



Chemical Composition and Antioxidant and Antimicrobial Properties of the Essential Oil of *Hyssopus officinalis* L

Fayegh Moulodi^{1*}, Arezou Khezerlou², Hajar Zolfaghari³, Ali Mohamadzadeh⁴ and Foad Alimoradi⁵

¹Department of Food Science and Technology, Faculty of Agriculture, Urmia University, Urmia, Iran

²Department of Food Science and Technology, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

³Department of Hygiene and Food Safety, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

⁴Healthcare Network of Mahabad, Urmia University of Medical Sciences, Urmia, Iran

⁵Kowsar Hospital, Kurdistan University of Medical Sciences, Sanandaj, Iran

*Corresponding author: PhD Student, Department of Food Science and Technology, Faculty of Agriculture, Urmia University, Urmia, Iran. Tel: +98-9143443884, Email: fayegh.molodi@yahoo.com

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Abstract

Background: Medicinal plants and their extracts are extensively used in nutrition, medicine and food industry. These plants are considered the most important natural antimicrobial antioxidants since they provide rich sources of phenolic compounds, and if resuscitated, they prevent the oxidation process.

Objectives: The present study was conducted to investigate the antimicrobial and antioxidant properties of *Hyssopus officinalis* essential oil as a chemical preservative.

Methods: The *Hyssopus officinalis* plant was collected from the mountainous regions of Kurdistan, Iran. The essential oil was extracted through distillation and analyzed and its components were identified using gas chromatography-mass spectrometry (GC-MS). The antioxidant effect was evaluated using the diphenylpicrylhydrazyl (DPPH) free radical-scavenging method. The antimicrobial property was assessed using the standard microdilution method to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Results: The major components of *Hyssopus officinalis* essential oil were found to be camphor (23.61%) and β -pinene (21.91%). The obtained results showed that the total phenolic content of this species was 23.16 mg of gallic acid per gram of the essential oil and its IC50 was 11.22 μ g/mL. MIC obtained for *Escherichia coli* was 156.25 and MBC 312.5 μ g/mL, while these figures were respectively 312.5 and 625 μ g/mL for *Listeria monocytogenes*.

Conclusions: The results obtained indicate the antioxidant activity and antimicrobial power of *Hyssopus officinalis* essential oil against pathogenic microorganisms. This essence can be used as an antimicrobial substance and a natural antioxidant in pharmaceutical and food industries.

Keywords: Antioxidant Activity, Antimicrobial Properties, Essence, *Hyssopus officinalis* L

1. Background

Artificial antimicrobial compounds have long been used to manage and control deterioration or contamination of foods. Today, concerns and negative reactions of consumers to artificial and commercial preservatives have increased the tendency to use more natural compounds such as essences. In fact, given a serious need for replacing chemical preservatives with healthy and effective compounds, efforts have been focused on the potential applications of herbal essences. Medicinal plants, essences and their constituents present antimicrobial, antifungal and preservative properties against a wide range of microbial pathogens, including bacteria, yeasts and molds (1, 2). The extract of many plants such as Ziziphora, rosemary, thyme,

lavender, mint, cumin, caraway, marjoram, pennyroyal, hyssop and ajwain contain compounds such as phenylpropanoid glycosides, polyacetylene, flavonoids, ditropan, polyphenols and flavone glucoside, which are involved in antimicrobial and antioxidant activities and widely used in traditional medicine (3-5).

