Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (3) 2010, 99-103

# Chemical Composition, Antimicrobial and Antioxidant Activities of Hyssop (*Hyssopus officinalis* L.) Essential Oil

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**Abstract**

The essential oil of hyssop is widely used in food, pharmaceutical and cosmetic industries throughout the world. Therefore, it is very important to know the chemical characteristics of the oil for economic use and enhanced performance of the end products. This study was carried out to determine antimicrobial and antioxidant activities of the essential oil of *Hyssopus officinalis* (L.) (*Lamiaceae*) collected from wild in the Southeast Anatolian, Turkey. Chemical compositions of hydrodistilled essential oils obtained from hyssop leaves were analyzed by gas chromatography-mass spectrometry (GC-MS). For antimicrobial activity, disc diffusion tests were carried out on *Escherichia coli* line ATCC25922, *Pseudomonas aeroginosa* line ATCC27853, *Staphylococcus aureus* line 25923, *Staphylococcus pyogenes* line ATCC19615 and *Candida albicans* line ATCC10231, and the antioxidant activity was determined by using the diphenylpicrylhydrazyl (DPPH) radical-scavenging method. It was determined that hyssop essential oil contained *iso*pinocamphone (57.27%), (-)-β-pinene (7.23%), (-)-terpinen-4-ol (7.13%), pinocarvone (6.49%), carvacrol (3.02%), *p*-cymene (2.81%) and myrtenal (2.32%) as major components. As shown by treatments with 5 and 10 µl of oil; which exhibited strong antimicrobial activity against *S. pyogenes, S. aureus*, *C. albicans* and *E. coli*, but not against *P. aeruginosa*. The antioxidant activity of *H. officinalis* essential oil was lower compared to butylated hydroxytoluene (BHT) and ascorbic acid. These results demonstrated that hyssop essential oil has relatively low antioxidant activity and good antimicrobial activity against some test organisms.

***Keywords:*** *Hyssopus officinalis* L., essential oil, *iso* pinocamphone, biological activity

## Introduction

The perennial *Lamiaceous* herb hyssop (*Hyssopus officinalis* L.), is an important medicinal plant extensively cultivated in Russia, Spain, France and Italy (Omidbaigi, 2000). The plant is a typical xerophyte and is well adapted to drought and low input conditions (Hornok, 1992). It is mainly used for antispasmodic, stomachic, antifungal and cough treatments. The essential oils isolated from hyssop are popularly used as food and drink additives as well as cosmetic materials (Murakami *et al.*, 1998).

Garg *et al.* (1999) found that the main volatile constituents of *H. officinalis* essential oil from a variety of locations included β-pinene, limonene, β-phellandrene, 1,8-cineole, pinocamphone, *iso* pinocamphone, pinocarvone, germacrene-D and methyleugenol. They also found that the oils extracted from different subspecies or plant populations of varying origin or morphology differed in percentage composition of the major volatile constituents. Similarly, Kizil *et al.* (2008) found that the main compounds of the hyssop oil were *iso* pinocamphone, β-pinene, 4-carvomenthenol, γ-terpinene, carvacrol, and pinocarvone, and these six components together constituted 75-81% of total essential oil.

It is well-known that most spices possess a wide range of biological and pharmacological activities. These volatile compounds are widely used in cosmetics as fragrance components, in the food industry to improve the aroma and the organoleptic properties of different types of foods, and in a variety of household products. In addition to their particular aroma, many essential oils and their isolated components also exhibit muscle relaxant, antibacterial and antifungal activities (De Martino *et al.*, 2009).

There is currently a large interest in substituting synthetic food preservatives and synthetic antioxidants for substance that can be marketed as natural. Synthetic antioxidants such as gallates, butylated hydroxytoluene (BHT), tert-butyl hydroxyanisole (BHA) and tert-butyl hydroquinone (TBHQ) were the first preservatives designed for widespread industrial use. However, some physical properties of BHA and BHT, such as their high volatility and instability at elevated temperatures, strict legislation on the use of synthetic food additives, and consumer preferences, have shifted the attention of manufacturers from synthetic to natural antioxidants (Dapkevicius *et al.*, 1998).

The present study is aimed at assessing the antimicrobial and antioxidant activities of *H. officinalis* which naturally grow in Southeast Anatolian, Turkey.

## Materials and methods

*Plant material*

Hyssop plants were grown at the Medicinal Plant Collection Garden of Department of Field Crops, Agriculture Faculty, Dicle University, Diyarbakir (latitude 37°53′N, longitude 40°16′E, altitude 680 m above sea level), Turkey, and were harvested at full flowering period (June 2009). Fresh herbage was dried under shadow for one week. Voucher specimens of *H. officinalis* are stored at the herbarium of medicinal and aromatic plants, located in the Faculty of Agriculture (DUZF 0040), Dicle University.

