August 2007 *Volume 3 Issue 2*

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 3(2), 2007 [87-91]

Chemical Composition And Biological Activities Of *Hyssopus Officinalis* Cultivated In Iran

Noushin Rastkari[1]*, Nasrin Samadi[2], Reza Ahmadkhaniha[3], Rozita Alemi[4], Leila Afarin[4]

[1]Center for Environmental Research, Tehran University of Medical Sciences, Tehran, (IRAN)

[2]Department of Drug & Food Control, School of Pharmacy, Tehran University of Medical Sciences, Tehran, (IRAN)

[3]Department of Medicinal Chemistry, School of Pharmacy, Tehran Universityof Medical Sciences, Tehran, (IRAN)

[4]Iran Color Research Center, Tehran, (IRAN)

Tel : +98-21-66406757 ; Fax : +98-21-66461178

E-mail : n_rastkari@ yahoo.com

Received: 12th March, 2007 ; Accepted: 17th March, 2007

ABSTRACT

Hyssopus officinalis L.has been known as a culinary and medicinal herb for hundreds of years. The present study was designed to investigate the chemical composition, antimicrobial and free radical-scavenging activity of *H.officinalis* ethanolic extracts which is cultivated in Iran and has not been studied previously. Forty four compounds were identified by gas chromatography-mass spectrometry analysis. The ethanolic extract of aerial parts of *Hyssopus officinalis* which is cultivated in Iran inhibited the growth of some of examined microorganisms including different kinds of bacteria. The highest antimicrobial activity was observed against *Bacillus cereus* and *Pseudomonas aeruginosa* but the extract was inactive against the fungi, *C.albicans* and *A.niger*. The ethanolic extract of aerial parts of *Hyssopus officinalis* was evaluated as DPPH free-radical scavenger. The antioxidant potential of extract was evaluated by FRAP assay and its ability to inhibit lipid peroxidation was also determined by TBARS test in rat. *In vivo* evaluation of antioxidant effects of *H.officinalis* with these two methods showed that the extract of *H.officinalis* has a moderate ability to inhibit lipid peroxidation and has a moderate antioxidant power with all doses comparing to control ($p<0.05$).

KEYWORDS

Hyssopus officinalis;
Antimicrobial activity;
MIC;
Free radical-scavenging activity;
Chemical composition.

INTRODUCTION

Hyssopus officinalis L.is a perennial and polimorphous species belonging to the Lamiaceae family, native to the Mediterranean area, southern Europe and Asia Minor and cultivated in the United States and Russia[1]. The aerial parts of the plant is used for its aromatic scent and is generally employed in perfumery, in the food industry as flavoring agent, in liqueurs and cosmetic products, and in the pharmaceu-

Full Paper

tical field in several antiseptic preparations[2]. A crude extract of dried leaves has been reported to have anti-HIV activity[3, 4], and the aerial parts are used as ingredients in blood-pressure-increasing preparations and tea blends for cough and asthma relief[5]. The *in vitro* antimicrobial and cytotoxic activity of the oil of *H.officinalis* was shown on *Staphylococcus aureus* and *Streptococcus sanguis*[6]. In another study[7], the effect of *H.officinalis* oil was tested on several Gram positive and Gram negative bacteria. Several investigations on oil of *H.officinalis* have been done, but there is no report about chemical composition, antimicrobial effect and free radical-scavenging activity of *H.officinalis* ethanolic extract. The aim of this study is to identify the chemical composition of ethanolic extract of the aerial part of the plant by gas chromatography-mass spectrometry (GC/MS) analysis and to evaluate its free radical-scavenging activity. The antibacterial and antifungal activities of the extract were examined on Three Gram-positive bacteria(*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* ATCC 6633), three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* PTCC 1111, *Escherichia coli* ATCC 8739) and two fungi(*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404).

MATERIAL AND METHOD

Plant material

Hyssopus officinalis was collected from Karaj (Iran) and identified at the Department of medicinal plants of shaheed Beheshti university,Tehran, Iran.

