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# Composition and antimicrobial activity of the essential oil of *Hyssopus seravschanicus* growing wild in Tajikistan

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**ABSTRACT**

*The volatile compounds from* Hyssopus seravschanicus *Pazij, a plant growing wildly near the high ridge mountainous regions of Varzob, Northern Dushanbe in Tajikistan at an altitude approximately 2500 meters above sea level, were extracted and analyzed using gas chromatography-mass spectrometry (GC-MS). Eighty-seven chemical components of the essential oil were found and characterized representing approximately 95.4% of the oil. The most abundant components were* cis*-pinocamphone (57.0-88.9%), β-pinene (0.4-6.0%), 1,8-cineole (1.8-3.6%), camphor (0.5-4.0%), and spathulenol (0.1-5.0%). The essential oil from* Hyssopus seravschanicus *showed notable antimicrobial activity against* Bacillus cereus *and* Staphylococcus aureus.

**Keywords:** *Hyssopus seravschanicus*, essential oil composition, antimicrobial activity, *cis*-pinocamphone

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## INTRODUCTION

*Hyssopus seravschanicus* Pazij takes the local Tajiki name *ushnondoru* and is a perennial, branched, semi-shrub that can be found growing wildly in the northwestern part of Tajikistan. The genus *Hyssopus* belongs to the Lamiaceae and consists of over 15 species worldwide. *Hyssopus* is a source of volatile oils and its constituents are mostly sesquiterpenes, bicyclic monoterpenes, and some acids [1]. The essential oils of this genus have been studied for different chemotypes and phenotypes. Specifically, *Hyssopus officinalis* L. has been studied for the abovementioned traits with reference to development stages (cultivated and growing wild), the parts of the plant that are used, and the origin of the species. Although only very little previous work on *H. seravschanicus* has appeared [2], *H. officinalis* has been extensively studied. It is a perennial herb that is native to the Mediterranean region but has also spread throughout the Eastern part of Russia, the Caucasus region, and middle Asia. The chemical compositions and bioactivities of this species have been attributed to geography, climate, and stages of development [3,4].

The hyssop plant is an important culinary and medicinal plant located mostly in Central Asia, South Europe and North Africa [5]. It is rich in essential oils, flavonoids, tannins, and marrubiin and has been used as a healing herb in the Turkish culture for centuries. It has also been used for its antispasmodic, stomachic, antifungal and expectorant properties [5]. Recently, there has been a growing demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics, etc. [3,6]. Medicinal plants such as hyssop add to our pharmacopeia

and help to expand drug research. For this reason, we have recently examined several aromatic medicinal plants traditionally used in Tajikistan [7-14].

In 1974, Zotov and co-workers [15] reported that an *H. seravschanicus* essential oil was made up of the following compounds: α-pinene (1.8%), sabinene (16.3%), β-pinene (0.5%), myrcene (5.3%), *p*-cymene (2.8%), γ-terpinene (3.5%), *trans*-pinocamphone (23%), thymol (1.7%), carvacrol (1.2%), α-terpinene (0.8%), limonene (13.8%), terpinolene (0.7%), fenchone (0.5%), menthone (1.1%), *cis*-pinocamphone (= isopinocamphone) (12.1%), verbenone (3.7%), pinocampherol (2.2%) and isopinocampherol (3.4%). It is worth pointing out that the most abundant compounds that were isolated from the essential oil were *trans*-pinocamphone, sabinene, limonene, and *cis*-pinocamphone.

A number of years later in 1990, Dzhumaev *et al.* [16] reported that the major components of the leaf of *H. seravschanicus* essential oil were α-pinene (0.2%), sabinene (1.3%), β-pinene (8.6%), myrcene (1%), *p*-cymene (1.3%), 1,8-cineole (6.4%), *cis*-β-ocimene (1.4%), *trans*-β-ocimene (0.7%), γ-terpinene (0.2%), *trans*-pinocamphone (71%), $C_{10}H_{16}O$ (0.4%), thymol (0.2%), carvacrol (1.6%), caryophyllene (0.3%) and $C_{15}H_{24}$ (0.6%). In this study, the most abundant chemical component was also *trans*-pinocamphone (see above), but β-pinene and 1,8-cineole were also abundant rather than sabinene, limonene, and *cis*-pinocamphone. In this present work, we present the chemical compositions and antimicrobial activities of the essential oil of *H. seravschanicus* and compare it with previous studies on this and other species of hyssop.

