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Essential Oil Composition and Antibacterial Activity of *Hyssopus Officinalis* L. Grown in Iran.

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ABSTRACT

This study was conducted for the first time on plant pathogens and was carried out to the determine antibacterial activities and chemical compositions of the essential oil of aerial part of Hyssopus officinalis obtained from the hydro – distillation. The chemical composition of essential oil was determined using gas chromatography/mass spectrometry (GC/ MS). Antibacterial activity of the essential oil was evaluated the micro broth dilution and disc diffusion methods. The oil showed high antibacterial activity against Erwinia amylovora and Klebsiella sp. But on the other bacteria tested is ineffective. Thirty nine components were identified in H. officinalis oil that thymol (18.95%), ß-bisabolol (10.62%), carvacrol (7.73%), n–Dodecan (5.23%), caryophyllene (4.96%), ortho – acetanisol (4.72%), camphor (3.47%), cumin aldehyde (3.22%) and spathulenol (3.02 %) as major components in essential oil.

Key words: *Essential oil, GC/MS, Erwinia amylovora, Klebsiella sp.*

INTRODUCTION

Hyssop (*Hyssopus officinalis*), family Lamiaceae is an important perennial culinary and medicinal plant. It is native to the Caucasus, North western Iran, North Eastern Turkish Black sea region, and southern Anatolia [1]. The herb is an evergreen perennial plant with small, linear leaves and purplish-blue flowers [2]. It has a very strong spicy taste and intensive flavor. It is commonly used in folk medicine. *H. officinalis* extracts and oil may be found as flavor ingredient in many food products, mainly sauces and seasonings, also in bitters and liqueurs. The oil is a fragrance component in soaps, cosmetics and perfumes, especially eau-decologne and oriental bases [3]. The plant is atypical xerophyte and is well adapted to drought and low input conditions [4]. As a medicinal herb, hyssop is used in viral infections such as colds, coughs, sore throats, bronchitis and asthma. A tea made from the herb is effective in nervous disorders and toothache [5]. The flowering tops of hyssop produce a pleasant volatile oil responsible for most of biological activities of the plant. 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 food, and variety of house hold products. In addition to their particular aroma, many essential oils and their isolated components also exhibit muscle relaxant, antibacterial and antifungal activities [6].

The present study is aimed at assessing the antibacterial activities of *H. officinalis* which naturally grow in Southwestern in Iran.

MATERIALS AND METHODS

Plant material

Hyssopus officinalis was collected in March 2012 at full flowering stage from the Sepidan Mountains in Southwestern Iran. Then the plant material was dried in the shade for 15 days.

Essential oil extraction

The air- dried parts of *H. officinalis* were subjected to hydro-distillation using a Clevenger-type apparatus (w/w) for 3 h. Then the oils drying by anhydrous sodium sulfate. The isolated oils were stored in tightly closed vials at 4°c until analysis.

Essential oil analysis

Essential oil was analyzed by Hewlett – Packard GC/MS (model 6890 series II) operating at 70e V ionization energy, equipped with a HP–5 capillary column phenyl methyl siloxane (30m' 0.25mm, 0.25 µm film thickness) with helium as the carrier gas and a split ratio of 1:20. The retention indices for all the components were determined according to the Van Den Dool method [7] using n–alkanes as standard. The compounds were identified by comparison of retention indices (RRI–HP–5) with those reported in the literature and by comparison of their mass spectra with the Wiley and Mass finder 3 libraries or with the published mass spectra [8].

Micro –organisms

Human pathogens used in the study obtained from Persian Type Culture Collection (PTCC) in Iranian Research Organization for Science and Technology and plant pathogens were isolated from samples of plant disease and identified by Faculty of Agriculture, Islamic Azad university, Shiraz, Iran and then were used in this experiment. (Table 2). *Pseudomonas fluorescens*, *Xanthomonas axonopodis* pv *citri*, *Erwinia amylovora*, *Acidovorax* sp., *Streptomyces scabies*, *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella* sp. Were used in the study.

Antibacterial activity

In vitro antibacterial activity of the essential oil of *H. officinalis* was evaluated by micro broth dilution and disc diffusion method with determine of minimum inhibitory concentration, minimum bactericidal concentration and inhibition zones. The tested bacteria were cultured $^{CFU}/_{ml}$ ient agar medium for period of time required. Then the suspension was prepared with a concentration of 10^8 bacteria, purified on NA medium.