Compounds

DPPH(1, 1-diphenyl-2- picrylhydrazyl) was purchased from Fluka(Barcelona, spain). All other reagents and solvents were of analytical grade and supplied by Merck(Darmstadt, Germany).

Microorganisms

Three Gram-positive bacteria(*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* ATCC 6633), three Gram-negative bacteria(*Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* PTCC 1111, *Escherichia coli* ATCC 8739) and two fungi(*Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) were used in the experiments. The test microorganisms were stocks of the Department of Drug and Food Control, School of Pharmacy, Tehran University of Medical Sciences.

Preparation of the extract

The dried aerial parts of H.officinalis 200gr were extracted with an ethanol water solution(80:20). Extraction was carried out by replacing(three times) the solution every 24h with fresh solvent. The mixture was filtered through a paper filter and then was evaporated under vacuum at 50^0C to dryness as a dark brown mass(46gr).

GC&GC/MS analysis

1. Gas chromatography

The ethanolic extract of aerial parts of *Hyssopus officinalis* was analyzed and the relative peak area for individual constituents averaged. Quantification was computed as the percentage contribution of each compound to the total amount present. The relative percentages were determined using a HP 6890 gas-chromatography(HP, Palo Alto, USA) equipped with a FID detector and a HP-5 capillary column(30m ×0.25mm i.d.×0.25μm film thickness). Operating conditions were as follows: injector temperature, 280^0C; FID temperature, 280^0C; carrier gas(Helium), flow rate 2ml/min and split injection with split ratio 1:10. Oven temperature was programmed from 50^0C to 280^0C at a rate of 4^0C/min. 1μl of ethanolic extract(10μg/ml) which was prepared from dried residue was injected. The percentage composition of the ethanolic extract was computed by the normalization method from the GC peak areas, calculated by means of three injections of extract.

2. Gas chromatography/Mass spectrometry

The extract was analyzed by a Hewlett-Packard 6890 GC gas chromatograph coupled to HP 5973 Mass selective detector(HP, Palo Alto, USA) equipped with the same capillary column and operating under the same conditions as described above for GC. The MS operating parameters were as follows: ionization potential, 70 ev; ionization current, 2A; scan speed, 1 scan s^{-1}; ion source temperature, 200^0C. The mass scan rang was 50-550 Atomic Mass Units(AMU). Compound were identified by comparison of their GC retention indices calculated by linear interpolation relative to retention times of a series of n-alkanes(C7-

C25) with those reported in the literature[8], and by comparison of mass spectra from the Wiley 275.L and Nist 02 mass spectral database.

Pharmacology

1. Free radial scavenging activity

A DPPH radical-scavenging activity of the total ethanolic extract was measured by using the method of Blois[9] with a slight modification. A 0.1-mM solution of 1,1-diphenyl-2-picryl hydrazyl(DPPH) in ethanol was prepared and 1ml was added to 3ml of sample solutions in ethanol at a range of concentrations(100-400μg/ml). After 30min, the absorbance was measured at 517nm. All data are an average of triplicate analyses. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. This activity is shown as % DPPH radical-scavenging that is calculated by the equation:

% DPPH radical- scavenging activity

=(Control absorbance-sample/Control absorbance)×100

The DPPH solution without sample solution was used as control (Figure 1).

2. *In vivo* study protocol

In the *in vivo* study protocol, male Sprague-Dawley rats weighing 120-180gr were randomly distributed into 6 groups within 6 animals in each group. The groups were divided into control, Torolox treated and *H.officinalis* extract treated in 4 different doses groups. The animals in the treated groups were orally administered 50, 75, 100, 200mg/kg/day body weight of hydro alcoholic solution of *H.officinalis* extract and control animals received only hydro alcoholic solution to observe ethanol effect as gavage vehicle. Torolox was selected as a reference drug (10mg/kg/day) in, in vivo experiments. All treatments were continued for 10 days after which blood samples were collected following an overnight fast.

The antioxidant capacity of plasma was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} established as named FRAP test and described previously[10]. The complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593nm.

Thiobarbituric acid reactive substances (TBARS) measurement was used to measure the rate of lipid peroxidation. In this method plasma samples were mixed with trichloroacetic acid 20%(w/v) and the precipitate was dispersed in H_2SO_4(0.05 M). 2-thiobarbituric acid (0.2% in 2M sodium sulfate) was added and heated for 30 min in boiling water bath. TBARS adducts were extracted by n-butanol(4ml) and absorbance was measured at 532nm[11].

3. Minimum inhibitory concentration (MIC) determination

The MIC values of the ethanolic extract was determined by conventional agar dilution method[12,13] with respect to different test microorganisms including three Gram-positive bacteria(*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633), three Gram-negative bacteria(*Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* PTCC 1111, *Escherichia coli* ATCC 8739)and two fungi(*Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404). Two-fold dilution of the extract was prepared in dimethylsulfoxide (DMSO; 1 mL). Each dilute was added to molten Mueller-Hinton (MH) agar (19mL) at 50^0C to give the final concentrations of 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.781μg μl^{-1}. The bacteria inocula were prepared by suspending overnight colonies from Muller-Hinton(MH) agar media in 0.9% saline. The *C.albicans* and *A.niger* inocula were prepared by suspending colonies from 48h and 72h old sabouraud dextrose(SD) agar cultures in 0.9% saline respectively. The inocula were adjusted photometrically at 600nm to a cell density equivalent to approximately 0.5 McFarland standards(1.5×10^8CFU/mL). The suspensions were then diluted in 0.9% saline to give 10^7 CFU/mL. The plates were spot-inoculated with 1μL of each prepared bacterial suspension(10^4CFU/spot); including a control plate containing 1mL DMSO without any antibacterial agent. The plates containing bacteria were incubated at 30-35^0C for 24h and those containing fungi were incubated at 20-25^0C for 48h. The MIC was determined as the lowest concentration of the agent that completely inhibits visible growth of the microorganisms.

Statistical analysis

The results are reported as mean ±SE. Statistical analysis of data was carried out by computer using SPSS version 11.5 software. One-way ANOVA and Tukey post hoc multiple comparison tests were used to analyze data. P-values less than 0.05 were

Full Paper

TABLE 1 : Composition of *H.officinalis* ethanolic extract

Compound name	Retention index(KI)	Percentage
α-Thujene	930	1.01
α-Pinene	937	0.90
β-Pinene	978	2.00
Myrcene	990	0.05
Limonene	1030	0.2
1,8- Cineole	1032	6.30
Methyl benzoate	1091	15.78
Linalool	1097	6.50
Undecane	1099	1.16
Pinocarveol (trans)	1140	0.09
Camphor	1145	2.5
Pinocamphone	1160	6.28
Benzoic acid	1170	0.81
Pinocarvone	1164	5.40
Ethyl benzoate	1173	3.50
Isopinchampone	1176	10.1
Dodecane	1199	1.82
m-Thymol	1290	1.95
Methyl decanoate	1326	0.8
Tridecane	1299	0.60
α- Terpinyl acetate	1349	0.90
Methyl cinnamate	1379	0.12
1- Tetradecene	1390	0.61
Tetradecane	1398	1.2
α-Cedrene	1411	1.06
Cinnamic acid	1454	0.12
α-Humulene	1455	0.65
Ethyl cinnamate (E)	1467	0.09
Germacrene-D	1484	0.90
Methyl dodecanoate	1526	0.20
Dodecanoic acid	1567	0.15
Caryophyllene oxide	1581	0.70
Ethyl dodecanoate	1595	0.10
Hexadecane	1600	0.97
Octadecene	1793	0.26
Octadecane	1800	0.28
Nanodecene	1883	0.64
Nanodecane	1900	0.30
Methyl palmitate	1922	6.50
Ethyl palmitate	1993	1.20
Methyl stearate	2125	2.14
Palmitic acid	2150	2.50
Ethyl stearate	2197	0.90
Stearic acid	2180	2.10

considered significant.

RESULTS & DISCUSSION

The chemical composition of the ethanolic extract of *H.officinalis* sample was assessed by GC/MS analysis.The identified compounds have listed in TABLE 1. The ethanolic extract contained 44 components, representing 92.34% of the total extract. The major components of the extract were methyl benzoate(15.78%), isopinochampone (10.3%), linalool(6.5%), methyl palmitate(6.5%),1,8-cineole(6.3%), pinocamphone(6.28%), and pinocarvone (5.4%). The composition of our extract was in agreement with data reported in literature[14-17]. In fact pinocamphone and isopinocamphone are generally the compounds characterizing the oils of *H.officinalis*[18]. The DPPH radi-

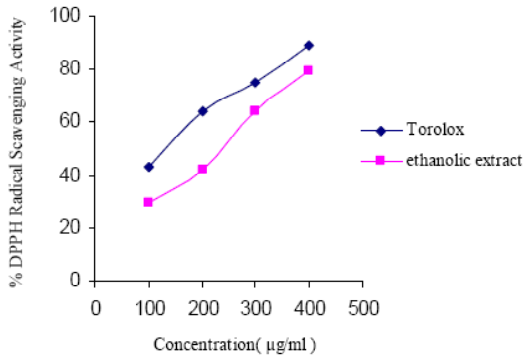
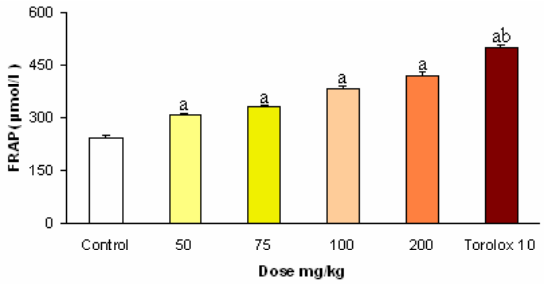
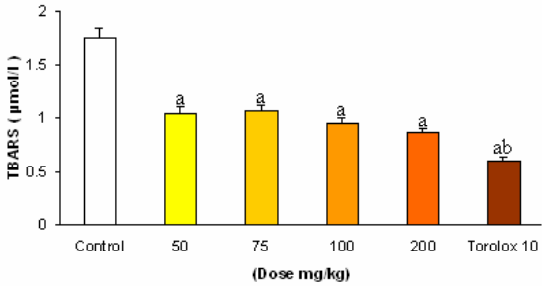


Figure 1 : DPPH radical-scavenging activities of ethanolic extract of *H.officinalis*



[a]Means that the difference between control and treated groups is significant at p<0.05. [b]Means that the difference between Torolox and treated groups is significant at p<0.05

Figure 2 : Effect of different doses of *H.officinalis* and Torolox on rat blood antioxidant power



[a]Means that the difference between control and treated groups is significant at p<0.05,[b]Means that the difference between Torolox and treated groups is significant at p<0.05.

Figure 3 : Effect of different doses of *H.officinalis* and Torolox on rat blood lipid peroxidation

TABLE 2: Minimum inhibitory concentrations (MICs) of ethanolic *H.officinalis* extract

Microorganism	MIC(μg/μl)
Bacillus cereus	1.562
Staphylococcus aureus	>50
Staphylococcus epidermidis	>50
Pseudomonas aeruginosa	3.125
Serratia marcescens	6.25
Escherichia coli	>50
Candida albicans	>50
Aspergillus niger	>50

cal-scavenging activity, the total antioxidant power and lipid peroxidation inhibiting power are shown in figures 1, 2, 3 respectively. Comparison of Torolox and different dose groups of *H.officinalis* ethanolic extract showed that all doses have less activity than Torolox but more activity than control group($p<0.05$).

Antibacterial and antifungal activities of *H.officinalis* ethanolic extract were examined on Gram-positive(*S.aureu, S.epidermidi, B.subtilis*) and Gram-negative (*E.coli, P.aeruginosa, S.marcescens*) and fungal strains (*C.albicans, A.niger*). Results of minimum inhibitory concentration(MIC) determination have shown in TABLE 2. The ethanolic extract of H.officinalis exhibited an inhibition in the growth of all examined microorganisms. The highest antibacterial activity was observed against Gram-positive bacteria such as *B.subtilis* with the MIC of 1.562μg/μl and a moderate inhibitory effects against Gram-negative such as *Pseudomonas aeruginosa* and *Serratia marcescens* with the MIC of 3.125 and 6.25μg/l, respectively. But it was inactive against the fungi, *C.albicans* and *A.niger*.