*Essential oil extraction*

Essential oil of 30 g dry herb samples was extracted by hydro-distillation for 2.5 h under continuous steam using a Clevenger-type apparatus (v/w). The isolated oils were stored in tightly closed vials at 4°C until analysis.

*GC/MS analysis*

The essential oils of *H. officinalis* samples were analysed by using a GC Clarus 600-MS Clarus 600 C (Perkin Elmer) equipped with an auto sampler. One microlitre of sample volume was injected using split method. Chromatographic separations were accomplished with a Elite 5-MS capillary column (5% Diphenyl)-Dimethylpolysiloxane, 0.25 mm i.d.x30 m, film thickness 0.25 μm) with injections in the split mode with 20 split ratio. Analysis was carried out using helium as the carrier gas with flow rate of 1.0 ml/min. The column temperature was initially kept at 60°C for 3 min then gradually increased to 130°C at 4°C/min$^{-1}$ rate, held for 2 min, and finally raised to 240°C at 20°C/min$^{-1}$. The injection port temperature was 240°C. The ionization voltage applied was 70 eV with mass range m/z of 20/550 a.m.u. The separated components were identified tentatively by matching with EI-MS results of National Institute of Standards and Technology (NIST), WILEY 8$^{th}$ edition and NBS mass spectral library data because hyssop essential oil reference reagents were not available. The quantitative determination was carried out based on peak area integration.

*Antimicrobial activity*

*Microbial strains*

The essential oil was tested against microorganisms including *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* 25923, *Staphylococcus pyogenes* ATCC19615, *Candida albicans* ATCC10231. Bacterial strains were cultured overnight in Nutrient Broth (NB) at 37°C, with the exception of *C. albicans* cultured at 30°C.

*Antimicrobial screening*

The agar disc diffusion method was employed for the determination of antimicrobial activity of the essential oil in question (NCCLS, 1997). Briefly, a suspension of the tested microorganism (0.1 ml of $10^8$ CFU/ ml) was spread on the solid media plates. Sterile filter paper discs (6 mm diameter) were impregnated with 5 and 10 μl of the oil and placed on the inoculated plates. These plates, after standing at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeast. Imipenem (IMP) was used as a positive control. The diameters of the inhibition zones were measured in millimetres. All tests were performed in triplicate. Values are presented as means ± SD of three parallel measurements.

*Antioxidant activity*

*DPPH assay*

Hydrogen atoms or electrons donation ability of the corresponding oils was measured from the bleaching of purple coloured methanol solution of 2, 2′-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometric assay uses the stable radical DPPH as a reagent (Cuendet *et al.*, 1997; Burits and Bucar, 2000). Fifty micro liter of the oil in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. The same procedure was repeated with the synthetic antioxidants butylated hydroxylanisole (BHA), butylated hydroxyltoluene (BHT), and ascorbic acid as positive controls. Inhibition free radical DPPH in percent (I%) was calculated in the following way:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

where $A_{blank}$ is the absorbance of the control reaction (containing all reagents except the test compound), and $A_{sample}$ is the absorbance of the test compound. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against extract concentration. For the calculation of these values, Microsoft Excel software was used.

*Statistical analysis*

All experiments were done in triplicate and mean values are presented. Statistical analysis was performed by SPSS 12.0.

## Results and discussion

*Essential Oil Components*

Tab. 1 reports the chemical composition of hyssop oil. Thirty-four components were identified in the hyssop oil representing 99.83% of the total weight. The major components were *iso* pinocamphone (57.27%), β-pinene (7.23%), terpinene-4-ol (7.13%), pinocarvone (6.49%), carvacrole (3.02%), *p*-cymene (2.81%) and pinocamphone (2.59%). These seven components constitute 86.54% of total oil. In the literature, *iso*pinocamphone, pinocamphone, β-pinene and pinocarvone were reported to be the most

Tab. 1. Essential oil components of *H. officinalis*

| Oil components | % |
|---|---|
| 3-Thujene | 0.15 |
| Pinene | 0.31 |
| Camphene | 0.07 |
| 4(10)-Thujene | 0.85 |
| -(-) β-pinene | 7.23 |
| 1-Octen-3-ol | 0.54 |
| beta Myrcene | 0.52 |
| *Iso* terpinolene | 0.12 |
| *p*-Cymene | 2.81 |
| D-Limonene | 0.60 |
| Cineole | 0.30 |
| Linalool | 0.77 |
| β-thujone | 0.14 |
| 4-Carvomenthol | 0.14 |
| 1R)-(+)-Norinone | 0.77 |
| Pinocamphone | 2.59 |
| Pinocarvone | 6.49 |
| *Iso* pinocamphone | 57.27 |
| (-)-Terpinen-4-ol | 7.13 |
| p-Cymen-8-ol | 0.29 |
| Myrtenal | 2.32 |
| Pinanediol | 0.35 |
| Thymol | 0.64 |
| Carvacrol | 3.02 |
| (-)-Myrtenyl acetate | 0.98 |
| Neryl acetate | 0.17 |
| Eugenol methyl ether | 0.24 |
| Caryophyllene | 0.35 |
| cis-Pinonic acid | 0.63 |
| β-bisabolene | 0.57 |
| Elemol | 0.55 |
| Spathulenol | 0.59 |
| Caryophyllene oxide | 0.21 |
| *Iso* propyl myristate | 0.12 |
| Total | 99.83 |
| Essential Oil Content (%) | 1.26 |

abundant components in hyssop oil (Kizil *et al.*, 2008; De Martino *et al.*, 2009).

The composition of the essential oil of *H. officinalis* has been examined previously by Svoboda *et al.* (1993), Veres *et al.* (1997), Vallejo *et al.* (1997), Mazzanti *et al.* (1998), Garg *et al.* (1999), Jankovsky and Landa (2002), Mitic and Dordevic (2000), Ozer *et al.* (2005) and Kizil *et al.* (2008). Veres *et al.* (1997) reported that hyssop essential oil could be categorised depending upon their percentage composition of β-pinene, limonene, pinocamphone, and *iso* pinocamphone. These results are in agreement with some of the previously published data. Different compounds have been identified as the main component in hyssop oil by other researchers: methyleugenol (38%) by Gorunovic *et al.* (1995), 1,8-cineole (53%) by Vallejo *et al.* (1995), pinocamphone (49.1%) by Garg *et al.* (1999), *iso* pinocamphone by Mitic and Dordevic (2000), and pinocarvone (36.3%) by Ozer *et al.* (2005).

*Antimicrobial activity*

The antimicrobial activity of hyssop essential oil was assayed using 5 and 10 µl volumes. Hyssop essential oil showed antimicrobial activity against *S. pyogenes, S. aureus*, *C. albicans* and *E. coli*, but no antimicrobial activity against *P. aeruginosa* was detected in these assays (Tab. 2). As should be expected, the diameters of the inhibition zones in the presence of 5 µl of oil were smaller than in the presence of 10 µl, indicating a concentration dependent effect. Kizil and Uyar (2006) also reported that a lower essential oil amount produced a smaller inhibition zone compared to a higher one. Such an activity could be strictly related to the chemical composition of oil: in fact, *iso* pinocamphone, is reported for its good antibacterial effect. Similar results are reported by Saeedi and Morteza-Semnani (2009) for the essential oil of *H. anqustifolius*.

Essential oils seem to have no specific targets because of their large number of constituents (Carson *et al.*, 2002). They pass through the cell wall and cytoplasmic membrane like typical lipophiles and disrupt the structure of their layers. Moreover, they can coagulate the cytoplasm (Gustafson *et al.*, 1998) and damage lipids and proteins (Ultee *et al.*, 2002; Burt, 2004). It has earlier been reported that the culture medium, the technique of testing, the botanical source of the plant, the age of the plant, regional conditions, such as altitude and climatic conditions, the state of plant material used (dried or fresh), the quantity of the oil used for the test and the isolation technique are some factors implicated in the great variation of the activity of the essential oil (Delaquis *et al.*, 2002; Daferera *et al.*, 2003). Some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and their antimicrobial activity (Ipek *et al.*, 2005). In addition, other authors also report activity of the main components of essential oils is probably modulated by other minor molecules (Franzios *et al.*, 1997; Santana-Rios *et al.*, 2001; Hoet *et al.*, 2006).

It is most likely that numerous components of the essential oils play role in defining the features of oils, lipophilic or hydrophilic attraction and fixation on cell wall and membranes, and cellular distribution (Cal, 2006). This feature is crucial because, depending on their com-

Tab. 2. Antimicrobial activity of *H. officinalis* essential oil

| Test bacteria | Inhibition zone diameter (mm) | |
|---|---|---|
| | 5 µl | 10 µl |
| *S. pyogenes* | 19.0±0.1 | 23.6±0.5 |
| *S. aureus* | 18.0±1.7 | 21.7±1.5 |
| *E. coli* | 20.3±1.8 | 23.3±1.7 |
| *P. aeruginosa* | nd* | nd |
| *C. albicans* | 15.0±1.0 | 20.0±1.1 |

*nd: not detected

ponent, the distribution of the oil in the cell determines the different types of radical reaction produced (Bakkali *et al.*, 2008). In that sense, for biological purposes, it is more informative to study the entire oil rather than some of its components because the concept of synergism appears to be more meaningful.

