Essential oil composition of hyssop (*Hyssopus officinalis* L.) cultivated in north-western Poland

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Summary

Essential oil from the herb of *Hyssopus officinalis* L. was obtained by steam distillation, simple hydrodistillation and hydrodistillation in Dean-Stark apparatus. The chemical composition of the oil was determined by GC-MS. The analysis revealed the presence of 31 compounds in steam distilled oil, 36 compounds in hydrodistilled oil and 27 compounds in essential oil obtained by hydrodistillation in Dean-Stark apparatus. Isopinocamphone was the dominating component (40.07–45.45%) in the all analysed oil samples.

Key words: *hyssop, herb of hyssop, steam distillation, hydrodistillation, hydrodistillation in Dean-Stark apparatus, GC, MS, elemol, isopinocamphone*

INTRODUCTION

Hyssopus officinalis L. is an important medicinal plant native to central and Southern Europe, Western Asia, and North Africa. The herb is an evergreen perennial plant with small, linear leaves and purplish-blue flowers [1].

It has a very strong spicy taste and intensive flavour. It is commonly used in folk medicine. Hyssop extracts and oil may be found as flavour ingredient in many food products, mainly sauces and seasonings, also in bitters and liqueurs. The oil is a fragrance component in soaps, cosmetics and perfumes, especially eau-de-cologne and oriental bases [2].

As a medicinal herb, hyssop is used in viral infections such as colds, coughs, sore throats, bronchitis and asthma. A tea made from the herb is effective in nervous disorders and toothache [3-5].

The flowering tops of hyssop produce a pleasant volatile oil responsible for most of biological activities of the plant.

Extracts of the leaves are antimicrobial, mildly spasmolytic and exhibit strong antiviral activity against HIV [6-7]. Antibacterial and antifungal property of hyssop has been attributed to the presence of pinocamphone, isopinocamphone and β-pinene. Antiviral activity has probably been attributed to the presence of caffeic acid, tannins and unidentified high molecular weight compounds [7-8].

High amounts of phenolic acids which were found in methanol extract of *H. officinalis* L. revealed possible antioxidant activity of hyssop [9]. Moreover, fresh herb of hyssop is also characterized by a high content of vitamin C: 63.6 $mg \cdot 100g^{-1}$ f.w.[10-11].

In agriculture, determination of essential oil composition is necessary for quality control, cultivation and production of aromatic plants.

Essential oils are pleasant smelling, volatile mixtures extracted from different parts of the plant – leaves, stems, flowers, fruits, roots, barks or other elements of a plant-by distillation (most frequently by steam or water). The efficiency and yield of oil depend on the length of distillation time, the temperature and quality of the plant material [12, 13]. The aim of our study was to compare three different distillation techniques for isolating volatile oil from the flowering aerial parts of *Hyssopus officinalis* L. cultivated in the Department of Vegetable Crops, West Pomeranian University of Technology, Szczecin, Poland.

MATERIAL AND METHODS

Chemicals

Dichloromethane (pure p.a.) was purchased from Chempur and used as received.

Plant

The research material was produced at the Horticultural Experiment Station (Dołuje), which belongs to the Department of Vegetable Crops of the West Pomeranian University of Technology, Szczecin.

Hyssop herb was collected in August 2009, air-dried and stored in sealed bags in a dark and cool place.

Isolation of the essential oil

Steam distillation

The dried plant material weighing 5 g was placed in round bottom flask containing 500 ml water and distilled for 2 hours. Steam was generated separately in a steam boiler and was passed through the distillation flask. The distillate was saturated with NaCl and transferred to a separator funnel where was extracted with dichloromethane. The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield yellowy-green oil (0.02 g; 0.4%).

Simple hydrodistllation

The dried plant material (5 g) was suspended in water (450 ml) and hydrodistilled for 2 hours to obtain a yellowish oil. The aqueous layer from the distillate was extracted with dichloromethane and dried over anhydrous sodium sulfate. After removing of the solvent, the oil (0.04 g, 0.8%) was stored in a refrigerator prior to analysis.