Determination of minimum inhibition concentration (MIC)

Initially is removed 100µl of Nutrient broth, and were dumped in to small Welles of the first six rows of micro plate. Then essential oil was added to the first small well of each row. After mixing the contents of the first small wells, 100 µl was removed and added to the next small well and so. A serial dilution twice of essential oil was prepared to twelfth small wells and was discarded 100 µl of twelve of the small wells. For test of each row were considered a row control and essential oil was not added to control lines. Then was added 100µl of bacteria suspension to the test small wells of all rows [9].

Determination of minimum bactericidal concentration (MBC)

To the determination the MBC, of all small wells without turbidity were cultured in NA medium. Then the medium at the proper temperature for each bacterium was placed inside the incubator. After the time required for bacterial growth. The lowest concentration of essential oil that 99/9% of bacteria have not growth, were considered as bactericidal concentration [9].

Determination of antibacterial activity by the disc diffusion method

The agar disc diffusion method was employed for the determination of inhibition zone for MIC and MBC. The suspension was prepared with a concentration of 10^8 cfu/ml bacteria. Then paper disc with a diameter of 5mm were prepared and was inoculated with values of MIC and MBC. Bacterial suspension was culture to slimy layer method by a sterile cotton swab on NA mediums. The sterile paper discs (6 mm in diameter) were individually impregnated with 25 µl to 100 µl of the oil and then placed on the agar plates which had previously been inoculated with the tested microorganisms and after the time of required for bacterial growth, inhibition zone for values of MIC and MBC were recorded with a special ruler. At least experiments were repeated for four times separate[10].

Statistical analysis

All data were done in four replicate and analyzed by analysis of variance (ANOVA) and mean values were compared with dun cans multiple range test using SPSS software.

RESULT AND DISCUSSION

Essential oil components

The chemical composition of the essential oil of *H. officinalis* and the retention indices are presented in Table 1. Thirty – nine components were identified in the *H. officinalis* essential oil representing 99.82% of the total weight. The major components were thymol (18.95%), β- bisabolol (16.62%), carvacrol (7.73%%) , n-Dodecane (5.23%), caryophyllen (4.96%) , ortho - acetanisol (4.72%), camphor (3.47%), cumin aldehyde (3.22%) and spathulenol

(3.02%). These nine components constitute 67.92% of total oil. The composition of the essential oil of *H. officinalis* has been examined previously by other researchers. Kizil *et al*., [11] reported, Isopinocamphone (57.27%) was the main component of *H. officinalis* essential oil. cis-pinocamphone (44.77%) by Wesolowska *et al*., [12], isopinocamphone (43.29%) by Jasmina *et al*. [13], 1,8-cineole (53%) by Vallejo *et al*., [14], Pinocamphone (49.1%) by Garg *et al*., [15], Pinocarvone (36.3%) by Ozer *et al.,* [16]. These differences in the essential oil compositions can be attributed to several environmental factors such as climatic, seasonal and geographical or ontogenesis variations [17-18].

Table 1. Essential oil components of *Hyssopus officinalis* L. analysis by (GC/MS)

Percentage in oil	RI[a]	compound	No
0.34	932	? -pinene	1
11.76	999	n-Decane	2
0.92	1009	? -3-Carene	3
0.32	1022	p-Cymene	4
3.03	1026	Limonene	5
1.18	1029	1,8-Cineole	6
0.26	1040	Benzene acetaldehyde	7
0.28	1055	? -Terpinene	8
0.95	1098	Linalool	9
3.47	1142	Camphor	10
1.04	1179	Naphthalene	11
1.04	1188	? -Terpineol	12
5.23	1198	n-Dodecane	13
3.22	1237	Cumin aldehyde	14
1.63	1241	Carvone	15
1.31	1252	Piperitone	16
4.72	1283	ortho-Acetanisole	17
18.95	1291	Thymol	18
7.73	1299	Carvacrol	19
1.08	1338	Piperitenone	20
1.61	1354	Eugenol	21
0.40	1382	? -Bourbonene	22
1.56	1397	n-Tetradecane	23
1.59	1416	(E)-Caryophyllene	24
0.37	1450	? -Humulene	25
0.22	1454	(E)- ? -Farnesene	26
1.31	1478	Germacrene D	27
0.67	1483	trans-? -Ionone	28
0.57	1493	Bicyclogermacrene	29
0.32	1500	cis- ? -Bisabolene	30
0.39	1505	? -Bisabolene	31
0.90	1518	Myristicin	32
0.37	1561	(E)-Nerolidol	33
3.02	1574	Spathulenol	34
4.96	1580	Caryophyllene oxide	35
0.41	1650	Muurolol<epi-? ->	36
0.56	1662	ar-Tumerone	37
10.62	1676	? -Bisabolol	38
1.51	1839	6,10,14-trimethyl,2-pentadecanone	39
99.82			Total