## MATERIALS AND METHODS

*Plant Materials:* Aerial parts of *Hyssopus seravschanicus* Pazij were collected from the Varzob region of Tajikistan (2200-2500 meters above sea level). Sample #1 (fresh material), sample #2 (dry material), and sample #3 (leaf, dry material) were collected on 22 May 2011 (during the budding period). Sample #4 (dry material), and sample #5 (leaf, dry material) were collected on 24 June 2011 (during the flowering period). The plants were identified by F.S. Sharopov and a voucher specimen (TJ2011-001) has been deposited in the herbarium of the Chemistry Institute of the Tajikistan Academy of Sciences. The air-dried samples were crushed and hydrodistilled for 3 hours to give the white-yellow essential oils, with a 0.8-1.0% yield.

*Gas Chromatography – Mass Spectrometry Analysis (GC-MS):* A gas chromatography-mass spectrometry analysis (GC-MS) was performed on the essential oils of *H. seravschanicus* using an Agilent 6890 GC with Agilent 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/s), and a fused silica capillary column (HP-5ms, 30 m × 0.25 mm) coated with 5% phenyl-polymethylsiloxane (0.25 µm phase thickness). The carrier gas was helium with a flow rate of 1 mL/min and the injection temperature was 200°C. The oven temperature was programmed to initially hold for 10 minutes at 40°C, then ramp to 200°C at 3°C/min and finally to 220°C at 2°C/min. The interface temperature was 280°C. A 1% w/v solution of each sample in $CH_2Cl_2$ was prepared, and 1 µL of each sample was injected using a splitless injection technique. Identification of the essential oil components was based on their retention indices, which were determined by a reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [17], and stored on the MS library [NIST database (G1036A revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The chemical compositions of the *H. seravschanicus* oils are summarized in Table 1.

*Antimicrobial Screening:* The five essential oil samples from the aerial parts of *H. seravschanicus* were screened for antibacterial activity. The essential oils were screened against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213) as well as Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique. Dilutions of the crude extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 µL of 1% w/v solutions of crude extracts in DMSO plus 50 µL CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately $1.5 \times 10^8$ colony forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hr. The final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Geneticin® was used as a positive antibiotic control; DMSO was used as a negative control. Antifungal activity was determined as described above using *Candida albicans* (ATCC No.10231)

in yeast-nitrogen base growth medium with approximately $7.5 \times 10^7$ CFU/mL. Amphotericin B was the positive control. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control. The antimicrobial activities of the essential oils are summarized in Table 2.