*Antioxidant activity*

The antioxidant activity of the hyssop essential oil was assessed by employing DPPH free radical-scavenging and the results are shown in Tab. 3. The DDPH free radical is considered a simple and very fast method for determining antioxidant activity. The effect of antioxidant on DPPH radical scavenging was tough due to their hydrogen donating ability or radical scavenging activity. The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen donating antioxidant (Viuda-Martos *et al.*, 2010).

Natural and synthetic antioxidants such as ascorbic acid, BHT and BHA were compared with the antioxidant activity of *H. officinalis* essential oil (Tab. 3). Lower $IC_{50}$ value indicates higher antioxidant activity. According to the results, it was observed that hyssop essential oil ($IC_{50}$ = 16.37±0.001 μg/ml) presented lower antioxidant activity than ascorbic acid ($IC_{50}$ = 10.94±0.94 μg/ml). The lowest activity was obtained from BHT (37.14±0.05 μg/ml); hyssop essential oil and BHA showed the same activity.

Tab. 3. Antioxidant activity of some synthetic antioxidants and *H. officinalis* essential oil

| Samples | DPPH $IC_{50}$ (μg/ml) |
|---|---|
| Ascorbic Acid | 10.94±0.94 |
| BHA | 16.37±0.004 |
| BHT | 37.14±0.05 |
| *H. officinalis* | 16.37±0.001 |

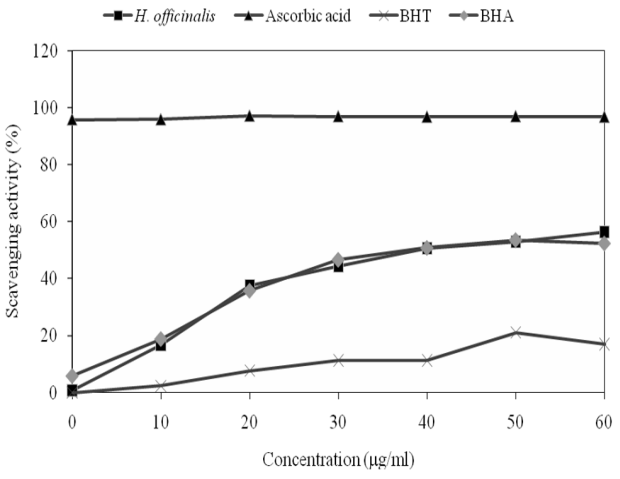


Fig. 1. The DPPH radical scavenging activity of some synthetic antioxidants and *H. officinalis* essential oil

DPPH radical scavenging activity of test samples was in the following order; ascorbic acid > BHA > *H. officinalis* essential oil > BHT (Tab. 3; Fig. 1).

The results show that *H. officinalis* essential oil possesses weak radical scavenging activity. Similar results were reported by Dapkevicius *et al.*, (1998). Generally, antioxidant properties of essential oils depend on their components, especially phenolic compounds. *Iso* pinocamphone is the main component of hyssop oil, whose antioxidant activity has not yet been reported in the literature. The activities of essential oils such as antioxidants depend on their structural features but also on many other factors, such as concentration, temperature, light, type of substrate and physical state of the system, as well as on microcomponents acting as a pro-oxidant or synergists (Yanishlieva-Maslarova, 2001). It has been described that the kinetic behaviour of some compounds can affect radical scavenging abilities (Bondet *et al.*, 1997). For slow reacting compounds, the influence was attributed to the complex reacting mechanism. In our study, most likely, the constituents from *H. officinalis* essential oil are involved in one or more secondary reactions, which resulted in the slower reduction of DPPH solutions.

## Conclusions

GC-MS analysis of hyssop essential oil showed major compounds as *iso* pinocamphone, β-pinene, terpinene-4-ol, pinocarvone, carvacrole, *p*-cymene and pinocamphone. There were many other compounds in minor amounts. Hyssop oil possesses good antibacterial activity. It is clearly apparent that the essential oil of hyssop possesses compounds with antimicrobial properties, which could be used as antimicrobial agents in new drugs for therapy against human infectious diseases. Furthermore, the study also revealed that even low amounts of the hyssop essential oil had detectable antioxidant activity.

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