromatogram of compounds present in the tested essential oil is shown in Figure 1.

Table 1 presents the percentage composition of this oil. Components are listed in order of their elution from the DB-5 capillary column.

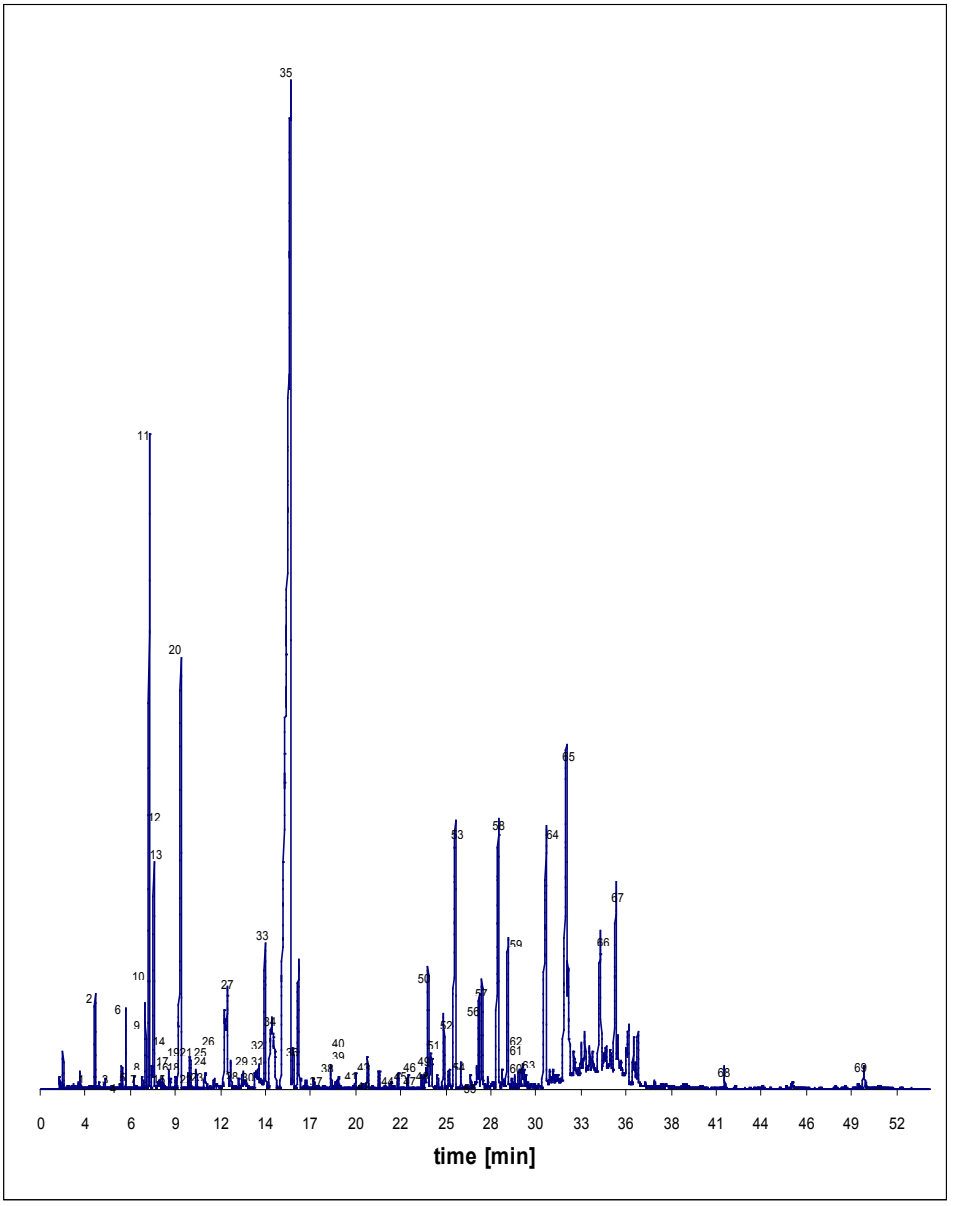


Fig. 1. GC chromatogram of chemical compounds present in essential oil of *Hyssopus officinalis L.* – compounds are marked in accordance with Table 1

