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Investigating the Effect of Height on Essential Oils of *Urtica diocia* L. (Case Study: Ramsar, Mazandaran, Iran)

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ABSTRACT

Urtica Diocia L. from the Urticaceae family is a plant of herbal value and of a noticeable distribution in the north of Iran. The growth of different plants in various natural environments and ecosystems seems to be affected by factors such as the height (from sea surface). To investigate the effect of height on *Urtica Diocia* L. medicine compounds in its natural environment, three areas with the height of zero, 800 and 1800m were selected. The samples were randomly gathered three times and were dried; also, their compounds was extracted using the Clivenger with the water-distilling method. To determine the medicine compounds, the GC-MS as well as the GC machines were used. The analysis of variance was done in the form of the random-full-block design. The results indicated that there was a significant difference between the percent of EOs in the selected heights; however, such difference was not significant within each height. From among the eight flavors of the study, the Phytol compound was more in terms of percentage. By increasing the height the percent of EOs would decrease. lower heights could be considered most appropriate for producing the studied effective materials despite of the moistened climate and soil there.

Key words: *Urtica Diocia* L, Height, EOs.

INTRODUCTION

Iran in terms of climate and location in growing herbs is a one of the best parts of the world. Mazandaran province in north of Iran is one of the prone areas of the country with suitable habitats and varied for different types of drug eruption such as nettle. This is one of the most abundant weed species in there. While in Western Europe was imported items, add to it has also many domestic uses (Baradaran, 2009). over 50 species is growing in the wild (Janke and DeArmond, J., 2004). Urticaceae family generally consists of grass plants, this plants are multi-year with height 10-8 cm and most shoot of the air is covered with hairs like hool and cone-shaped (Zargari,1996). Among the different varieties of nettle *Urtica dioica* L. and *U. urens* L. As a medicinal plant has been important from very long time (Kavalali et al, 2003).

Pharmaceutical compounds are the important products in plants that have very broad

and extensive application in various industries such as pharmaceutical industry, Food and cosmetics. Varieties used in this study is *Urtica dioica* L. Extracts of this plant is a good substitute for chemical additives in controlling plant fungal diseases. Fungi on nettle extract has lower growth (Hadizadeh et al, 2009). EOs[1] of *Urtica dioica* L. regulate blood fat disorders and improve chemical structures in the liver of animals (Alisic et al, 2008). Pay attention this subject and also growing investment on their and their production resources, research in the field of EOs of medicinal plants is very important.

In agricultural and natural ecosystems factors such as humidity, water, nutrients, light, height above sea level are the main factors determining the quality and quantity of plants (Kocjaki and Hosseini, 1995; Lebaschi et al, 2003; Donhue, 2000 .(.. It should be noted that the agricultural product from a pharmaceutical plant when it is cost effective in terms of economic that its the amount of primary and secondary metabolites is reached to the optimal level(OmidBeigi, 2000; Zobayed et al, 2005) So by choosing appropriate plant varieties and environmental factors can be achieved to the maximum amount of product. altitude as an environmental factor has a high correlation with some constituents of nettle plants (Chevalier, 1996). In the combination of chemical monet many changes have occurred given the chemical diversity even at small distances from each other (Blandel and Aroson, 1995(.The factors affecting the quality and quantity of pharmaceutical plants.

Thus, identifying factors that influence on the quality and quantity of pharmaceutical plants and more effective was considered quality of pharmaceutical compounds, Accordingly, researchers are trying to provide various methods for producing pharmaceutical composition with the most effective. Inspired by research conducted about the impact of environmental factors such as climate and the different ecological conditions on the EOs of medicinal plants, The present study examines the effect of altitude on the Eos of wild nettle in natural habitats and also identifies and defines the appropriate height to produce the highest amount of medicinal compounds.

MATERIAL AND METHODS

The studied area located in Ramsar city, Mazandran province in Iran. stretched between N 36°53'11.68" and E 50° 34'11.68" (figure1). The weather is rainy and snowy in late autumn and winter. Regarding information about weather provided from synoptic station in Ramsar, the maximum and minimum temperature are 32.6 ° C and -3 ° C, and the average yearly raining is 1107 ml. In order to determine the best height to produce the highest amount of essential oils in nettle plant was chosen three regions of the city with appropriate vegetation by GPS, respectively: Zero height of the Ramsar, 800 meters in the Javaher Deh village and 1800 meters located in the highlands of Javaher Deh.

Sampling was done on sunny days from late May to early June, Branches of Nettle in every region of length 10 to 25 cm from the tip of the plant were randomly as the sample was taken in three plot, Area of 1 m in each height class. identification of wild nettle plant (*Urtica dioica* L.) was done in herbarium .The samples collected were washed with distilled water, then, 4 days at 30 to 35 ° C was maintained in the dark place for dried. In order to better extract the essential oils from plants, the dried sample was milled.