**Table 1. Chemical compositions of *Hyssopus seravschanicus* Pazij essential oils from Tajikistan**

| RI[a] | Compound | Percent Composition | | | | |
|---|---|---|---|---|---|---|
| | | *H. z.* #1 | *H. z.* #2 | *H. z.* #3 | *H. z.* #4 | *H. z.* #5 |
| 932 | Tricyclene | --- | --- | --- | --- | tr[b] |
| 935 | α-Thujene | tr | tr | 0.1 | --- | 0.1 |
| 941 | α-Pinene | 0.2 | 0.2 | 0.5 | tr | 0.6 |
| 954 | Camphene | tr | --- | 0.2 | --- | 0.2 |
| 959 | Thuja-2,4(10)-diene | --- | --- | --- | --- | tr |
| 960 | 4-Methylpent-2-enolide | --- | tr | --- | --- | --- |
| 964 | Benzaldehyde | --- | --- | tr | --- | tr |
| 976 | Sabinene | 0.6 | 0.8 | 1.4 | tr | 1.4 |
| 979 | β-Pinene | 2.9 | 4.1 | 5.3 | 0.4 | 6.0 |
| 982 | 1-Octen-3-ol | --- | --- | --- | --- | tr |
| 993 | Myrcene | 0.2 | 0.5 | 0.8 | 2.6 | 1.0 |
| 1024 | *p*-Cymene | 0.4 | 0.4 | 0.6 | tr | 0.8 |
| 1028 | β-Phellandrene | 0.7 | 1.6 | 2.1 | 0.1 | 2.1 |
| 1031 | 1,8-Cineole | 2.8 | 2.2 | 1.8 | tr | 3.6 |
| 1033 | Benzyl alcohol | --- | --- | 0.2 | --- | 0.1 |
| 1038 | (*Z*)-β-Ocimene | tr | 0.3 | 0.6 | tr | 0.6 |
| 1048 | (*E*)-β-Ocimene | --- | --- | tr | tr | tr |
| 1058 | γ-Terpinene | --- | --- | tr | tr | tr |
| 1067 | *cis*-Sabinene hydrate | 0.7 | 0.4 | 1.6 | tr | 0.6 |
| 1072 | *cis*-Linalool oxide (furanoid) | --- | --- | tr | --- | tr |
| 1088 | *trans*-Linalool oxide (furanoid) | --- | --- | tr | --- | 0.1 |
| 1094 | 6,7-Epoxymyrcene | --- | --- | tr | --- | tr |
| 1098 | *trans*-Sabinene hydrate | --- | --- | 0.2 | --- | 0.1 |
| 1100 | Linalool | 0.8 | 0.6 | 1.0 | tr | 0.9 |
| 1105 | *cis*-Thujone (= α-Thujone) | tr | --- | 0.2 | --- | 0.3 |
| 1116 | *trans*-Thujone (= β-Thujone) | --- | --- | 0.1 | --- | 0.2 |
| 1119 | Dehydrosabinaketone | --- | --- | tr | --- | tr |
| 1121 | *cis*-*p*-Menth-2-en-1-ol | --- | --- | 0.2 | --- | 0.2 |
| 1125 | Chrysanthenone | --- | --- | tr | --- | tr |
| 1134 | (*Z*)-Epoxyocimene | --- | --- | tr | --- | tr |
| 1137 | Nopinone | --- | --- | 0.4 | --- | 0.2 |
| 1139 | *trans*-Pinocarveol | 0.3 | tr | 0.6 | --- | 0.3 |
| 1143 | Camphor | 4.0 | 0.5 | 1.0 | 1.0 | 0.8 |
| 1158 | Unidentified | 4.5 | 4.5 | 5.3 | 2.1 | 5.1 |
| 1159 | *trans*-Pinocamphone | 0.9 | 0.9 | 1.2 | tr | 0.5 |
| 1162 | Pinocarvone | 0.2 | tr | --- | 3.8 | 0.7 |
| 1167 | δ-Terpineol | tr | tr | --- | tr | --- |
| 1173 | *cis*-Pinocamphone | 73.9 | 79.7 | 57.0 | 88.9 | 64.3 |
| 1176 | Terpinen-4-ol | 1.5 | 1.1 | --- | 0.4 | --- |
| 1185 | Cryptone | 0.8 | 0.2 | 1.1 | 0.1 | 0.5 |
| 1190 | α-Terpineol | 0.6 | 0.5 | 1.3 | tr | 0.7 |
| 1195 | Myrtenal + Myrtenol | 1.0 | 0.5 | 2.3 | 0.3 | 1.0 |
| 1198 | Estragole (= Methyl chavicol) | --- | --- | tr | --- | tr |
| 1201 | *cis*-4-Caranone | --- | --- | tr | --- | tr |
| 1206 | *trans*-Piperitol | --- | --- | 0.1 | --- | 0.1 |
| 1217 | *trans*-Carveol | --- | --- | tr | --- | tr |
| 1227 | *m*-Cumenol + Citronellol | --- | --- | 0.2 | --- | 0.1 |
| 1236 | Cuminaldehyde | tr | --- | 0.4 | tr | 0.2 |
| 1243 | Carvone | --- | --- | 0.1 | tr | tr |
| 1246 | *trans*-2-Hydroxypinocamphone | 0.9 | 0.4 | 0.9 | 0.3 | 0.7 |
| 1252 | Geraniol | --- | --- | tr | --- | tr |
| 1261 | *cis*-Chrysanthenyl acetate | --- | --- | --- | --- | 0.3 |
| 1268 | *trans*-Ascaridol glycol | tr | --- | --- | --- | --- |
| 1274 | *cis*-Tetrahydrojasmone | tr | --- | --- | tr | --- |
| 1282 | α-Terpinen-7-al | --- | --- | 0.1 | --- | tr |
| 1285 | Isobornyl acetate | 0.2 | --- | 0.1 | --- | 0.1 |
| 1289 | *p*-Cymen-7-ol | 0.1 | --- | 0.3 | --- | 0.2 |
| 1292 | Thymol | --- | --- | 0.1 | --- | --- |
| 1292 | *trans*-Sabinyl acetate | --- | --- | --- | --- | 0.2 |
| 1296 | Methyl myrtenate | 0.3 | tr | 1.0 | --- | 0.5 |

