STUDY OF THE ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL EFFECT OF ESSENTIAL OIL AT DIFFERENT GROWTH STAGES OF PLANT HAUSSKNECHTIA ELYMAITICA BOISS AS ENDEMIC SPICES OF IRAN.

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Abstract. The vegetative (stem and leaves) and generative (flowers and fruits) parts of H. elymaitica (Apiaceae), which is endemic of Iran, were collected during the growth stages from Takhtan Mountain area around Dehloran (Ilam province) and The essential oil was obtained by using hydrodistillation method. The antioxidant potential of the samples was evaluated using free radical scavenging method 2, 2-diphenyl-1picrylhydrazyl (DPPH) method in comparison with Ascorbic acid. The results showed that inhibition percentage of different parts of essential oil increased with increasing concentration similar to ascorbic acid also there was a considerable difference between antioxidant activities in 200 and 400 p.p.m concentrations their considerable difference is appeared.

Keywords: *Haussknechtia elymaitica*, antioxidant activity, antibacterial activity, essential oil

1 Introduction

Use of medicinal compounds with plan origin has been always considering by human. Medicines, which are largely used today to treat different types of diseases such as bacterial, viral, and fungal infections as well as different types of metabolic diseases and even cancer, have natural origin (Tchakam, et al., 2012). Free radicals are molecules with a single electron shell and are generated in organisms' body naturally, but when generation of these radicals is more than the normal level then substrates such as cell membranes, proteins and nucleic acids will be exposure to oxidation. In this case, degradation of macromolecules such as DNA and protein degradation occurs. Antioxidant compounds can neutralize free radicals and protect the body (Oke et al., 2009). According to the warnings of World Health Organization about indiscriminate use of antibiotics and serious concerns in relation with increased resistance against pathogenic microorganisms, many studies have been started in field of antibacterial materials affecting these resistant microorganisms (WHO 2011, Bhullar et al., 2012).

Antioxidant and antibacterial activity of plant essential oil has been studying for several years but the highest concentration on this issue has occurred during past 30 years. In this period, plants with traditional use have been studied specially in China and USA. Whereas, reports related to endemic plants in Iran have few (Suffredini, 2006).

Umbelliferae (Apiaceae) family is one of the largest plant families with about 300 genera and 3000 aromatic herbal species. There are various medicinal species among plants of Umbelliferae and many of these plants are used in traditional medicine; also, leaves and fruit of these plants are used as food (Zargari, 1980). *Haussknechtia* or white celery, which has perennial specie in Iran called *H.* called *H. elymaitica,* mostly grows in mountain and oak forests of Zagros west of Iran. This endemic plant grows in Iran not seen in any other places in world (Mozaffarian, 1994). This unique specie is a perennial plant with alternate leaves, numerous cuts and divisions ending to sheathed petioles that covers the stem in the junction place. This plant has an erect, smooth and glabrous and mostly has one-three stems beside each other in one base of plant and these stems might be with or without branches with height of 3 meter. White celery grows in west of Iran in some provinces and local people use the leaves and different parts of this plant as food spice traditionally (Mozaffarian, 1996, Mozaffarian, 1987). This plant is named as a medicine for diabetes, blood pressure, anti-stress and anti-tension in many of scientific texts (Ghorbani, 2013). Three important medicinal compounds are discovered from photochemical examinations that these compounds are two Phenylpropanoids called Trans- methyl eugenol and Trans-Asaron as well as a derivative of benzoic acid called Asarunic acid. Habibi et al., (2012) studied chemical compositions of aerial part of this plant using Column chromatography and GC/MS methods, isolated, and identified 12 different parts of the essential oil of this plant such as α – zingiberene, β bisabolene, sesquiphellandrene, and alpha-phellandrene (Mohammadi et al., 2012). Other studies were conducted on the essential oil of H. *elymaitica* fruit and 10 types of chemical materials were isolated and purified that amount of the β bisabolene was more than Trans Asaron and lavandulyl acetate (Mehregan and Ghannadi, 2013). According to the presence of various photochemical materials in H. *elymaitica* plant, there was not any reports in scientific references about the evaluation of antioxidant and antibacterial activities in this plant. Considering the mentioned properties, the purpose of this study is to examine antioxidant and antibacterial potential and activity of essential oil in vegetative (leaf and stem) and generative (flower and fruit) parts.

2 Materials and Methods

2.1 Plant collection and preparation

During spring and summer 2015, we went to growth location of examined plant in mountain area of Takhtan in Dehloran (Ilam Province), Iran and removed vegetative and generative organs of plant. We dried and mixed these organs at the environment temperature in the shade and placed them in closed containers far from direct sunlight until the essence extraction.

2.2 Producing essential oil

The essential oil of mixed and dried herbal materials of *H. elymaitica* was extracted using hydro distillation method and Clevenger apparatus for 4 hours, material was put into a dark closed container at the 4°C temperature until the use time after filtering and dewatering using Sodium Sulfur.