Hydrodistillation in Dean-Stark apparatus

Essential oil was obtained through hydrodistillation of dried plant material (5 g) using Dean-Stark apparatus for 4 hours. The aqueous layer from distillate was extracted with dichloromethane and dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure in a rotary evaporator at 35^{o}C.

Light yellow oil with characteristic smell was obtained (0.02 g, 0.4%).

GC-MS analysis

Gas chromatography-mass spectrometry analysis (GC-MS) was performed using an HP 6890 gas chromatograph coupled with HP 5973 Network mass selective detector operating at 70eV electron impact mode. Compounds were separated on 30 m long capillary column (HP-5MS), 0.25 mm in diameter (methylsiloxane modified with phenyl groups) in the 0.25 μm active phase layer.

The GC oven temperature was programmed from 40^{o}C (kept constant for 5 minutes) to 280^{o}C (kept constant for 30 minutes) at a rate of 9^{o}C/min. The duration of analysis time was 61.67 minutes.

Flow rate of helium was 4.1 mL/min at 9.7 psi. The volume of sample injected was 5 μL and split injection was used (split ratio: 5.4:1).

The injector and detector temperature was 280^{0}C, the ion source temperature was 230^{o}C , the solvent delay was 3 minutes and the mass scan range was *m/z* 29 – 800.

The oil samples were injected in dichloromethane.

Compound identification

Compounds were identified using NBS75K.L Hewlett-Packard Mass Spectrometer ChemStation library search and manually rechecked with NIST/EPA/NIH Mass Spectral Library (2002 version).

RESULTS AND DISCUSION

Thirty one compounds were identified in the oil obtained by steam distillation from dried flowering aerial parts of *Hyssopus officinalis* L. representing 90.85% of the oil constituents.

The main compounds found in the oil were characterized as isopinocamphone (41.97%) and elemol (17.21%). The other components of the oil are listed in table 1.

The oil was rich in sesquiterpene alcohols (31.31%): elemol, spathulenol, α-eudesmol, γ-eudesmol, virdiflorol, hedycaryol and monoterpene ketones (42.13%): isopinocamphone and cis-jasmone.

Hydrodistillation of the dried flowering aerial parts of *Hyssopus officinalis* L. gave 0.04 g (0.8%) of light yellowish oil. As shown in table 2, thirty-six components were identified in this oil, accounting for 91.73 % of the total oil composition.

Monoterpene ketones (45.56%): isopinocamphone, cis-jasmone, sesquiterpene alcohols (25.69%): elemol, guaiol, spathulenol, virdiflorol, hedycaryol, α-eudesmol, γ-eudesmol and sesquiterpene hydrocarbons (8.54%): γ-gurjunene, β-bourbonene, α-gurjunene, caryophyllene, (-)-alloaromadendrene, (+)-aromadendrene, γ-cadinene, valencene, α-selinene, (+)-epi-bicyclosesquiphellandrene were dominated components of the isolated oil.

The essential oil obtained after hydrodistillation in Dean-Stark apparatus gave an average yield of 0.4%, similarly to the steam distillation. The main constituents were isopinocamphone (40.07%) and elemol (11.50%) (tab. 3).

Sesquiterpene hydrocarbons (13.98%), sesquiterpene alcohols (23.92%) and monoterpene ketones (40.35%) dominated in the oil.

The oil was separated to 27 components, representing 93.92 % of total yield.

Table 1.

Composition of the steam distilled essential oil of *Hyssopus officinalis* L.

peak	Rt (min)	component	area %
1	9.58	β-pinene	0.20
2	9.81	1-octen-3-ol	0.25
3	10.81	eucalyptol	0.61
4	11.18	benzeneacetaldehyde	0.22
5	11.62	cis-β-terpineol	0.50
6	12.27	(R/S)-linalool	0.31
7	12.35	linalool	1.31
8	12.74	(E)-p-menth-2-en-1-ol	0.18
9	13.09	trans-pinocarveol	0.13
10	**13.79**	**isopinocamphone**	**41.97**
11	13.95	α-terpineol	0.72
12	14.06	myrtenol	2.71
13	15.87	carvacrol	0.15
14	16.60	eugenol	0.32
15	17.18	cis-jasmone	0.16
16	17.24	eugenol methyl ether	0.80
17	17.49	caryophyllene	0.17
18	18.08	(-)-alloaromadendrene	0.11
19	18.36	β-cubebene	0.64
20	18.57	(5E)-2,5-dimethyl-3-methylene-1,5-heptadiene	0.44
21	19.38	elemol	17.21
22	19.73	(-)-spathulenol	2.63
23	19.79	7-(1-methylethylidene)bicyclo[4.1.0] heptane	1.23
24	20.04	virdiflorol	1.58
25	20.12	hedycaryol	1.96
26	20.25	α-selinene	1.75
27	20.40	γ-eudesmol	3.70
28	20.50	8-isopropyl-5-methyl-2-methylene-1,2,3,4, 4a,5,6,7-octahydronaphthalene	3.46
29	20.69	α-eudesmol	4.23
30	20.93	γ-gurjunene	1.12
31	24.73	hexadecanoic acid	0.08
identified			**90.85**
unidentified	(13 compounds)		9.15

Table 2.

Composition of the hydrodistilled essential oil of *Hyssopus officinalis* L.

peak	Rt (min)	component	area %
1	9.58	β-pinene	0.23
2	9.82	1-octen-3-ol	0.25
3	10.81	β-thujene	0.86
4	11.18	benzeneacetaldehyde	0.19
5	11.62	cis-β-terpineol	0.29
6	12.27	(R/S)-linalool	0.31
7	12.40	linalool	1.51
8	**13.81**	**isopinocamphone**	**45.45**
9	14.06	myrtenol	2.13
10	14.77	cumic aldehyde	0.13
11	16.61	eugenol	0.14
12	16.98	β-bourbonene	0.20
13	17.18	cis-jasmone	0.11
14	17.24	eugenol methyl ether	0.55
15	17.33	α-gurjunene	0.14
16	17.50	caryophyllene	1.03
17	17.98	α-caryophyllene	0.29
18	18.08	(-)-alloaromadendrene	0.61
19	18.38	(+)-epi-bicyclosesquiphellandrene	2.59
20	18.48	(+)-aromadendrene	0.10
21	18.59	(5E)-2,5-dimethyl-3-methylene-1,5-heptadiene	1.79
22	18.81	γ-cadinene	0.09
23	18.92	4,11,11-trimethyl-8-methylenebicyclo [7.2.0]undec-4-ene	0.48
24	19.10	dihydroactinidiolide	0.21
25	19.37	elemol	13.16
26	19.58	guaiol	0.40
27	19.72	(-)-spathulenol	2.21
28	19.78	valencene	1.03
29	20.05	virdiflorol	1.69
30	20.11	hedycaryol	1.56
31	20.25	α-selinene	1.57
32	20.39	γ-eudesmol	2.37
33	20.50	2-isopropyl-5-methyl-9-methylenebicyclo [4.4.0]dec-1-ene	2.73
34	20.69	α-eudesmol	4.30
35	20.93	γ-gurjunene	0.89
36	29.71	methyl 9-(2-[(2-butylcyclopropyl)methyl] cyclopropyl)nonanoate	0.14
identified			91.73
unidentified	(15 compounds)		8.27

Table 3.

Composition of the essential oil of *Hyssopus officinalis* L. obtained by hydrodistillation (Dean-Stark apparatus)

peak	Rt (min)	component	area %
1	12.27	(R/S)-linalool	2.50
2	13.34	2,6,6-trimethylbicyclo[3.1.0]heptan-3-one	0.28
3	**13.62**	**isopinocamphone**	**40.07**
4	13.94	p-menth-1-en-8-ol	1.20
5	14.02	myrtenol	5.96
6	16.97	β-bourbonene	0.84
7	17.24	eugenol methyl ether	0.97
8	17.32	(-)-aristolene	0.74
9	17.49	caryophyllene	3.13
10	17.77	(+)-aromadendrene	0.14
11	17.98	α-caryophyllene	0.94
12	18.08	(-)-alloaromadendrene	2.38
13	18.27	γ-muurolene	0.77
14	18.36	β-cubebene	1.48
15	18.55	viridiflorene	1.22
16	18.80	γ-cadinene	0.70
17	18.90	δ-cadinene	0.76
18	19.29	elemol	11.50
19	19.76	caryophyllene oxide	2.11
20	20.03	ledol	0.72
21	20.10	patchoulane	0.44
22	20.24	δ-selinene	0.44
23	20.38	γ-eudesmol	3.65
24	20.48	2-isopropyl-5-methyl-9-methylenebicyclo [4.4.0]dec-1-ene	1.48
25	20.64	β-eudesmol	4.26
26	20.67	α-eudesmol	3.79
27	28.14	9-octadecenamide	0.97
identified			93.92
unidentified	(3 compounds)		6.08