The essential oil extracted from nettle leaves with the most common method is steam distillation method of global standards. For this purpose, the research Corticchiato et al in 1998, The oil extraction process was done by Hydrodistillation system and Clivenger that includes a 2-liter balloon, distillation column and heating device. 100 g of dry powder was weighed , 300 ml of distilled water was added to the balloon and extraction was done. There is some water in EOs obtained. In order to water extraction was used sodium sulfate. By freezing, The oils was removed by syringe from the frozen water. To determine the percentage of oil, The experiment was repeated. Then, for qualitative analysis of samples and determine the main compounds was used of GC and GC-MS sets. specifications and models of gas chromatography set are listed in Table 1.

Column temperature programmed in two device with initial temperature 50 C ° in oven and stop temperature for 5 minutes, Temperature round 3° C per minute, Increasing temperature 240 at 15 ° C degrees per minute and then increase the temperature to ° C 300 and 3 minute stop at this temperature. Injection chamber temperature was 290 ° C. helium as the carrier gas was used with flow rate 0.8 ml min. Mass spectrograph was used with ionization voltage of 70 electron volts, ionization method was EI and ionization source temperature was 220 C °.

Spectra were identified by Inhibition index and it comparison with indexes in reference books, Articles, by using the mass spectra of standard compounds and the information contained in the library computer.

Statistical analysis in a randomized block design was done by MINITAB 13 software.for meanes compare was used the Tukey test at 0.05 level.

RESULTS

Pay attention to values obtained from two stages of EOs extraction the essence of the nettle herb was 0.5%. In this study 8 EOs was studied because of the highest percentage of compound. They are respectively: Phytol, Pentadecanone, α-Phellandren, Isoelemicin, Linalool, Menta- Diene, Ethyl Hexanoate and Benz aldehyde.

Results of statistical studies show (table 2),there is quite significant differences between the percentage of EOs of three height classes zero, 800. 1800 in 0.01 probability level. It means, the percentage of EOs are altered under the influence of height. This difference is not significant only in composition percentage of Linalool. The resultes of analysis of variance inside each height class in 3 plot don't show significant difference between the percentage of EOs except of Menta-Diene.

The result of comparing the average percentage of EOs studied in the nettle plant by using the Tukey test in table 3 also indicates that there is quite significant differences between them in three height classes in 0.05 probability level. only average comparison of Linaloo don't show significant difference between height classes. Pay attention to table 3, phytol) 27.73%) has highest percentage at zero height. This EO is highest than other compounds.

Pentadecanone (12.76%), Isoelemicin (8.27%), á-Phellandren (8.92%), Linalool(2.67%) and Menta- Diene (1.33%) also have the highest amount in tne zero height. á-Phellandren and Menta- Diene in zero height don't have significant difference with a height of 1,800 meters. But their differences is significant with height class of 800 meters. Benz aldehyde (2.55%) and Ethyl Hexanoate (1.74%) have the highest amount in the height of 800 meters. Average of Ethyl Hexanoate EO percentage in 800 height show significant differences with two hieght classes of zero and 1800 but in the Benz aldehyde composition this difference is significant only by zero height class and have the lowest in this height.

DISCUSSION

The EOs in medicinal plants are mainly influenced by natural variations in their environment. Although the amount of secondary metabolites is controlled by genes but amount and their accumulation is significantly influenced by environmental conditions (Abu Darvish and Abu Dieyeh, 2009; Yanive and Palevitch, 1982). The best

Table 1: GC and GC-Ms specifications for analysis of pharmaceutical compounds in *Urtica dioica* L

Model MS-GC	Agilent 6890
Model GC	Younglin Acm600
MS model	Agilent 5973
Column length	30M
Inside diameter of the column	0/25mm
Layer thickness	0/25 µ
Column type	Hp-5Ms
The initial temperature of column	50° C
The final temperature of column	300 ° C
Carrier gas type	He

Table 2: the result of Analysis of variance the effect of altitude on the percentage of EOs of *Urtica Diocia* L.