With a scientific name "*Hyssopus officinalis*", this plant is commonly known as hyssop and belongs to the Lamiaceae family, and widely grows in southern Europe, Central Asia, Russia and Iran. Hyssop has thick ramified roots and several 20 - 60 cm high wooden stems. This plant has small, paired, pointed and very fragrant leaves and purplish dark-blue, white and occasionally red flowers (6), and is widely used in pharmaceutical, hygiene and food industries. Hyssop has long been used to treat rheumatic pains, bruising,

wounds, blood pressure fluctuations and neurological disorders such as depression and anxiety (7, 8). The aerial shoots of this perennial herbaceous plant are useful for the treatment of respiratory diseases, including asthma, bronchitis and coughs, as they contain chemicals such as terpenes, flavonoids, volatile oils, tannins and resin. The amount of essential oil is 0.3% - 1% in the vegetative body of hyssop and reportedly 0.1% - 1.8% in literature. Hyssop essential oil is bitter, spicy and dry, and its main constituents include pinocampone, α and β -pinene, camphene and sesquiterpene alcohol. This plant's vegetative body contains flavonoid, tannin (5% - 8%), bitter substances (3% - 6%) and other substances such as diosmin, hyssopin and mucilaginous compounds (9, 10).

Research confirms the antimicrobial and antifungal effects of hyssop, and shows that in addition to hyssop essence, its extract prevents the oxidation of fat and decomposition of pigments caused by cooking and preservation, and it can be used as a useful additive for meat processing and prevention of its oxidation and discoloration (11). The ethanol extract of hyssop has been recently reported to present protective properties for the gastric tract based on the adhesive stomach mucosal compounds, and also significant anticoagulant and antioxidant properties through reducing the production of free radicals (12). Research suggests that hyssop essence has special antimicrobial effects on bacteria such as *Streptococcus pyogenes*, *Candida albicans*, *E. coli*, and *Staphylococcus aureus* (13). In 2012, Marino et al. studied the effect of the main compounds of hyssop essence on six gram-positive and nine gram-negative bacteria, and reported that phenolic compounds and their most important ones carvacrol and thymol are very effective herbal compounds in microorganisms (14). Researchers associated the proper and effective dose of hyssop essence to several factors, including the consumer's age and health status. Further scientific information is, however, needed for determining the proper dose range for hyssop essential oil. Medicinal essences and plants provide a wide range of antimicrobial and antioxidant properties. Using these compounds is also very effective as they lack the side-effects of chemicals. Identifying and investigating the amount of effective compounds contained in native essences and medicinal herbs is therefore crucial. Given that this amount differs with climate and in different parts of the plant, several experiments are required to determine the amount of these compounds in different regions.

2. Objectives

The present study was therefore conducted to analyze and determine the chemical compounds of the ethanol extracts of *Hyssopus officinalis* collected from the mountainous regions of Kurdistan, and to assess their antioxidant

and antimicrobial properties and therefore provide a better description for these natural products and make optimal use of them.

3. Methods

3.1. Collecting the Plant and Extracting the Essence

After being collected from the mountainous regions of Kurdistan and identified and confirmed, hyssop was used in experiments to extract the plant essential oil. To extract essence, the plant was dried and milled. The volatile essential oil of the plant was then extracted using a Clevenger apparatus and through aqueous distillation. The essence obtained was dried and dehydrated using sodium sulfate, and placed in a dark glass container away from sunlight at 4 °C after passing through a 0.45- μ m filter.

3.2. Bacterial Strains

Standard strains were used to evaluate the antibacterial effects of hyssop essence. These strains were collected from the Bacteriology Laboratory of Faculty of Veterinary Medicine, Tabriz University, Iran, and included *E. coli* (25922 ATCC) and *Listeria monocytogenes* (19117 ATCC). These strains were then identified using culture media and biochemical tests.

3.3. Essence Decomposition

The essence sample was decomposed using a gas chromatography device (GC-MS, model Agilent6890) equipped with a capillary column of a 30 m length, 0.25 mm internal diameter and 0.25 μ m internal layer thickness. The column temperature schedule included: 2 minutes at 70°C, increasing to 220°C at a rate of 15°C /min followed by increasing and holding the temperature at 300°C for two minutes. The essence constituents were identified by comparing their mass spectrum with the bank spectrum and comparing their inhibition coefficients with reference and standard values. The relative values of the constituents were calculated by the machine software based on the total area of the peaks (15).

