Journal of Biologically Active Products from Nature

ISSN: 2231-1866 (Print) 2231-1874 (Online) Journal homepage: https://www.tandfonline.com/loi/tbap20

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To cite this article: Mohaddeseh Mahboubi , Ghasem Haghi & Nastaran Kazempour (2011) Antimicrobial Activity and Chemical Composition of *Hyssopus officinalis* L. Essential oil, Journal of Biologically Active Products from Nature, 1:2, 132-137, DOI: 10.1080/22311866.2011.10719080

To link to this article: https://doi.org/10.1080/22311866.2011.10719080

Published online: 24 Jun 2013.

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Journal of Biologically Active Products from Nature
ISSN Print: 2231-1866 Online: 2231-1874
www.jbappn.com

Antimicrobial Activity and Chemical Composition of *Hyssopus officinalis* L. Essential oil

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Received 13 January 2011; accepted in revised form 18 February 2011

Abstract: *Hyssopus officinalis* L. has been traditionally used for its antiseptic properties in treatment of infectious disorders. In order to confirm its antiseptic properties with respect to traditional uses, we have screened the antimicrobial activity of aerial parts of *H. officinalis* L. essential oil against different microorganisms. The oil was obtained using hydro distillation and analyzed by GC and GC-MS. Antimicrobial activity of *H. officinalis* L. essential oil was screened by disc diffusion and micro broth dilution assays. Analysis of essential oil revealed the presence of isopinocamphopinone (39.3 %) and isopinocamphone (22.1 %) as the major components. The results exhibited significant activity against Gram-positive bacteria with inhibition zone and minimal inhibitory concentration value in the range of 7-16 mm and 0.5-1 µl/ml, respectively. Moreover, the oil showed inhibitory effect against fungi. This work confirmed the traditional uses of *H. officinalis* as antimicrobial agent for treatment of infectious disorders. Further clinical trials are required to validate its uses as therapeutic, alternative agent for treatment of infections.

Key words: Antimicrobial activity; *Hyssopus officinalis*; Isopinocamphone; Isopinocamphopinone.

Introduction

H. officinalis L. is one of the most important plants from Labiatae family has known as culinary and medicinal herb for hundreds of years. The flowering aerial parts of *H. officinalis* have traditionally used for treatment of chronic coughs, laryngitis, bronchitis, and wound infections [5]. Some pharmacological effects of its oil such as antihelmintic, antituberculosis activity [4], antifungal activity [7], muscle relaxing [8] and its spasmolytic action [10] were confirmed. The ingredients of *H. officinalis* oil have shown a difference in its constituents depending on variety, growth stage, date of collection, and climatic conditions and have been some variation in constituents from different countries [1,3]. *H. officinalis* oil from Montenegro contains methyl eugenole (38.3 %)[3]; from Spain; 1, 8-cineole (52.9 %) [16]; from Turkey; pinocarvone (36.3 %), pinocamphone (19.6 %), β-pinene (10.6 %), 1,8-cineole (7.2 %), isopinocamphone (5.3 %) [15]. *H. officinalis* oil from France contains linalool (51.7 %) with greater antimicrobial activity than isopinocamphone type from Italy [11]. The *H. officinalis* oil from two different localities of Italy contain pinocamphone (34 and 18.5 %), isopino-

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camphone (3.2 and 29 %), and β-pinene (10.5 and 10.8 %) [1]. Antifungal activity of *H. officinalis* oil with major components of L-bornyl acetate, isopinocampheol and pinocamphone completely inhibited growth of plant pathogenic fungi [4]. The effect of several supercritical fluid extraction (SFE) parameters (pressure, temperature, modifier, dynamic and static extraction times) was investigated on chemical compositions of *H. officinalis* oil from north of Iran in the supercritical carbon dioxide extraction. Main components of these extracts under different SFE conditions were sabinene (4.2 - 17.1 %), *iso*-pinocamphene (0.9 - 16.5 %) and pinocamphene (0.7-13.6%) [6].

However, the flowering aerial part of this plant commonly is used for treatment of different kinds of infections [17], but there is no report about the antibacterial activity of *H. officinalis* oil. The aim of this study was to identify the chemical composition and determine the antimicrobial activity of Iranian *H. officinalis* oil against different microorganisms in order to evaluating its traditional uses.