| RI[a] | Compound | Percent Composition | | | | |
|---|---|---|---|---|---|---|
| | | *H. z.* #1 | *H. z.* #2 | *H. z.* #3 | *H. z.* #4 | *H. z.* #5 |
| 1299 | *trans*-Pinocarvyl acetate | --- | --- | --- | --- | 0.1 |
| 1301 | Carvacrol | --- | 0.2 | 0.1 | --- | tr |
| 1315 | 4-Hydroxycryptone | --- | --- | tr | --- | tr |
| 1324 | Myrtenyl acetate | --- | --- | 0.1 | --- | 0.2 |
| 1330 | 3-Oxo-*p*-menth-1-en-7-al | --- | --- | tr | --- | tr |
| 1365 | Neryl acetate | --- | --- | 0.2 | --- | 0.1 |
| 1375 | α-Copaene | --- | --- | tr | --- | 0.1 |
| 1383 | β-Bourbonene | --- | --- | 0.1 | --- | 0.1 |
| 1390 | β-Cubebene | --- | --- | tr | tr | tr |
| 1392 | β-Elemene | --- | --- | tr | --- | tr |
| 1399 | (*Z*)-Jasmone | --- | --- | 0.2 | --- | 0.1 |
| 1406 | Methyl eugenol | --- | --- | 0.1 | --- | tr |
| 1409 | (*Z*)-Caryophyllene | --- | --- | 0.1 | --- | tr |
| 1412 | Unidentified | --- | --- | --- | --- | 0.6 |
| 1419 | (*E*)-Caryophyllene | --- | --- | 0.5 | tr | 0.3 |
| 1428 | β-Copaene | --- | --- | tr | --- | tr |
| 1453 | α-Humulene | --- | 0.2 | 0.2 | --- | 0.1 |
| 1458 | (*E*)-β-Farnesene | --- | 0.1 | --- | --- | --- |
| 1460 | Alloaromadendrene | --- | --- | 0.2 | --- | 0.1 |
| 1472 | (2*E*)-Dodecenal | --- | --- | --- | --- | 0.1 |
| 1481 | Germacrene D | --- | --- | 0.5 | --- | 0.2 |
| 1497 | Bicyclogermacrene | --- | --- | 0.6 | --- | 0.2 |
| 1578 | Spathulenol | 1.5 | 0.2 | 5.0 | 0.1 | 1.5 |
| 1584 | Caryophyllene oxide | --- | --- | 1.0 | tr | 0.3 |
| 1610 | Humulene epoxide II | --- | --- | 0.2 | --- | tr |
| 1635 | Isospathulenol | --- | --- | 0.5 | --- | 0.2 |
| 1641 | τ-Cadinol | --- | --- | 0.1 | --- | --- |
| | Total Identified (%) | 95.5 | 95.5 | 94.3 | 97.9 | 93.8 |

[a] *RI = "Retention Index" on an HP-5ms column determined with reference to a homologous series of n-alkanes.*
[b] *tr = "trace" (< 0.05%).*

**Table 2. Antimicrobial activity (MIC, µg/mL) of the *H. seravschanicus* essential oil**

| Organism | *H. z.* #1 | *H. z.* #2 | *H. z.* #3 | *H. z.* #4 | *H. z.* #5 |
|---|---|---|---|---|---|
| *Bacillus cereus* | 625 | 625 | 625 | 312 | 156 |
| *Staphylococcus aureus* | 156 | 312 | 312 | 312 | 312 |
| *Pseudomonas aeruginosa* | 625 | 625 | 625 | 625 | 625 |
| *Escherichia coli* | 625 | 625 | 625 | 625 | 625 |
| *Candida albicans* | 625 | 625 | 1250 | 625 | 625 |
| *Aspergillus niger* | 625 | 625 | 1250 | 625 | 625 |

## RESULTS AND DISCUSSION

The volatile compounds from *Hyssopus seravschanicus* growing in Tajikistan were obtained by hydrodistillation and analyzed using GC-MS. Eighty-seven compounds of the essential oils were identified. The most abundant components were *cis*-pinocamphone (57.0-88.9%), β-pinene (0.4-6.0%), 1,8-cineole (1.8-3.6%), camphor (0.5-4.0%) and spathulenol (0.1-5.0%). In comparison with other reports of *H. seravschanicus*, Zotov *et al*. reported 23% *trans*-pinocamphone [15] whereas Dzhumaev *et al.* reported 71% pinocamphone [16]. By contrast, in this present study *cis*-pinocamphone (= isopinocamphone) was the dominant component in *H. seravschanicus* oil while *trans*-pinocamphone was present in much smaller concentrations (trace-1.2%).

In general, the dominant components in essential oils of cultivated *H. officinalis* plants are *trans*-pinocamphone, *cis*-pinocamphone, β-pinene and pinocarvone with compositions of up to 63, 43, 23, and 12%, respectively [3]. Kizil *et al*. in 2010 showed that the major components of Turkish *H. officinalis* oil were *cis*-pinocamphone (57.3%), β-pinene (7.2%), terpinene-4-ol (7.1%), pinocarvone (6.5%), carvacrole (3.0%), *p*-cymene (2.8%) and *trans*-pinocamphone (2.6%) [5]. These seven components constituted 86.5% of total oil. In most other literature reports, *cis*-pinocamphone, *trans*-pinocamphone, β-pinene and pinocarvone were found to be the most abundant chemical components of hyssop oil [6,18].

The composition of essential oil can vary by species although there are subtle similarities within the Lamiaceae. Essential oils of hyssop plants, either growing wildly or cultivated, mostly contain large amounts of compounds with

pinane carbon skeletons. These include β-pinene, *trans*-pinocamphone, and *cis*-pinocamphone, with percent compositions ranging from ~3 to 70% [3]. Similarly, Kizil *et al.* reported that the essential oils of Turkish *H. officinalis* were dominated by *cis*-pinocamphone (47.9-51.4%) [5]. Their research also demonstrated that hyssop oil compositions were greatly affected by the environmental conditions and phenological stages, with the highest oil content in the post-blooming stage [5]. Notably, the oil content and compositions of many plants, not just hyssop, are considerably affected by blooming stage [12,19-21]. In general, the dominant chemical components in essential oils of cultivated hyssop plants have been *trans*-pinocamphone, *cis*-pinocamphone, β-pinene and pinocarvone with percent compositions of up to 63, 43, 23, and 12%, respectively [3].

It is worth noting that pinane monoterpenoids are persistent constituents of the essential oils of hyssop, regardless of species. The percent compositions changed for each study as well as for each species but generally the same substances were always present. There have also been cases in which other substances were dominant. For example with *H. officinalis*, there is cineole-rich oil (52.9%) from Spain and a methyleugenol-rich oil (38.3%) from Montenegro [2]. These are good examples of how the same species can change in chemical composition in relation to location, climate, water provisions, and other environmental properties.

The essential oils of hyssop have demonstrated antibacterial, antifungal, and antiviral activities [3,6,22-24]. In this present study, *H. seravschanicus* essential oil showed notable antibacterial activity against the Gram positive *Bacillus cereus* and *Staphylococcus aureus*, but lower activities against Gram-negative bacteria or fungi. The major component, *cis*-pinocamphone, has been reported as having an antibacterial effect [5].

Normal cellular growth mechanisms can be influenced by both physical and chemical factors which lead to changes in cell composition [25]. The reason for antimicrobial action exhibited by hyssop oils could be because of the low molecular weight and lipophilic constituents of the essential oil which allows it to diffuse across a cell membrane. This allows for the biological activity of the chemical compounds that make up the oil and give it the antibacterial, antifungal and antimicrobial properties [26]. *cis*-Pinocamphone was the most abundant component in most of the *Hyssopus* oils, and it is very likely that this compound is responsible for the antimicrobial activity.

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