Eos	Source Changes	sum of squares (SS)	Mean Square (SS)	Degrees (DF) of freedom	F
Phytol	Between height classes	508.65	252.32	2	2793.4**
	Inside height classes	0.39	0.19	2	2.15 ns
	Error	0.36	0.09	4	
Pentadecanone	Between height classes	106.8	53.4	2	3.7 **
	Inside height classes	0.0004	0.0002	2	0.88 ns
	Error	0.0058	0.0014	4	
á-Phellandren	Between height classes	19.67	9.84	2	850.5 **
	Inside height classes	0.032	0.016	2	1.39 ns
	Error	0.046	0.012	4	
Isoelemicin	Between height classes	19.66	9.83	2	1.3 **
	Inside height classes	0.00082	0.0004	2	0.53 ns
	Error	0.0031	0.00078	4	
Linalool	Between height classes	0.0065	0.0032	2	2.77 ns
	Inside height classes	0.0033	0.0016	2	1.4 ns
	Error	0.0047	0.0012	4	
Menta- Diene	Between height classes	0.037	0.018	2	59.6 **
	Inside height classes	0.048	0.024	2	76.75 **
	Error	0.001	0.0003	4	
Ethyl Hexanoate	Between height classes	0.804	0.4	2	25.23 **
	Inside height classes	0.006	0.003	2	0.2 ns
	Error	0.064	0.016	4	
Benz aldehyde	Between height classes	9.78	4.8	2	477.9 **
	Inside height classes	0.033	0.034	2	1.65 ns
	Error	0.04	0.04	4	

** significance in 0.01 leve

Ns no significant differences in height classes

Table 3: Comparison of the mean percentage of EOs of Urtica Diocia L. (there is No significant differences between means with with at least one letter in common by using the Tukey test at 0.05 level)

EOs	Height		
	0.00	**800**	**1800**
Phytol	27.52 ± 1.094 a	12.6 ± 0.44 b	6.35 ± 0.05 c
Pentadecanone	12.60 ± 0.038 a	8.63 ± 0.03 b	4.17 ± 0.03 c
α-Phellandren	8.38 ± 0.098 a	5.26 ± 0.16 b	7.89 ± 0.06 c
Isoelemicin	8.24 ± 0.021 a	6.61 ± 0.02 b	3.81 ± 0.03 c
Linalool	2.61 ± 0.032 a	2.630 ± 0.03 a	2.68 ± 0.05 a
Menta- Diene	1.33 ± 0.106 a	1.18 ± 0.09 b	1.27 ± 0.08 ab
Ethyl Hexanoate	1.13 ± 0.125 b	1.74 ± 0.12 a	1.09 ± 0.07 c
Benz aldehyde	0.2 ± 0.17 b	2.46 ± 0.08 a	2.36 ± 0.03 a

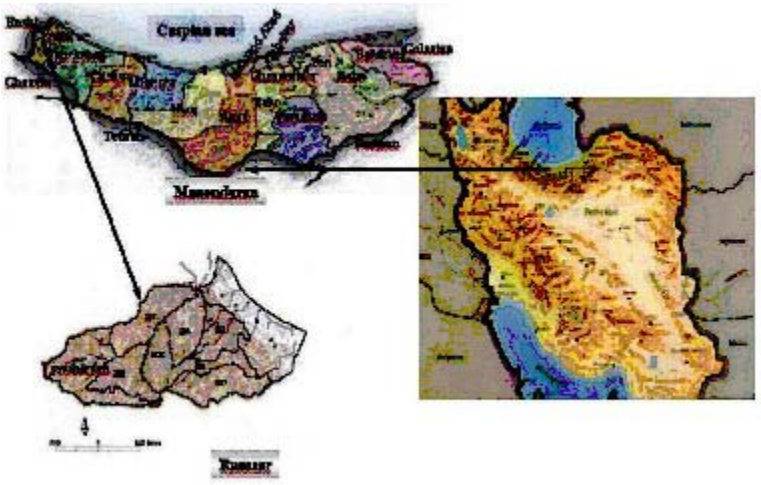

Fig. 1: Map of the study area located in Javaherdeh, Ramsar, Mazandaran, Iran

time to harvest the plants is between vegetative phase and before the reproductive phase (Kofidis. and Bosabalidis. 2008). So in late May to June is the best time for harvesting wild nettle plant with a maximum of drug combinations.

According to research Habibi et al, 2006 and Jamshidi et al, 2006; height is among environmental factors that is cuse of change the EOs of medicinal plants. Pay attention to the result of this study, of the eight EOs, Most of the compounds percentage in (Urtica dioica L.) was in the zero height class. Only Ethyl Hexanoate and Benz aldehyde had the highest in the height of 800 meters (table 3). It seems this is due to wet weather and proper soil for optimum production of EOs in Nettel.

Species studied in the Himalayas at altitudes of 350 to 3500 is also growing. Bharmauria research et al in 2009 showed, samples of this plant in low height have less genetic similarity with high height. According to research Sakata and Yokoi in 2002, Nettle is shade-friendly but will be adapted to different light conditions. So, there is no significant effect of Light on the amount of EOs in nettle. But the height will impact on its physiology. In environments with high humidity and 4 to 5 percent of the amount of organic material is high the amount of EOs of this plant (Janke and DeArmond, 2004). Low height in the study area with more temperature provides these favorable conditions. But height up to 1800 m is with reducing the moisture content, