

## Genus *Hyssopus* L. – recent knowledge

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**ABSTRACT:** The genus *Hyssopus* L. – the hyssop is a source of volatile oil whose constituents are sesquiterpene alcohols, many terpene carbonyl compounds and some acids. As documented by the cited data, volatile oil composition depends not only on the species but also on the variety, date of drug harvest and many external factors including the quality of plant nutrition. The paper summarizes the knowledge of literary sources published over ca. the last twenty years that dealt with the hyssop (*Hyssopus officinalis* L.); the aim is to stimulate the search for other possibilities of application of hyssop volatile oil in ecological agriculture and alternative or official medicine.

**Keywords:** *Hyssopus* L.; volatile oil; isolation; composition; efficiency; other use

Among the medicinal or aromatic plants the hyssop (*Hyssopus officinalis* L.) is a plant that has not been studied very much. Both wild form and cultivated form of this perennial, branched semi-shrub is used. Its leaves are used in (folk) medicine, they are a common part (condiment) of European cuisine and are suitable for decorative purposes. Herba hyssopi is an official drug with indication of expectorant, stomachic and diuretic.

Yield per one hectare of hyssop (*Hyssopus officinalis*) is 2–3 tons of fresh leaves and 10–20 kg of volatile oil (DZHUMAEV 1986). Recent findings are rather different when differences in the production of plant fresh and dry matter were revealed by a comparison of 13 European sources of hyssop. The yield of fresh leaves ranged from 5–32 tons/ha, that of dry leaves from 0.67 to 3.26 tons/ha (SVOBODA et al. 1993).

### VOLATILE OIL ISOLATION

Common methods such as steam distillation or extraction with water-free solvents are used for industrial isolation of hyssop volatile oil (JIRÁSEK et al. 1986). To study the efficiency of different solvents and their effect on the quantity and composition of volatile oil hyssop was extracted with petroleum ether, dichloromethane and ethylene oxide. The length of extraction was from 1 to 90 minutes at temperatures of 50, 20 and –5°C. The best result was obtained with petroleum ether at 50°C for 90 minutes. The method was more efficient than previous methods, the volatile oil yield of hyssop increased from 0.92 to 1.49%, i.e. by 61.8% (SHLYAPNIKOV, SHLYAPNIKOVA 1972).

The extracted oily liquid (0.15–0.76%) is light yellow in color, of pleasant spicy, herb-like, slightly camphoraceous smell with  $\rho = 0.917\text{--}0.965$ ;  $\alpha = -6^\circ$  to  $-25^\circ$ ; acid

number  $\leq 4$  (VONÁŠEK et al. 1987). According to some literary sources the hyssop (*Hyssopus officinalis*) contains more than 1% of volatile oil with maximum content at the onset of flowering stage (among others HLAVA et al. 1983). The content of volatile oils (0.90–0.92%) in plants of *Hyssopus officinalis* var. *seravschanicus* Illus grown in the area Sukhandarya in Russia is almost in agreement with the latter reference. Leaves had the highest content of volatile oil at the stage of bud formation (DZHUMAEV 1981). Newer papers report higher contents – up to 7% – in relation to genotype as demonstrated by analyses of the hyssop (*Hyssopus officinalis*) population grown in 1985–1990 (DIACONU, BIRZA 1991). The volatile oil content in 6 phenotype representatives (corolla color from dark blue to white) was conditioned by flower color (higher content was found in darker-color individuals). Shoots without leaves and non-green plant parts did not contain any volatile oil.

### VOLATILE OIL CHARACTERISTICS

In general, the hyssop volatile oil is considered to contain these constituents:  $\alpha$ -pinene,  $\beta$ -pinene, camphene, pinocampheol, L-pinocamphone, sesquiterpene alcohols, methylmyrtenate, 2-hydroxyisopinocamphone, cis-pinonic acid. Camphene and pinene are spasmolytics, pinene has bactericidal effects.