Materials and method

Plant materials

Flowering aerial parts of *H. officinalis* were collected from Kashan area (Isfahan Province, center of Iran) in June 2008. The voucher specimen was identified by Dr Mozaffarian, Research Institute of Forest and Rangelands, Tehran, Iran, and deposited at the Herbarium of Agriculture Department, Medicinal Plants Research Center of Jundi Shapour, Kashan Iran, under number 152/1.

Extraction, isolation and identification of the oil

The dried flowering aerial parts of *H. officinalis* was hydrodistilled for 3 h using a Clevenger type apparatus and a yield of 1.1% (w/w) was obtained. The oil analysis was carried out using GC and GC-MS. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 40°C and held for 1 min then raised up to 230°C at a rate of 3°C/min held for 10 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. GC-MS analysis was conducted on a HP 6890 GC system coupled with a 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50, injector and oven temperature programmed was identical to GC.

The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, and NIST (National Institute of Standards and Technology).

Microorganisms

Staphylococcus aureus ATCC 25923, *Staphylococcus saprophyticus* ATCC 13518, *Bacillus cereus* ATCC 1247, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404 were used as tested microorganisms.

Antimicrobial susceptibility testing

Antimicrobial activity of oil was determined using disc diffusion and micro broth dilution assays. The bacteria inoculate was prepared by suspending the overnight colonies from nutrient agar media in normal saline. The *C. albicans* and *A. niger* inoculate were prepared by suspending colonies from 48 and 72 h sabouraud dextrose agar (SDA) cultures in buffered RPMI 1640 medium (Sigma-Aldrich chemie GmbH, Steinheim, Germany) with 0.165 M morpholine propane sulfonilic acid (Merck KGaA, Darmstadt, Germany). This inoculate was adjusted to 0.5 McFarland (1×10^7 - 1×10^8 CFU/ml). Suspensions were cultured individually on Muller Hinton Agar and SDA using a sterile cotton swab. Subsequently, filter discs (6 mm in diameter) (Padtan Teb Co, Tehran, Iran) were saturated with one and two μl of oil that dissolved in 10 μl dimethyl sulfoxide (DMSO). Antibiotic discs and disc containing DMSO were used as controls. The plates were incubated at 37°C for 24 and 48 h for bacteria and fungi respectively.

The inhibition zone diameters (IZ) were recorded in millimeters (mm) [12]. The minimal inhibitory concentrations (MICs) and minimal bactericidal

concentrations (MBCs) were determined by micro broth dilution assay.

The oil was two-fold serially diluted with 10 % DMSO that contains 8 - 0.0125 µl/ml of oil. Buffered RPMI 1640 and cation adjusted Muller Hinton broth were used as broth media for fungi [9] and bacteria [13], respectively. After shaking, 100 µl of oil were added to each well. The suspension of each organism were adjusted to 1×10^5 -1×10^6 CFU/ml and then 100 µl was added to each well and incubated at 35°C. MICs were defined as the first tube showing no growth and MBCs were the first tube that showing no growth on solid media.

Results

Chemical composition of essential oil

Analysis of essential oil by GC-MS revealed forty three compounds. The prominent components were isopinocamphopinone (39.3 %), isopinocamphone (22.1 %) as is shown in Table 1. Moreover, linalool, pinocarveol, limonene, 1,8-cineole and methyl eugenol were trace (<0.1 %) and bornyl acetate, camphor and isopinocampheol were not detected.

Table 1. Chemical composition of *Hyssopus officinalis* aerial part essential oil

Compounds	RI [a]	(%) [b]
2,3-Dimethyl-1,3-pentadiene	644	0.1
4-methyl-3-penten-2-one	761	0.5
α-pinene	906	0.2
sabinene	946	0.8
β-pinene	952	2.9
cymene	997	1.2
limonene	1000	0.2
1,8-Cineole	1004	0.2
trans-Sabinene hydrate	1041	0.9
cis-Linalool oxide	1044	0.3
trans-Linalool oxide	1060	0.2
linalool	1078	0.7
β-Thujone	1090	0.2
trans-Pinocarveol	1126	0.3
isopinocamphone	1143	22.1
isopinocamphopinone	1166	39.3
β-fenchyl alcohol	1169	0.5
myrtenal	1176	2.0
carvotanacetone	1221	0.4
(+)-(1R,2R)-2-Hydroxy-2,6,6-trimethylnorpinan-3-one	1233	5.4
4-methyl-3-pentenal	1246	0.4
thymol	1252	0.2
carvacrol	1258	0.5
p-cymene alpha-ol	1261	0.2
methyl p-anisate	1343	0.3
β-Bourbonene	1361	1.7
methyleugenol	1367	0.4
s- (+)-5-(1-Hydroxy-1-Methylethyl)-2-cyclohexan-1-one	1402	0.8
germacrene D	1405	0.2

table 1. (continued).