2.3 Measuring antioxidant properties using DPPH method

In this test, the ability to free hydrogen atom or electron by different essential oils or compositions is measured through discoloring de-purple solution 2-diphenyl-1picrylhydrazyl in methanol. In this method, DPPH material (Sigma-Aldrich) as an indicator for stable radical combination. In this regard, 50 microliter of different concentrations of essential oil of control sample and Acid Ascorbic added to 5 ml 0/044% solution of DPPH in methanol. After 30 minutes remaining in oven at room temperature, the optical absorbance of samples was red at 517-nanometer wavelength against blank disc. Percentage of scavenging of free radicals in DPPG was calculated using the following formula (Burits and Bucar, 2000):

$$I\% = (Ablank- Asample / Ablank) \times 100$$

Where, A_{blank} indicates optical absorbance of negative control without essential oil and A_{sample} indicates optical absorbance of different concentrations of essential oil.

2.4 Antibacterial activity

Bacterial strains:

The bacteria used in this study were given by Biotechnology Research Center in University of Baqiyatallah. General list of these bacteria with their symbol codes are described in table 1.

Table 1. Standard bacteria used in antibacterial effect test

Bacteria name	Symbol	Properties
Staphylococcus aureus	ATCC 25923	Gram-positive cocci
Staphylococcus epidermidis	ATCC 14990	Gram-positive cocci
Pseudomonas aeruginosa	85237 ATCC	Gram-negative bacilli
Escherichia coli	ATCC 25922	Gram-negative bacilli

Test method of Antibacterial effect was chosen based on Disc Diffusion Method. To prepare bacteria suspension, one-night culture of microorganism with concentration equal to 0.5 McFarland standard was used. Then, bacteria sample was cultured on the Mueller-Hinton agar. To examine antibacterial effects, 5 concentrations including 70mg/ml, 80mg/ml, 90mg/ml, 100mg/ml, and 110mg/ml prepared from vegetative (leaves and stem) and generative (flower and fruit) parts in 5% DMSO solvent (Dimethylsulfoxide) and then sterilized paper discs were added to plate contained bacteria and each of them was inoculated with 15µl of mentioned dilutions. All of examinations were conducted as three parallel replications. Gentamicin was used to compare antibacterial effects of essential oil and Dimethylsulfoxide (DMSO) was used as negative control. At the end, diameter of inhibition zone was measured after plates heating for 24 hours at 37°C temperature and 3 repetitions were determined averagely (Elgayyar et al., 2001).

To determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the examined essential oils, serial dilution method was used. To determine MIC, serial dilutions 70, 80, 90, 100, 100, 120, and 200mg/ml were prepared in NB environment (Wiegand et al., 2008). After preparing serial dilutions, 20µl of 0.5 McFarland bacteria suspension was added to positive control (positive environment of essential oil). Finally, the inoculated serials were incubated for 24 hours at 37°C temperature. The lowest dilution of essential oil without any impurity considered as MIC. To determine MBC, some sample of serials without any growth was cultured on Mueller-Hilton agar medium. The inoculated mediums were placed at 37°C temperature for 24 hours. After incubation, the lowest concentration of essential oil without any growth considered as MBC. Nutrient Broth medium with bacteria-less essential oil and medium without bacteria-containing essential oil were used as positive and negative controls, respectively.

3 Results

3.1 Examining antioxidant properties using DPPH method

In figure 1, scavenging level of DPPH radical in concentrations 25, 50, 75, 100, 200, and 400-m.p.p essential oil and Acid Ascorbic are presented. Results indicate that inhibitory percentage of essential oil in vegetative and generative parts has been increased with increasing concentration amount and this effect has been more on generative organs (flower and fruit) than leaves and stem; also, in low concentrations, inhibitory effect of generative part is more than Acid Ascorbic and the difference is more significant in concentrations 200-400 m.p.p of essential oil in flower and fruit compared to low concentrations. The most percent of free radicals scavenging was recorded to 80/75% in concentration of 400 mg/ml. Results obtained from free radical scavenging by different concentrations are indicated in table 2.

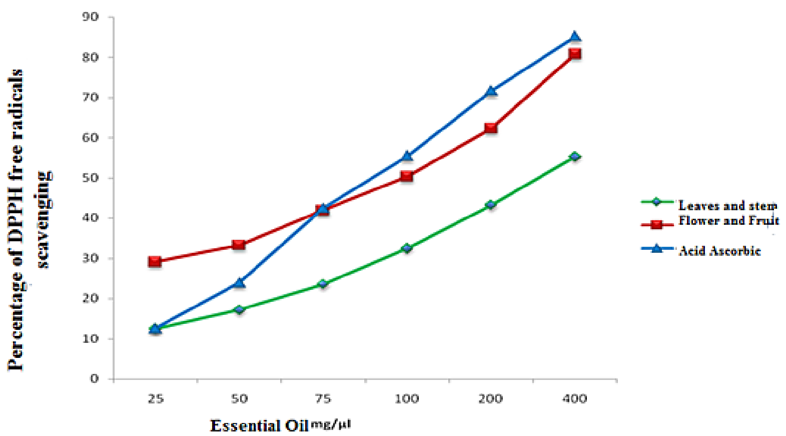

Figure 1. Comparing DPPH radical scavenging by different concentrations of essential oil in vegetative and generative parts with Acid Ascorbic antioxidant in 517nm

Table 2. Comparison of DPPH radical scavenging by different concentrations of essential oil in vegetative and generative parts with Acid Ascorbic antioxidant in 517nm

Examined part of plant	Essential oil concentrations					
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml
percentage of free radical scavenging in generative part (flower and fruit)	29.12	33.25	41.88	50.33	62.21	80.75
percentage of free radical scavenging in vegetative part (leaves and stem)	12.45	17.12	23.52	32.45	43.23	55.24

percentage of free radical scavenging of Acid Ascorbic	12.56	23.88	42.35	55.42	71.59	85.12

Diameter of inhibition zones in disk diffusion method (table 3) for essential oil of generative part of *Haussknechtia elymaitica* indicated that Staphylococcus epidermidis was affected in 110mg/ml concentration with average diameter of inhibition zone equal to 25/32±2. In similar conditions, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa indicated average diameter of inhibition zone to 23/90±1, 18/33±2, and 16/42±1, respectively (table 3).

As can be seen in table 3, the most effect of essential oil of different parts of H. *elymaitica* was on Staphylococcus epidermidis and the lowest effect was on Pseudomonas aeruginosa.

Table 3. Mean± Standard deviation of diameter of inhibition zone (mm) obtained from different concentrations of essential oil in vegetative (leaves and stem) and generative (flower and fruit) parts for studied bacteria

Bacteria type	Examined part	Essential oil concentration					Gentamicin 10mg/ml
		70 mg/ml	80 mg/ml	90 mg/ml	100 mg/ml	100 mg/ml	
Staphylococcus aureus	A	17.82±1	19.32±2	20.10±1	21.72±3	23.90±1	25.90
	B	14.24±2	16.34±1	17.18±1	18.92±1	20.10±2	
Staphylococcus epidermidis	A	19.18±1	20.87±2	21.72±2	23.36±1	25.32±2	27.84
	B	17.10±1	18.73±2	19.54±2	20.18±1	22.75±1	
Pseudomonas aeruginosa	A	10.31±2	11.52±1	13.00±1	14.38±2	16.42±1	20.54
	B	8.52±1	10.83±2	12.38±1	13.82±2	15.22±1	
Escherichia coli	A	12.90±2	14.00±1	15.10±1	16.16±1	18.33±2	22.62
	B	10.20±1	11.84±1	13.10±1	14.25±2	16.14±1	

A: Essential oil of generative part (flower and fruit)
B: Essential oil of vegetative part (leaves and stem)

Also, results obtained from diameter of inhibition zone show that growth inhibitory effect of examined parts on positive-gram bacteria has been more than this effect on negative-gram bacteria. Also, with increase in concentration of essential oil the diameter of inhibition zone will increase.

Results obtained from MIC and MBC indicated that Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of essential oil of flower and fruit is more that essential oil of leaves and stem. The MIC equal to 70mg/ml was observed in essential oil of generative parts of Staphylococcus epidermidis bacteria that this concentration was equal to 90mg/ml in essential oil of vegetative parts. Results related to MIC and MBC (table 4) indicate that Pseudomonas aeruginosa has more resistance against the essential oils extracted from H. *elymaitica* plant compared to other studied strains; MIC and MBC of bacteria in essential oil of flower and fruit indicated concentrations 100 and 120mg/ml, respectively. In essential oil of leaves and stem, concentration for MIC was equal to 120mg/ml and for MBC to 200mg/ml.

Table 4. Results of MIC and MBC of essential oil in vegetative (leaves and stem) and generative (flower and fruit) parts for studied bacteria

Essential oil	Bacteria	MIC µg/mm	MBC µg/mm
generative part (flower and fruit)	Staphylococcus aureus	80	100
	Staphylococcus epidermidis	70	100
	Pseudomonas aeruginosa	100	120
	Escherichia coli	90	110
vegetative part (leaves and stem)	Staphylococcus aureus	100	120
	Staphylococcus epidermidis	90	110
	Pseudomonas aeruginosa	120	200
	Escherichia coli	100	120