Volatile oil composition varies in dependence on variety, growth stage on the date of collection, climatic conditions, admixture of foreign plants and extraction technology (VONÁŠEK et al. 1987). The study of 15 species and more than 20 hybrids of hyssop at Sukhumi experimental station was described in a paper containing fundamental information on the volatile oil composition. It demonstrated the presence of sesquiterpenes,

aldehydes, ketones, phenols, hydrocarbons and lipidic components with other constituents such as geraniol, safrole, citronellol, linalool, etc. Volatile oil yield ranged from 0.1 to 1.8% (ESVANDZHIYA 1976), which was in agreement with DZHUMAEV's (1981) data published later. Shoots of *Hyssopus officinalis* var. *seravschanicus* contained 0.84–0.89% volatile oil during bud formation and flowering.

A more detailed French paper was published almost parallelly (JOULAIN 1976). Of 52 substances, the following constituents were identified for the first time on the basis of analysis and study of hyssop (*Hyssopus officinalis*): myrtenolmethylether, methylmyrtenate, cis-pinic acid, hydroxyisopinocampone and cis-pinonic acid.  $\alpha$ -pinene,  $\beta$ -pinene, camphene, pinocampone and isopinocampone, of them pinic, pinonic and myrtenic acid, were determined by gel isotachopheresis. The constituents previously identified in volatile oil accounted for 69% of the mixture. Fourteen constituents have not been identified. The effect of the ontogenetic stage was not taken into account in these studies, only flowers and other plant material were distinguished.

It is necessary to assume variations in the content of volatile oil and its composition during ontogenesis. First experiments of this type were conducted by SCHULZ and STAHL-BISKUP (1991), who took hyssop samples at three stages. The first stage was in May (before flower setting), the second at the beginning of July (onset of flowering), the third in early September (after flowering termination). The total content of volatile oil was found to range from 0.03 to 0.16% (very low values) with maximum at the second stage. Besides the major constituents such as pinocampone, camphor and beta-pinene other 15 compounds were detected: among them were identified isopinocampone, alpha and beta-phellandrene, germacrene D and some derivatives of myrtenol. Although the proportion of glycoside-bound constituents of volatile oil is very low (0.01–0.06%), after hydrolysis with beta-glucosidase other constituents were identified: 3-octanol, linalool, cis-nerolidol, benzyl alcohol, phenyl ethanol, eugenol and o-vanillin. Small amounts of bicyclic terpenes myrtenol and verbenol were detected in leaves.

In general, the result confirmed data obtained in Egyptian hyssop (*Hyssopus officinalis*), but the presence of  $\Delta^3$ -carene reported in a previous paper was not proved (HILAL et al. 1978).

Fifty constituents, of them forty were identified and their values correspond to HILAL's values, were detected in hyssop oil by GC-MS analysis of volatile oil of *Hyssopus officinalis* L. and *Cymbopogon iwarancusa* (Jones) Schult. (MAHESHWARI, CHANDEL 1988).

Hyssop (*Hyssopus officinalis*) leaves harvested in the environs of Khandiza in 1986 contained 0.34% of volatile oil in dry weight, which is less than a half of the amount reported in other papers (see above) (DZHUMAEV et al. 1990). Volatile oil contained 71% pinocampone, 8.6% beta-pinene and 6.4% of 1,8-cineole. Sabinene content was 1.3% only.

D-pinocarvone at an amount of 44.3% was found by the analysis of *H. cuspidatus* Boriss volatile oil (DUNYAN et al. 1990). Of the other 32 constituents, the quantities of 1-pinocampone, beta-pinene and 1,8-cineole were highest.

In an experiment carried out at Lucknow hyssop (*Hyssopus officinalis*) plants were grown from seeds planted in early December 1997 (GARG et al. 1999). Volatile oil was obtained during flowering in May 1998. Its amount was 0.25% in fresh leaves and 1.18% in dry leaves. GC-MS analysis indicated the presence of 21 constituents representing 65.6% of volatile oil. These were 7 monoterpene hydrocarbons, 5 oxygen monoterpenes, 1 phenol and 6 sesquiterpenes. Pinocampone, beta-pinene and isopinocampone were the main constituents of volatile oil smelling after camphor.