3.4. Measuring the Antioxidant Effect

The ability of extracts and essences tested to combine with hydrogen or electronize was measured based on the discoloration of a purple methanol DPPH solution. The stable radical DPPH was used as the reactive agent in this spectrophotometric evaluation. Fifty μ L of different concentrations of the essence was added to 5 mL of a 0.004% methanol solution of DPPH and kept at room temperature for 30 minutes. Optical absorption was then read at a wavelength of 517 nm compared to the sham value. The inhibition of DPPH free radical was calculated in I% as follows:

$$I\% = \left(\frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100 \quad (1)$$

Where, A_{blank} is the absorption of the sham solution (containing all the reactive agents except for the essence) and A_{sample} is the absorption of the solution containing different concentrations of the essence. The synthetic BHT antioxidant was also used as a positive control, and the tests were repeated three times, and their mean was announced as the desired value. The essence antioxidant activity is expressed as IC_{50} , which indicates an essence concentration that causes a 50% inhibition in radical capacity (16).

3.5. Determining the Amount of Total Phenol

Phenolic compounds are measured based on Folin reagent reduction by phenolic compounds in an alkaline medium producing a blue complex showing a maximum absorption at a wavelength of 760 nm. The total amount of phenolic compounds was measured using the Folin-Ciocalteu method. Different concentrations of the essence were first prepared and 0.1 mL of each concentration was added to an Erlenmeyer flask, and 46 mL of distilled water was mixed with 1 mL of Folin-Ciocalteu reagent. Three mL of a 2% sodium carbonate solution was added to the obtained solution after 3 minutes. The solution was then placed on a moderately shaking disc at room temperature for two hours, and the absorption was read against the sham value at a wavelength of 760 nm using a spectrophotometer. Gallic acid (0 - 1000 μg per 0.1 mL) was used to plot the standard curve. The total amount of phenolic compounds contained in the essence was calculated using the equation derived from the standard curve, and the results were expressed in micrograms of gallic acid per milliliters of the essence (17).

3.6. Antimicrobial Activity Measurement

MIC and MBC were measured using microdilution. One hundred μL of the liquid medium and 100 μL of the prepared bacterial suspension (with a bacterial concentration of 1.5×10^6 per mL) were added to each well. One hundred μL of the essence or extract was then added to the first well in each row. After mixing its content, 100 μL was removed from the first well and added to the next well. A control row was considered for each test row associated with an essence or extract, but no bacteria were added to the control row. After 24 hours of incubation at the right temperature, the results were assessed in terms of opacity. The last well with no observed growth was considered the MIC. To determine MBC, 10 μL of the wells' content were cultured on Mueller Hinton agar (Merck-Germany) after 18 hours of incubation. A petri dish was incubated for 18 hours to investigate the bacterial growth. The minimum concentration of the essence or extract in which 99.9% of the bacteria presented no growth was ultimately reported as MBC. All the tests were repeated three times (18, 19).

4. Results

Table 1 present the analysis results of the essence using the GC device. Fourteen compounds comprising 71.45% of the total constituents were identified in the essence of *Hyssopus officinalis*. The major components of this essence were camphor (23.61%) and β -pinene (21.91%). Other important constituents included 1,8-cineole (14.25%), α -pinene (4.09%) and bicyclo hept-2-en-2-meta. The proportion of other compounds was below 2%.

Table 1. Chemical Compounds of *Hyssopus Officinalis* Essential Oil

Peak N°	Component	Peak Area, %
1	Camphor	23.61
2	1,8-Cineole	14.25
3	β -pinene	21.91
4	α -pinene	4.09
5	β -caryophyllene	0.98
6	Bicycloheptane	1.8
7	Bicyclohepta.2-n	2.98
8	Caryophyllene	0.93
9	Camphene	0.34
10	β -elemene	0.24
11	α -humulene	0.16
12	Hexanal	0.05
13	Pentadecane	0.08
14	Naphthalene	0.03
Total		71.45

Table 2 present the results of evaluating the inhibitory strength of free radicals and the total phenolic content of the essence. The results show that, 50IC equals 11.23 $\mu\text{g}/\text{mL}$ for this essence, which is weaker compared to butylated hydroxytoluene (0.7 $\mu\text{g}/\text{mL}$).