CONCLUSION

Most recent studies have shown that some of the widely used synthetic antioxidants such as butylated hydroxytoluene promote development of cancer cells in rat[19] and might have some undesirable effects in man too[20] then natural antioxidants have gained popularity day by day. Consumers think that the natural food ingredients are better and safer than synthetic ones. Many of these compounds, such as plant phenolics, often exhibit anticarcinogenic, anti HIV, etc.[21,22], therefore the addition of these compounds into food products may be helpful to consumers' health and also to the stabilization of food products. Due to the presence of some of these effective compounds in H.officinalis extract it might be used as a mild antioxidant and antimicrobial preservative which not only may prolong the shelf life of some food but also may contribute to the health benefit of consumers.

REFERENCES

[1] R.Piccaglia, L.Pace, F.Tammaro; J.Essent.Oil Res., **11**, 693 **(1999)**.
[2] L.Hornok; 'Cultivation and processing of medicinal plants', John Wiley & Sons, Chichester, UK, 196-211 **(1992)**.
[3] W.Kreis, M.H.Kaplan; Antiviral.Res., **14**, 323 **(1990)**.
[4] L.M.Bedoya, S.S.Palomino, M.J.Abad; Phyttherap. Res., **16**, 550 **(2002)**.
[5] M.D.Steinmetz, P.Tognettiand; Plantes.Mad.Phytoth, **14**, 34 **(1980)**.
[6] G.Renzini, F.Scazzocchio, M.Lu, G.Mazzanti, G. Salvatore; Oil.J.Essent Oil Res., **11**, 649 **(1999)**.
[7] G.Mazzanti, L.Battinelli, G.Salvatore; J.Flav.Fragr., **13**, 289 **(1998)**.
[8] L.Giamperi, D.Fraternale, D.Ricci; J.Essent.Oil.Res., **14**, 312 **(2002)**.
[9] M.S.Blois; Nature, **26**, 1999 **(1958)**.
[10] I.F.Benzie, J.J.Strain; Ann.Biochem., **239**, 70 **(1996)**.
[11] K.Satho; Clin.Chim.Acta, **90**, 37 **(1978)**.
[12] 'European Committee for Antimicrobial Susceptibility Testing(EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Clin Microb Infect', Eucast Definitive Document E. Def, **6**, 509 **(2000)**.
[13] M.Fazeli , Gh.Amin, M.Ahmadian Attari; Food Control, In press, **(2006)**.
[14] B.M.Lawrence; 'Chemical composition of Labiatae oils and their exploitation', Royal Botanic Gardens, 399-346 **(1992)**.
[15] E.T.Tsankova, A.N.Konaktchiev, E.M.Genova; J.Essent.Oil Res., **5**, 609 **(1993)**.
[16] S.N.Garg, A.A.Naqvi; Flav.Fragr J., **14**, 170 **(1999)**.
[17] M.Tognolini, E.Barocell; Life Sciences, **78**, 1419 **(2006)**.
[18] J.C.Chalchat, D.Adamovic, M.S.Gorunovic; J.Essent.oil Res., **13**, 419 **(2001)**.
[19] E.N.Frankel; Food Chemistry, **57**, 51 **(1996)**.
[20] N.Nakatani, F.Shahidi (Ed.); 'Antioxidants from spices and herbs. In Natural Antioxidants', Chemistry, health effects. Champaign, IL: AOCS Press, **(1997)**.
[21] J.E.Kinsella, E.Frankel; Food Technology, **47**, 85 **(1993)**.
[22] S.Gollapudis, H.A.Shara; Biochemical and Biophysical Res.Commun., **210**,145 **(1995)**.