A comparison of volatile oils of *Hyssopus officinalis* var. *decumbens* from France (Banon) and hyssop (*Hyssopus officinalis*) from Italy (Piedmont) revealed large differences (SALVATORE et al. 1998). An Italian sample contained 4.4% pinocampone and 43.3% isopinocampone, which is in accordance with ISO Standard No. 9841. On the contrary, the quantities of both camphones in a French sample were low. Dominant constituents were linalool (49.6%), 1,8-cineole (13.3%) and limonene (5.4%).

Available are results of analyses of hyssop (*Hyssopus officinalis* L.) volatile oil from India. The highest quantities were found in isopinocampone 38.1%, pinocarvone 20.3%, 1,8-cineole 12.2% and beta-pinene 10.2%. These values contrast with data on volatile oil from the hyssop grown in Northern America that approximated Italian values (SHAN 1991).

The composition of volatile oil of hyssop (*Hyssopus officinalis* subsp. *aristatus*) shoots from the environs of Sofia (TSANKOVA, KONTAKTCHIEV 1993) obtained in bud and flowering stages was interesting. The main constituents were 1,8-cineole (48.2 and 39.6%), isopinocampone (16.3 and 28.0%) and beta-pinene (11.4 and 9.4%).

The results of a Yugoslavian paper contribute to variability in available values. Plants of *Hyssopus officinalis* subsp. *aristatus* from Petinice area contained 57 constituents in flowering shoots, among them eugenol (38.3%), limonene (37.4%) and beta-pinene (9.6%) were major ones (GORUNOVIC et al. 1995).

In comparison with a commercial sample of *H. officinalis* whose major constituents are isopinocampone (40.2%),  $\beta$ -pinene (14.2%), pinocampone (10.3%) and  $\beta$ -phellandrene (9.5%), a large difference is evident in the latter case. 1,8-cineole was not found in the sample. The analytical results of volatile oil of hyssop (*Hyssopus officinalis*) growing in Spain are in positive correlation with Bulgarian values. Remarkably high content of 1,8-cineole (52.89%) and lower content of  $\beta$ -pinene (16.82%) as major components are in agreement with the above-mentioned values (GARCIA-VALLEJO et al. 1995).

#### EFFECTS ON VOLATILE OIL COMPOSITION

Today almost historical sources (MRUK, SUREVICZ 1959) reported superphosphate applied at the flowering

stage when it increases volatile oil content as a suitable fertilizer. The effect of calcium saltpeter is similar. Papers published in the past years recommended application rates of ammonium fertilizer 10–12 g per 1 m<sup>2</sup> and potential irrigation in two-year plants (JURČENKO, VASILKEVIČ 1989).

It is a dose consistent with optimum level of fertilizers determined on sandy soils (CICUIUS, MARINICA 1994). When the variants of nitrogen rates 60, 90, 120, 150 and 180 kg per 1 ha were tested, the highest yield was achieved for the variant 180 kg N/ha, 40 kg/ha P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, i.e. at a high level of nutrients (except P).

A selected portion of hyssop (usually *Hyssopus officinalis*) seeds used for production of volatile oil in perfumery had a higher production of volatile oil after treatment with colchicine solution. Seeds were treated with 0.2% colchicine solution for 72 hours. Mutants had better-quality volatile oil and its production was higher than in untreated individuals by more than 70% (MEKHRAZ et al. 1989).

Irradiation of hyssop seeds with gamma rays from 100 to 700 grey will induce a change in the quantity and composition of volatile oil. The dose of 300 grey will induce individuals with typical volatile oil smell. This clone was found suitable for further breeding (MEKHRAZ et al. 1989).

#### OTHER HYSSOP CONSTITUENTS

Besides 233 plant species (160 varieties) hyssop (*Hyssopus officinalis*) was analyzed for flavonoid content in leaves, flowers, stalks and roots during full flowering (TSITSINA 1969). The highest content of flavonoids was found in flowers of *Hypericum perforatum* L. (11.71%) while the hyssop was not a plant with extreme (minimum or maximum) content of polyphenolics and flavonoids.