The total phenolic content of the essential oil reached 23.16 mg of gallic acid per gram of the essence.

The results obtained suggest an MIC of 156.25 $\mu\text{g}/\text{mL}$ and an MBC of 312.5 $\mu\text{g}/\text{mL}$ in the study bacteria (Table 3).

5. Discussion

The chemical composition of hyssop essential oil has been studied in literature. Fathiazad et al. reported myrtenyl acetate (74.08%), camphor (76.6%), germacrene (3.39%), spathulenol (2.10%), caryophyllene oxide (2.13%) and β -caryophyllene (2.1%) to be the main constituents of hyssop essential oil (20). A study conducted by Salma in Egypt found β -pinene (19.6%), pinocamphone (19.2%) and camphor (3.16%) to be the major constituents of the essence

Table 2. Antioxidant Activity Evaluated Using the DPPH Method and the Total Phenolic Content of *Hyssopus officinalis* Essential Oil

Sample	Antioxidant Activity (IC ₅₀ *, $\mu\text{g mL}^{-1}$)	Total Phenolic Content (mg GAE/L)
Hyssopus essential oil	11.23 \pm 0.02	23.16 \pm 0.05
BHT	3.01 \pm 0.7	

Table 3. MIC and MBC of the Essence of the Study Bacteria in $\mu\text{g/mL}$

Bacteria	MIC, mg/mL	MBC
<i>E. coli</i> O157:H7	156.25	312.5
<i>L. monocytogenes</i>	312.5	625

Abbreviations: MBC, minimal bactericidal concentration; MIC, minimum inhibition concentration

of this plant (21). Isopinocampone (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%) were also found to be the main chemical components of the essence of hyssop collected from the southeastern region of Anatolia, Turkey (22). Research suggests that several factors affect the composition of the essences extracted from a special plant species, including the region geographical conditions, harvesting season, plant's age, agricultural operations, plant growth stage and extraction methods and conditions (23).

DPPH free radical scavenging is considered a simple and very fast method for determining antioxidant activities. The antioxidant effect on inhibiting the DPPH radical is associated to the ability of releasing a hydrogen atom or eliminating free radicals. The inhibitory activity of free radicals depends on the concentration of essences and extracts, which present better protective effects with lower levels of 50IC. The antioxidant properties of essential oils can be generally attributed to the phenolic composition, especially camphor and β -pinene. In a study conducted by Fathiazad et al. in 2011, 50IC was reported to be 25 $\mu\text{g/mL}$ in hyssop (20), which shows a lower antioxidant strength compared to the present study hyssop, and can be explained by environmental and climate conditions. Hosseini et al. investigated the effects of the antioxidant strength of certain herbal essences, and showed that *Zataria multiflora* Boiss presents the highest inhibitory level with an inhibitory strength of 667 $\mu\text{g/mL}$, which is lower than the antioxidant strength of hyssop reported in the present study (24).

The antioxidant activity of essential oils depends on their structural properties and other factors, including concentration, temperature, lighting, type of substrate and the physical status of the system (25). Medicinal plants are rich sources of natural antioxidants, which can generally eliminate free radicals and superoxide and hydroxyl radicals by transferring free electrons. Phenolic

compounds have a high antioxidant power and constitute the secondary metabolites of many plants.

Ahmadi et al. examined the hyssop extract in terms of its phenolic content, and observed an average of 3.113 mL of gallic acid-based phenolic compounds per gram of this extract (26). Soleimani et al. reported the total phenolic content of hyssop extract to be 200 mg based on gallic acid (27).