4 Discussion

Results obtained from this research indicated that essential oil of both vegetative (leaves and stem) and generative (flower and fruit) contains compositions with antibacterial and antioxidant properties but these properties are different in these parts. Since there has not been any reports of antioxidant or antibacterial effects of essential oil of plant *H. elymaitica*, the results obtained from other genera of this family (Umbelliferae (Apiaceae)) used in this research. Examining of diameter of inhibition zone, it was found that essential oil of flower and fruit parts had more inhibitory effect on growth of tested microorganisms although inhibition zone was observed in growth of all used microorganisms; however, some bacteria such as Staphylococcus epidermidis and Staphylococcus aureus have more sensitivity toward herbal essential oil compared to negative-gram bacteria and negative-gram bacteria of Pseudomonas aeruginosa is more resistant. These results are matched with results obtained from study conducted by Pas et al., (2012) on antibacterial effect and antioxidant activity of essential oil of *Echinophora cinerea* Boiss plant from Umbelliferae family. In the mentioned study, Staphylococcus aureus was reported as sensitive bacteria and Pseudomonas aeruginosa as resistant bacteria. Essential oil of Echinophora cinerea Boiss indicated a considerable effect on Staphylococcus aureus and had inhibitory effect in dilution of MIC=0.16mg/ml and bactericidal effect in dilution of MBC=0.63 and these values were equal to MIC=87mg/ml and MBC=175mg/ml for resistant Pseudomonas aeruginosa (Pas et al., 2012). In other studies, antibacterial effect of *Dorema. ammoniacum* specie from Umbelliferae family, which are similar to *H. elymaitica* specie in terms of flower and fruit, on bacteria epidermidis, S. aureus., and E.coli was equal to 22, 17, and 14mm, respectively (Yousefzadi et al., 2011). This similar effect on bacteria might be because of common chemical compositions such as α-phellandrene and beta-bisabolene that effect of their antioxidant activity has been studied (Yousefzadi et al., 2011). Study on essential oil of genera of Ferula specie (Iranian Ferula and desert Ferula) indicated the most sensitivity among positive-gram bacteria Bacillus subtilis and Pseudomonas epidermis and negative-gram bacteria *E.coli* and lowest effect on Pseudomonas aeruginosa.

Results obtained from MIC, MBC were matched with findings of disc diffusion method, and it was found that essential oil of different parts had antibacterial effect on majority of bacteria. A study was conducted on antibacterial activity of *Cuminum cyminum* and *Alcea Digitata L.* and the obtained findings from MIC and MBC indicated the most sensitivity of bacteria *S. aureus;* negative-gram bacteria of Escherichia coli and Salmonella typhimurium were the most resistant bacteria (Zareii, et al., 2014). The difference between inhibitory levels of essential oils in different bacteria can be attributed to different mechanisms of microorganisms against factors limiting growth. The reason for sensitivity of positive-gram bacteria toward oil essentials might be raised from monolayer cell wall, while negative-gram bacteria have multilayer cell wall. In other words, negative-gram bacteria have an outer membrane and a periplasmic space, whereas positive-gram bacteria have none of them. Outer membrane of negative-gram bacteria is recognized as a barrier for penetration of antibiotic molecules. On the other hand, this membrane prevent from penetration of hydrophyte into the bacteria. Periplasmic space contains some enzymes that can decompose the external molecules entered from outer space.

Conducted examinations indicate that essential oil of flower and fruit has a better antibacterial and this point implies the presence of more and better antibacterial compositions in these parts. The mention point is matched with the results obtained from the study conducted by Mehregan and Ghanadi (2013) on chemical compositions of *H. elymaitica* plant. Conducted studies on white celery show that chemical compositions obtained from different parts of this plant has a good potential to treat some diseases such as epilepsy, hypertension (lowering cholesterol), diabetes, gallstones, ulcers and stress (Habibi et al., 2013). Also. These materials can be used in chocolate industry, the production of pesticides and cosmetics. However, pharmacological effects of scavenging of free radicals DPPH in essential oil of different parts of *Haussknechtia elymaitica* Boiss plant was determined and it was seen that inhibition percentage of essential oil of different vegetative and generative parts increases with an increase in concentration. However, radicals scavenging activity is one of distinguished mechanisms during activity and creation of synergistic effects. This activity in essential oils of flower and fruit parts was more than leaves and stem and this difference might be related to more percent of α-phellandrene and lavandulyl acetate compositions and Trans Arason in these parts that their antioxidant effects have been studied and approved in different plants (Dehghani, 2013).

5 Conclusion

Results of this study indicated that different parts of Haussknechtia elymaitica Boiss contains compositions with antioxidant and antibacterial properties and these effects in generative step (production of flower and fruit) is more than vegetative step. Therefore, in accordance with created pharmaceutical resistance and results obtained from study, it is recommended to isolate active pharmaceutical ingredients of studied plant and use them as a suitable and low-cost substituent for antibiotic and antioxidant medicines.

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