Besides seeds of other 22 species of the family *Lamiaceae* hyssop seeds were examined for the content of fatty acids in oil (PANEKINA et al. 1979). Hyssop triacylglycerols are of N<sub>3</sub> (3 acyls of unsaturated acids) and PN<sub>2</sub> type (P = acyl of saturated acid). The content of types PN<sub>2</sub> was maximally 2%, the type P<sub>3</sub> was not detected.

#### EFFECTS OF VOLATILE OIL AND OTHER EXTRACTS

Antioxidant properties of aromatic plant extracts were studied in Lithuania (DAPKEVICIUS 1998); the aim was to replace synthetic substances. After deodorization (volatile oil removal) by distillation with steam, supercritical CO<sub>2</sub> and acetone or methanol the antioxidant activity of hyssop (*Hyssopus officinalis*) extracts was very low in comparison with thyme, marjoram and sage.

In another study presenting the results of tests for antioxidant activity performed in 20% oil emulsion (AB-

DALLA, ROOZEN 1999) hyssop volatile oil was found to stimulate oxidation processes.

Hyssop (*Hyssopus officinalis*) infusions or extracts are used in folk medicine to alleviate digestive disorders, to accelerate wound healing, and they have curative effects in the case of laryngitis (JURČENKO, VASILKEVIČ 1989). A well-known fact is that hyssop is a part of sherbet and is added to some liqueurs.

Volatile oil of hyssop var. *decumbens* and its main constituents linalool, 1,8-cineole and limonene were applied to guinea-pig's small intestine. The intestine was contracted with acetylcholine and BaCl<sub>2</sub>. Acetylcholine was inhibited by volatile oil as well as by linalool. On the contrary, 1,8-cineole and linalool exhibited a weak spasmogenic activity (MAZZANTI et al. 1997).

A pharmacological study of hyssop (*Hyssopus officinalis*) volatile oil confirmed its anthelmintic and antituberculosis activity (HILAL et al. 1978).

Experiments with French hyssop (*Hyssopus officinalis* var. *decumbens* Banon) and Italian hyssop (*Hyssopus officinalis*) where dominant constituents were linalool, 1,8-cineole and limonene, were aimed at the antimicrobial and antibacterial activity of their volatile oils. Volatile oil was applied in disk tests against Gram-positive and Gram-negative bacteria. The antibacterial effect of *H. officinalis* L. was negligible, that of var. *decumbens* was higher. If volatile oil was applied to mold strains, both strains were inhibited. The effect of the French var. *decumbens* was rather bactericidal. This higher antibacterial activity can be caused by linalool and higher content of 1,8-cineole while limonene is apparently responsible for the effect of volatile oil against molds in both variants of hyssop (MAZZANTI et al. 1998).

Polysaccharide MAR-10 was isolated from hyssop plants grown in China: it inhibits the replication of HIV virus of type 1 at usual concentrations (GOLLAPUDI et al. 1995). This polysaccharide did not influence either function or evolution of lymphocytes.

An experiment with application of volatile oils to rat bodies was used as a proof of the presence of terpene ketones pinocamphone and isopinocamphone. Volatile oils of hyssop and sage produced epileptogenic and neurotic effects in the experimental animals (STEINMETZ et al. 1980).

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## Rod *Hyssopus* L. – novější poznatky

**ABSTRAKT:** Rod *Hyssopus* L. – yzop – je zdrojem silice, jejímiž složkami jsou zejména seskviterpenické alkoholy, řada terpenických karbonylových sloučenin a některé kyseliny. Jak vyplývá z citovaných údajů, složení silice je závislé nejen na druhu, ale i na odrůdě, době sklizně drogy a na řadě vnějších faktorů včetně kvality výživy rostlin. Práce shrnuje poznatky z literárních pramenů posledních dvaceti let zabývajících se převážně yzopem lékařským (*Hyssopus officinalis* L.) s cílem aktivizovat hledání dalších možností aplikace silice yzopu v ekologickém zemědělství a alternativní i oficiální medicíně.

**Klíčová slova:** *Hyssopus* L.; silice; izolace; složení; účinnost; jiné užití

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