Hamedi et al. conducted an *In vitro* and cheese model study of anti-listeria effects of the essences of mentha pulegium (pennyroyal), tarragon and mint alone and with monolaurin, and found the mint essence with an MIC of 0.2% to present the highest anti-listeria effect. The combination of monolaurin with each of these essences increased the anti-listeria effect, and MIC was found to be 0.66% in tarragon plus monolaurin. These results showed that using low concentrations of these substances in combination can have positive antimicrobial effects on foods (28), which is consistent with the present study.

Mendoza-Yepes et al. investigated the antimicrobial effects of a mixture of herbal essences (DMC) containing 50% rosemary, *Salvia officinalis* and Citrics plus 50% glycerol on *Listeria monocytogenes* and *E. coli* O157:H7 in Spanish soft cheese. According to the obtained results, DMC exerts bacteriostatic effects on *Listeria monocytogenes* at a concentration of 2500 ppm, although it is ineffective in controlling *E. coli* growth (29). In contrast, the present research revealed that the essence used exerts antimicrobial effects on both of the bacteria.

In line with the present study, the simultaneous effect of pennyroyal and mint on *E. coli* was investigated, and pennyroyal MIC was found to be 5000 $\mu\text{g/mL}$ on *Bacillus cereus* and 4166 $\mu\text{g/mL}$ on *E. coli*, and the mint essence was 100 mg/mL on both bacteria. Investigating the effects of three species of *Satureja* on *Salmonella paratyphi*, Sefidkon et al. found the *Satureja* essence to present inhibitory properties at concentrations of 2.5% and 5%, which presents lower antimicrobial properties compared to the hyssop essence (30).

The differences observed between different studies concerning the antimicrobial and antioxidant properties of different essences can be explained by the differences in the chemical compositions of plants, in methods and in their kinetics, which are affected by genetic factors, climate, environment and harvesting season. The amount of phenolic compounds was generally found to be positively associated with antioxidant activities.

5.1. Conclusion

Given the obtained results and the need for using natural preservatives, hyssop essential oil is recommended to be optimally used in the food industry to increase the shelf-life of food products and protect them against oxidizing and microbial factors and to control foodborne microbial diseases. Future studies are recommended to focus on this essence as a component of food models and in combination with other essences.

Footnotes

Conflict of Interests: No conflict of interests.