Table 1. Percentage composition of essential oil obtained from *Hyssopus officinalis L.*

| No | Compound | KI | Percentage | Method of identification | References |
|---|---|---|---|---|---|
| 1 | Hexanal | 794 | 0.11 | MS, RI | [1, 13] |
| 2 | *2(E)*-Hexenal | 844 | 0.46 | MS, RI | [1, 13] |
| 3 | *n*-Hexanol | 858 | tr | MS, RI | [1, 13] |
| 4 | Heptanal | 903 | tr | MS, RI | [1, 13] |
| 5 | *α*-Thujene | 926 | 0.09 | MS, RI | [1, 13] |
| 6 | *α*-Pinene | 934 | 0.32 | MS, RI, Co | [1, 13] |
| 7 | Camphene | 949 | 0.07 | MS, RI | [1, 13] |
| 8 | Thuja-2,4(10)-diene | 956 | 0.11 | MS, RI | [1, 13] |
| 9 | Benzaldehyde | 969 | tr | MS, RI | [1, 13] |
| 10 | Sabinene | 974 | 0.48 | MS, RI | [1, 13] |
| 11 | *β*-Pinene | 981 | 6.14 | MS, RI, Co | [1, 13] |
| 12 | 3-Octanone | 983 | 0.09 | MS, RI | [1, 13] |
| 13 | Myrcene | 991 | 1.26 | MS, RI | [1, 13] |
| 14 | dehydro-1,8-Cineole | 993 | tr | MS, RI | [1, 13] |
| 15 | *p*-Mentha-1(7),8-diene | 1008 | 0.12 | MS, RI | [1, 13] |
| 16 | *α*-Phellandrene | 1008 | tr | MS, RI | [1, 13] |
| 17 | *α*-Terpinene | 1017 | tr | MS, RI | [1, 13] |
| 21 | *p*-Cymene | 1025 | tr | MS, RI | [1, 13] |
| 19 | Limonene | 1030 | 0.71 | MS, RI, Co | [1, 13] |
| 20 | 1,8-Cineole | 1033 | 5.78 | MS, RI | [1, 13] |
| 21 | *Z-β*-Ocimene | 1036 | tr | MS, RI | [1, 13] |
| 22 | Benzene acetaldehyde | 1041 | 0.17 | MS, RI | [1, 13] |
| 23 | *γ*-Terpinene | 1058 | 0.08 | MS, RI | [1, 13] |
| 24 | n.i.* | 1068 | tr | – | – |
| 25 | Terpinolene | 1089 | tr | MS, RI | [1, 13] |
| 26 | *cis*-Linalool oxide | 1091 | tr | MS, RI | [1, 13] |
| 27 | Linalool | 1107 | 1.33 | MS, RI, Co | [1, 13] |
| 28 | *α*-Thujone | 1117 | 0.07 | MS, RI | [1, 13] |
| 29 | *β*-Thujone | 1122 | tr | MS, RI | [1, 13] |
| 30 | *cis-p*-Menth-2en-1-ol | 1129 | tr | MS, RI | [1, 13] |
| 31 | *trans*-Pinocarveol | 1149 | 0.29 | MS, RI | [1, 13] |
| 32 | *trans-p*-Menth-2-en-1-ol | 1151 | tr | MS, RI | [1, 13] |
| 33 | n.i.* | 1158 | 2.21 | – | – |
| 34 | *trans*-Pinocamphone | 1161 | 1.90 | MS, RI | [1, 13] |
| 35 | *cis*-Pinocamphone | 1219 | 48.56 | MS, RI | [1, 13] |
| 36 | *cis*-Piperitol | 1221 | tr | MS, RI | [1, 13] |
| 37 | n.i.* | 1223 | tr | – | – |
| 38 | Carvone | 1241 | 0.14 | MS, RI | [1, 13] |
| 39 | Carvotanacetone | 1250 | 0.07 | MS, RI | [1, 13] |
| 40 | *trans*-2-hydroxy-Pinocamphone | 1255 | tr | MS, RI | [1, 13] |
| 41 | *p*-Menth-1-en-7-al | 1284 | tr | MS, RI | [1, 13] |
| 42 | *α*-Terpinen-7-al | 1292 | tr | MS, RI | [1, 13] |
| 43 | *p*-Cymen-7-ol | 1298 | 0.22 | MS, RI | [1, 13] |
| 44 | *m*-Acetanisole | 1313 | 0.12 | MS, RI | [1, 13] |
| 45 | Eugenol | 1357 | 0.11 | MS, RI | [1, 13] |