Compounds	RI [a]	(%)[b]
cis-Pinonsaeure	1433	1.8
aromaderene	1439	0.5
γ-cadinene	1486	0.2
croweacin	1489	0.2
elemol	1523	1.7
spathulenol	1559	2.8
caryophyllene oxide	1565	1.2
veridiflorol	1572	0.2
α-Selinene	1577	0.1
ledol	1584	0.2
γ-Eudesmol	1807	0.3
α-Cadinole	1814	0.3
β-Eudesmol	1829	0.9
Hexahydrofarnesyl acetone	1797	0.2

[a] Retention Index
[b] Relative percentage obtained from peak area

Antimicrobial activity of oil

The results of antimicrobial activity of oil against different microorganisms are summarized in Table 2. In disc diffusion assay, two µl of oil exhibited a significant effect against *S. aureus* with IZ = 20 mm. The antibacterial activity of two µl of oil was comparable with vancomycin. The MICs and MBCs of oil against Gram-positive bacteria were the same and equal 0.5 µl/ml for *S. aureus* and 1 µl/ml for *B. cereus* and *S. saprophyticus*. In particular, the oil exhibited significant activity against *S. aureus* followed by *B. cereus* and *S. saprophyticus*. For Gram negative bacteria (*E. coli*, *P. aeruginosa*), MIC and MBC was 4 µl/ml. The Gram-negative bacteria were less sensitive than Gram-positive bacteria. The oil exhibited bactericidal activity against bacteria. The MIC and MBC was 1 and 2 µl/ml for *C. albicans* and 0.5 and 8 µl/ml for *A. niger.* The MBC of *A. niger* was fourfold of the MIC value. The oil showed inhibitory effect against *A. niger*.

Discussion

Gram-negative bacteria appear to be less sensitive than Gram-positive bacteria; it may be associated with the outer membrane of Gram-negative bacteria that acts as strong permeability barrier [14]. This oil had inhibitory effect against fungi and showed that the amount of oil necessary to inhibit spore germination was higher than needed to inhibit hyphal growth.

Other study showed that *H. officinalis* oil on growing hyphae of *A. fumigatus* reduces the total lipid and sterols and increases total phospholipids of cell wall [2]. The results obtained from oil analysis exhibited significant difference with previous investigation that could be attributed to geographical origin. The main components of Iranian *H. officinalis* oil were isopinocamphone and isopinocamphopinone while isopino-camphopinone was not found in oils from different countries.

The prevalence of antibiotic resistant strains is increasing and identification of safe and effective alternative agents for these infections are very important. This survey proved the antimicrobial activity of this oil. *H. officinalis* oil is a potential source of novel antimicrobial agent because of bactericidal effect on bacteria. Some studies should be done in order to evaluating the potency of oil and main components on clinical isolates of bacteria especially on *S. aureus*.

Acknowledgement
The authors are indebted to Barij Essence pharmaceutical Company for the opportunity to explore the interesting science.

Table 2. Antimicrobial activity of *Hyssopus officinalis* L. aerial part essential oil

Microorganisms	Inhibition Zone Diameters (mm)			Minimal concentration values	
	Essentialoil(µl/disc)		Antibiotics	Essential oil	
	1	2		MIC (µl/ml)	MBC (µl/ml)
Staphylococcus aureus	10	20	17[a]	0.5	0.5
Staphylococcus saprophyticus	7	16	18[b]	1	1
Bacillus cereus	9	19	26[b]	1	1
Escherichia coli	-	8	20[c]	4	4
Pseudomonas aeruginosa	-	-	23[c]	4	4
Aspergillus niger	-	-	9[d]	0.5	8
Candida albicans	-	10	17[d]	1	2

[a] Vancomycin (30 µg/disc), [b] Erythromycin (15 µg/disc),
[C] Gentamycin (10 µg/disc), [d] Amphotricin B (100U/disc)

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