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References

- Pandey AK, Singh P, Palni UT, Tripathi NN. In vivo evaluation of two essential oil based botanical formulations (EOBBFs) for the use against stored product pathogens and pests, *Aspergillus* species and *Callosobruchus* species (Coleoptera: Bruchidae). *J Stored Prod Res*. 2014;**59**:285–91. doi: [10.1016/j.jspr.2014.09.001](https://doi.org/10.1016/j.jspr.2014.09.001).
- Sonker N, Pandey AK, Singh P. Efficiency of *Artemisia nilagirica* (Clarke) Pamp. essential oil as a mycotoxicant against postharvest mycobiota of table grapes. *J Sci Food Agric*. 2015;**95**(9):1932–9. doi: [10.1002/jsfa.6901](https://doi.org/10.1002/jsfa.6901). [PubMed: [25199920](https://pubmed.ncbi.nlm.nih.gov/25199920/)].
- Sokmen M, Serkedjjeva J, Daferera D, Gulluce M, Polissiou M, Tepe B, et al. In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum acutidens*. *J Agric Food Chem*. 2004;**52**(11):3309–12. doi: [10.1021/jf049859g](https://doi.org/10.1021/jf049859g). [PubMed: [15161188](https://pubmed.ncbi.nlm.nih.gov/15161188/)].
- Bazzaz BS, Haririzadeh G. Screening of Iranian plants for antimicrobial activity. *Pharma Biol*. 2008;**41**(8):573–83. doi: [10.1080/13880200390501488](https://doi.org/10.1080/13880200390501488).
- Sadraei H, Asghari G, Hekmati AA. Antispasmodic effect of three fractions of hydroalcoholic extract of *Pycnocycla spinosa*. *J Ethnopharmacol*. 2003;**86**(2-3):187–90. doi: [10.1016/S0378-8741\(03\)00077-1](https://doi.org/10.1016/S0378-8741(03)00077-1). [PubMed: [12738085](https://pubmed.ncbi.nlm.nih.gov/12738085/)].
- Govaerts R. *World checklist of monocotyledons database in ACCESS: 1-71827. The board of trustees of the royal botanic gardens*. Kew; 2003.
- Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J Nutr*. 2003;**133**(5):1286–90. doi: [10.1093/jn/133.5.1286](https://doi.org/10.1093/jn/133.5.1286). [PubMed: [12730411](https://pubmed.ncbi.nlm.nih.gov/12730411/)].
- Steinmetz M, Tognetti P, Mourougue M, Jouglard J, Miller Y. Sur la toxicité de certaines huiles essentielles du commerce: Essence d'hysope et essence de sauge. *Plant Med Phytother*. 1980;**14**:34–45. French.
- Naghbi F, Mosaddegh M, Mohammadi Motamed M, Ghorbani A. Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. *Iran J Pharm Res*. 2010:63–79.
- Bernath J. *Wild growing and cultivated medicinal plants*. Budapest: Mezo Publication; 1993.
- Fernandez-Lopez J, Sevilla L, Sayas-Barbera E, Navarro C, Marin F, Perez-Alvarez JA. Evaluation of the antioxidant potential of hyssop (*Hyssopus officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) extracts in cooked pork meat. *J Food Sci*. 2003;**68**(2):660–4. doi: [10.1111/j.1365-2621.2003.tb05727.x](https://doi.org/10.1111/j.1365-2621.2003.tb05727.x).
- Saini A. To explore the ulceroprotective and antioxidant potential of *hyssopus officinalis* in ethanol-induced gastric ulcers in rats. *Brit J Pharm Res*. 2012;**2**(3):197–214. doi: [10.9734/bjpr/2012/1930](https://doi.org/10.9734/bjpr/2012/1930).
- Ghasemi Pirbalouti A, Gorgij A, Rahimmalek M, Hamed B. Phytochemical response of hyssop (*Hyssopus officinalis* L.) to foliar application of jasmonic acid. *J Herb Drugs*. 2013;**4**(1):7–14.
- Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. *Int J Food Microbiol*. 2001;**67**(3):187–95. doi: [10.1016/S0168-1605\(01\)00447-0](https://doi.org/10.1016/S0168-1605(01)00447-0). [PubMed: [11518428](https://pubmed.ncbi.nlm.nih.gov/11518428/)].
- Mahmoudi R. Effect of mentha longifolia. Essential oil on physicochemical properties of the bio-ayran. *J Essential Oil Bearing Plants*. 2014;**17**(1):56–66. doi: [10.1080/0972060x.2013.813205](https://doi.org/10.1080/0972060x.2013.813205).
- Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res*. 2000;**14**(5):323–8. doi: [10.1002/1099-1573\(200008\)14:5<323::AID-PTR621>3.0.CO;2-Q](https://doi.org/10.1002/1099-1573(200008)14:5<323::AID-PTR621>3.0.CO;2-Q). [PubMed: [10925395](https://pubmed.ncbi.nlm.nih.gov/10925395/)].
- Mahmoudi R, Nosratpour S. *Teucrium polium* L. essential oil: phytochemical component and antioxidant properties. *Int Food Res J*. 2013;**20**(4):1697.
- Kabudari A, Mahalleh SFRP. Study of antibacterial effects of *teucrium polium* essential oil on *bacillus cereus* in cultural laboratory and commercial soup. *Carpathian Food Sci Technol*. 2016;**8**(2):176–83.
- Jafari AA, Shohrati M, Mahmoudi R, Hoseini RH, Nosratpour S, Pajohi-Alamoti M. [Chemical composition and biological activities of *Scrophularia striata* extracts]. *J Minerva Biotech*. 2014;**26**(3):183–9. Persian.
- Fathiazad F, Mazandarani M, Hamedeyazdan S. Phytochemical analysis and antioxidant activity of *Hyssopus officinalis* L. from Iran. *Adv Pharm Bull*. 2011;**1**(2):63–7. doi: [10.5681/apb.2011.009](https://doi.org/10.5681/apb.2011.009). [PubMed: [24312758](https://pubmed.ncbi.nlm.nih.gov/24312758/)]. [PubMed Central: [PMC3845980](https://pubmed.ncbi.nlm.nih.gov/PMC3845980/)].
- Salma A. Chemical and physiological studies on anise hysop (*Agastache foeniculum* Pursh) and hyssop (*Hyssopus officinalis* L.) plants grown in Egypt as new spices. *J Bull Nation Res Centre*. 2002;**27**:25–35.
- Kizil S, Haşlımı N, Tolan V, Kilinc E, Karataş H. Chemical composition, antimicrobial and antioxidant activities of hyssop (*Hyssopus officinalis* L.) essential oil. *Not Bot Hort Agrobot Cluj*. 2010;**38**(3):99–103.
- Xu L, Zhan X, Zeng Z, Chen R, Li H, Xie T, et al. Recent advances on supercritical fluid extraction of essential oils. *Africa J Pharm Pharm*. 2011;**5**(9):1196–211. doi: [10.5897/ajpp11.228](https://doi.org/10.5897/ajpp11.228).
- Hosseini N, Malekiran A, Changizi Ashtiani S, Nazemi M. [Free radicals scavenging activity of essential oils and different fractions of methanol extract of *Zataria multiflora*, *salvia officinalis*, *rosmarinus officinalis*, *mentha pulegium* and *cinnamomum zeylanicum*]. *J Shahid Sadoughi Univ Med Sci*. 2012;**20**(1):28–38. Persian.
- Pokorny J, Yanishlieva N, Gordon M. *Antioxidants in food: Practical applications*. 1st ed. Boca Raton: CRC press; 2001. doi: [10.1201/9781439823057](https://doi.org/10.1201/9781439823057).
- Ahmadi E, Abdollahi A, Najafipour S, Meshki Baf MH, Fasihi Ramandi M. [Effect of phenolic compounds on antibacterial activity of medicinal plants extract: Evaluation of vitro in medicinal plants of Fasa-Fars city]. *J Fasa Univ Med Sci*. 2016;**6**(2):210–20. Persian.
- Soleimani H, Barzegar M, Sahari MA, Naghdi Badi H. [An investigation on the antioxidant activities of *Hyssopus officinalis* L. and *Echinacea purpurea* L. plant extracts in oil model system]. *J Med Plants*. 2011;**7**(37):61–72. Persian.
- Hamed H, Razavi-Rohani SM, Gandomi H. Combination effect of essential oils of some herbs with monolaurin on growth and survival of *Listeria monocytogenes* in culture media and cheese. *J Food Proc Preservation*. 2014;**38**(1):304–10. doi: [10.1111/j.1745-4549.2012.00778.x](https://doi.org/10.1111/j.1745-4549.2012.00778.x).
- Mendoza-Yepes MJ, Sanchez-Hidalgo LE, Maertens G, Marin-Iniesta F. Inhibition of *Listeria monocytogenes* and other bacteria by a plant essential oil (dmc) in Spanish soft cheese. *J Food Safe*. 1997;**17**(1):47–55. doi: [10.1111/j.1745-4565.1997.tb00175.x](https://doi.org/10.1111/j.1745-4565.1997.tb00175.x).
- Sefidkon F, Sadeghzadeh L, Teymouri M, Asgari F, Ahmadi S. [Antimicrobial effects of the essential oils of two *Satureja* species (*S. khuzistanica* Jamzad and *S. bachtiarica* Bunge) in two harvesting time]. *Iran J Med Aroma Plant*. 2007;**23**(2):174–82. Persian.