Comparison of distillation techniques used for isolation of volatile oil from hyssop

In general, the major components identified in the oil of hyssop obtained by different types of distillation were similar. Although, the highest amount of isopinocamphone (45.45%) was noticed in the oil obtained by simple hydrodistillation. The ISO standard with regard to the oil of hyssop recommends levels between 34.5 and 50% for cis-3-pinanone (isopinocamphone) [14].

The main differences observed were the content of elemol which was greater in steam distilled (SD) oil (17.21%) than in hydrodistilled (HD) oil (13.16%) and hydrodistilled oil in Dean-Stark apparatus (11.50%), the content of myrtenol: 5.96% (HD in Dean-Stark apparatus), 2.71% (SD) and 2.13% (HD), the content of (R/S) linalool: 2.50% (HD in Dean-Stark apparatus) and 0.31% (HD and SD), the content of caryophyllene: 3.13% (HD in Dean-Stark apparatus), 1.03% (HD) and 0.17% (SD) and the content of (-)-alloaromadendrene: 2.38% (HD in Dean-Stark apparatus), 0.61% (HD) and 0.11% (SD).

Eucalyptol (1,8-cineole), trans-pinocarveol, α-terpineol and carvacrol were identified in steam distilled oil only.

Caryophyllene oxide, β-eudesmol, δ-selinene, γ-muurolene, δ-cadinene, virdiflorene were detected in the oil obtained by hydrodistillation in Dean-Stark apparatus.

CONCLUSIONS

1. In general, 58 components were detected in the essential oil of *H. officinalis* L.
2. The largest yield of the oil (0.8%) and the maximum concentration of isopinocamphone (45.45%) were obtained by simple hydrodistillation.
3. The highest amount of elemol (17.21%) was detected in steam distilled oil.
4. The caryophyllene oxide (2.11%) was detected only in the oil isolated by hydrodistillation in Dean-Stark apparatus.
5. The highest amounts of monoterpene ketones (45.56%) and sesquiterpene alcohols (25.69%) were found in hydrodistilled oil.
6. The highest amounts of sesquiterpene hydrocarbons (13.98%) and monoterpene alcohols (9.66%) were detected in oil obtained by hydrodistillation in Dean-Stark apparatus.
7. Pinocamphone was not detected in the oil isolated by different distillation techniques from air-dried flowering aerial parts of *H. officinalis*.

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SKŁAD OLEJKU Z HYZOPU (*HYSSOPUS OFFICINALIS* L.) UPRAWIANEGO W PÓŁNOCNO-ZACHODNIEJ POLSCE

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Streszczenie

W badaniach wykorzystano suszone ziele hyzopu lekarskiego zebrane w okresie kwitnienia (sierpień 2009 r.). Materiał roślinny pochodził z eksperymentalnej uprawy Katedry Warzywnictwa Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. Olejek eteryczny wydzielono na drodze destylacji z parą wodną, destylacji prostej i destylacji z wykorzystaniem nasadki Deana-Starka. Skład olejku eterycznego określono metodą GC-MS. Zidentyfikowano 31 związków w olejku otrzymanym przez destylację z parą wodną, 36 związków w olejku otrzymanym na drodze destylacji prostej i 27 związków w olejku wydzielonym w nasadce Deana-Starka. Izopinokamfon stanowił główny składnik wszystkich analizowanych próbek (40,07–45,45%).

Słowa kluczowe: *hyzop, ziele hyzopu, destylacja z parą wodną, hydrodestylacja, hydrodestylacja z nasadką Deana-Starka, GC-MS, elemol, izopinokamfon*