[a]**RI, retention indices in elution order from HP – 5 column.**

Antibacterial activity

The MIC and MBC values of the essential oil against all microorganisms tested are reported in table 2. The results show that essential oils of *H. officinalis* possessed antibacterial activity against *Escherchia coli* and *Klebsiella* sp.because it shown the growth a zone diameter of inhibition on susceptible of the tested organism. that could be due to the present of compounds such as thymol and carvacrol. Because these compound due to the ability to penetrate cell membrane and rapid exit inter – cellular compounds have antibacterial properties. In general about mode of action of essential oil on the death of bacteria it has been suggested one of the important characteristics of these materials are hydrophobic nature that the resulting distribution in lipid of cell wall and mitochondria of bacteria and it is causing change and demolition of structure and permeability. That Which ultimately leads to the death of bacteria. But this Essential oil had no effect on the other tested bacteria. That probably due to the different sensitivity of bacteria to *H. officinalis* essential oil or due to the presence of resistance compounds in these bacteria to the plant oils [22].

Table 2. Antibacterial activity of *Hyssopus officinalis* Essential oil.

MBC [b]	MIC [a]	Bacteria	No
100µl	100µl	*Staphylococcus aureus*	1
50 µl	50 µl	*Escherichia coli*	2
100µl	100µl	*Klebsiella* sp.	3
25µl	25µl	*Bacillus subtilis*	4
50 µl	50 µl	*Xanthomonas axonopodis* pv *citri*	5
100µl	100µl	*Acidovorax* sp.	6
25µl	25µl	*Erwinia amylovora*	7
50 µl	50 µl	*Streptomyces scabies*	8
100µl	100µl	*Pseudomonas fluorescence*	9

a : Minimum inhibition concentration

b : Minimum bactericidal concentration

In the study, among bacteria the most susceptible is *Erwinia amylovora* and *Klebsiella* sp. However *H. officinalis* essential oil was not affected on other tested bacteria. (Table 3)

Table 3. Inhibition zone diameter for MIC and MBC

IZO for MBC	IZO for MIC	Bacteria
0.00 ± 0.00c	0.00 ± 0.00c	*Staphylococcus aureus*
0.00 ± 0.00c	0.00 ± 0.00c	*Escherichia coli*
6.00 ± 0.00b	6.00 ± 0.00b	*Klebsiella* sp
± 0.00d	0.00 ± 00.0c	*Bacillus subtilis*
0.00 ± 0.00c	0.00 ± 0.00c	*Xanthomonas axonopodis pv citri*
0.00 ± 0.00c	0.00 ± 0.00c	*Acidovorax* sp.
7.75 ± 0. 47 a	7.75 ± 0. 47 a	*Erwinia amylovora*
± 0.00d	0.00 ± 00.0c	*Streptomyces scabies*
± 0.00d	0.00 ± 00.0c	*Pseudomonas florescence*

In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test.

Some researchers reported that there is relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity.

Essential oils rich in phenolic compound such as Cumin aldehyde and Linalool are widely reported to possess high levels of antimicrobial activity [19,20].The variation on antibacterial activities of tested spice extracts maybe due to their essential oil content, such as linalool, carvone and thymol. It appears that there is relationship between the chemical structures of the most abundant compounds in the tested essential oil and the antibacterial activity [21-23]. Further research is needed in order to obtain information regarding the practical effectiveness of essential oils to protect the plants and human. But present study gives support for the application of certain essential oils to control plant and food pathogens such as *Erwinia amylovora* and *Klebsiella* sp..

CONCLUSION

In conclusion, present results suggested that the *H. officinalis* essential oil might be a source of antibacterial activity against plant and food borne pathogens. This study confirms that the essential oil of *H. officinalis* possesses high antimicrobial activities *in vitro* against *Klebsiella sp.* and *Erwinia amylovora* two important plant pathogens than the other and can be used in treatment of deseases of plant.

Present results suggest that the use of some spice and aromatic plants essential oils as antimicrobial agents maybe exploitable to prevent the deterioration of seeds by bacteria.

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