| No | Compound | KI | Percentage | Method of identification | References |
|---|---|---|---|---|---|
| 46 | n.i.* | 1340 | 0.21 | – | – |
| 47 | *α*-Copaene | 1373 | 0.07 | MS, RI | [1, 9, 13] |
| 48 | Menthyl *p*-anisate | 1380 | 0.08 | MS, RI | [1, 13] |
| 49 | *(E)-β*-Damscenone | 1386 | 0.10 | MS, RI | [1, 13] |
| 50 | *β*-Bourbonene | 1390 | 1.06 | MS, RI | [1, 9, 13] |
| 51 | *(E)*-Jasmone | 1395 | 0.14 | MS, RI | [1, 13] |
| 52 | Methyl eugenol | 1405 | 0.38 | MS, RI | [1, 13] |
| 53 | *(E)*-Caryophyllene | 1428 | 3.99 | MS, RI | [1, 9, 13] |
| 54 | *β*-Copaene | 1434 | 0.17 | MS, RI | [1, 9, 13] |
| 55 | n.i.* | 1449 | 0.07 | – | – |
| 56 | *α*-Humulene | 1459 | 0.82 | MS, RI | [1, 9, 13] |
| 57 | allo-Aromadendrene | 1467 | 0.84 | MS, RI | [1, 9, 13] |
| 58 | Germacrene D | 1489 | 3.37 | MS, RI | [1, 9, 13] |
| 59 | Bicyclogermacrene | 1503 | 1.34 | MS, RI | [1, 9, 13] |
| 60 | *β*-Bisabolene | 1511 | 0.06 | MS, RI | [1, 9, 13] |
| 61 | *γ*-Cadinene | 1521 | 0.09 | MS, RI | [1, 9, 13] |
| 62 | *δ*-Cadinene | 1521 | tr | MS, RI | [1, 9, 13] |
| 63 | *β*-Sesquiphellandrene | 1526 | 0.38 | MS, RI | [1, 9, 13] |
| 64 | Elemol | 1561 | 7.43 | MS, RI | [1, 13] |
| 65 | Caryophyllene oxide | 1590 | 5.17 | MS, RI | [1, 13] |
| 66 | *γ*-Eudesmol | 1639 | 1.25 | MS, RI | [1, 13] |
| 67 | *α*−Eudesmol | 1662 | 1.39 | MS, RI | [1, 13] |
| 68 | n.i.* | 2145 | 0.08 | – | – |
| 69 | Phytol acetate | 2219 | 0.17 | MS, RI | [1, 13] |
| Total | | | 99.67 | | |

*GC-MS 70eV, 200°C *m/z* (rel. int.): compound **24**: no M+, 43(100), 95(79), 79 (70), 81(50) 41(37), 39(36), 93(32), 67(30), 71(29), 53(27). Compound **33**: no M+, 91(100), 45(89), 93(64), 79(55), 92(51), 41(48), 39(32), 77(27), 119(26), 67(24). Compound **37**: no M+, 43(100), 79(97), 39(84), , 41(81), 94(64), 67(59), 91(56), 109(56), 99(48), 152(42). Compound **46**: no M+, 41(100), 39(85), 69(73), 83(67), 81(52), 96(38), 42(27), 53(25), 67(23), 95(19). Compound **55**: no M+, 41(100), 161(100), 91(83), 105(81), 93(70), 77(69), 79(60), 39(60), 81(58), 133(44). Compound **68**: no M+, 43(100), 41(58), 58(44), 110(39), 95(36), 55(33), 71(31), 59(28), 57(24), 39(23). Explanations: n.i., not identified; tr, trace amount (< 0.05%); KI, Kováts indices; MS, mass fragmentation; RI, comparison of Kováts indices with literature values; Co, Co-chromatography with authentic sample

In total 69 chemical compounds were found accounting for 99.7% of the sample and 63 were identified (97.1% of the sample). The main constituent was *cis*-pinocamphone (48.6%), followed by elemol (7.4%), *β*-pinene (6.1%), *1,8*-cineole (5.8%) and caryophyllene oxide (5.2%). A comparison of those results with the review of the published literature indicates that the composition of the essential oil isolated from Polish hyssop is similar to those obtained from hyssop in Serbia [12]. Furthermore, the level of *cis*-pinocamphone corresponded with ISO 984 Standard (1991 E) which recommends 34.5–50% for *cis*-pinocamphone. The level of the second isomer of pinocamphone (*trans*-pinocamphone) and the level of *β*-pinene are below the ISO Standard that demands 5.5–17.5% of *trans*-pinocamphone and 13.5–23% of *β*-pinene [11]. Investigations of essential oils obtained from hyssop plants that originate from

different locations are necessary because of their great diversity in chemical composition. For example, in essential oil obtained from hyssop grown in India, the main compound is *trans*-pinocamphone (49.1%); also, high levels of *β*-pinene (18.4%) and *cis*-pinocamphone (9.7%) are present [5]. The predominant compound in essential oil from *Hyssopus officinalis L. subsp. angustifolius (Bieb.) Arcangeli* (from Turkey) is pinocarvone. Moreover, high levels of *trans*-pinocamphone (19.6%), *β*-pinene (10.6%) and *1,8*-cineole (7.2%) are present, whilst the amount of *cis*-pinocamphone (5.3%) is relatively low [9]. The chemotype of hyssop from Spain has a unique composition, because of high amounts of *1,8*-cineole (52.89%) [20], whereas in essential oil from *Hyssopus officinalis var. decumbens* (from France) linalool (49.6%) predominates over other compounds. The characteristic feature of this oil is a very low level of monoterpenic ketones: *trans*-pinocamphone and *cis*-pinocamphone [17]. In addition to this, *Hyssopus officinalis L. subsp. aristatus*, an endemic plant growing in three regions of Italy – Popoli, Avezzano and Assergi – has low contents of these compounds. Furthermore, the chemotype from every region mentioned above has a unique chemical composition [22, 23].

## CONCLUSIONS

Polish hyssop that was the subject of our research belongs to the group of chemotypes rich in bicyclic monoterpenic ketones – *cis*-pinocamphone and *trans*-pinocamphone. The amount of essential oil obtained from air dried hyssop harvested during the flowering stage – 0.18% (v/w) – is relatively low in comparison with data published in literature – 1.18% (v/w) [5] and 1.13% (v/w) [14].

The quality of essential oil obtained from Polish hyssop does not come up to expectations of ISO 984 Standard (1991 E) [11], because of low content of *trans*-pinocamphone and *β*-pinene.

High levels of *cis*-pinocamphone suggests strong epileptogenic properties of oil obtained from Polish hyssop which were proven for several other rich in cis-pinocamphon chemotypes of hyssop [3, 4, 18]. That is why hyssop and the remedies that contain hyssop or its essential oil should not be used by children and those patients that suffer from epilepsy. What is more, allergic reactions to hyssop, as well as to the other species belonging to the *Labiatae* family, should also be taken under consideration while using hyssop [2]. Our experiment shows that Polish hyssop is poor in methyl eugenol (0.379%) that possesses carcinogenic properties [21].

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## SUMMARY

The plants of *Hyssopus officinalis L*. were grown in the herb garden at the Faculty of Pharmacy, Medical University of Lublin, Poland. The oil obtained with the use of steam distillation from the air-dried, aerial parts of hyssop was analysed by means of GC/MS and GC/FID techniques. Sixty-nine components were found, representing 99.7% of the essential oil and sixty-three of them were identified. The major constituents were identified as *cis*-pinocamphone (48.6%), elemol (7.4%), *β*-pinene (6.1%), *1,8*-cineole (5.8%) and caryophyllene oxide (5.2%).

*Keywords: Hyssopus officinalis L.*, hyssop, essential oil, cis-pinocamphone, GC/MS, GC/FID

STRESZCZENIE

Surowiec do badań stanowiło ziele hyzopu lekarskiego *Hyssopus officinalis L.*, uprawianego w Pracowni Roślin Leczniczych, Katedry i Zakładu Farmakognozji UM w Lublinie. Olejek eteryczny otrzymano poprzez destylację z parą wodną, a następnie analizowano metodą GC/MS oraz GC/FID. Oznaczono udział 69 składników olejku eterycznego (99,7%), z czego 63 zidentyfikowano. Głównymi składnikami olejku były *cis*-pinokamfon (48,6%), elemol (7,4%), *β*-pinen (6,1%), *1,8*-cineol (5,8%) oraz tlenek kariofilenu (5,2%).

*Słowa kluczowe: Hyssopus officinalis L.*, hyzop, olejek eteryczny, cis-pinokamfon, GC/MS, GC/FID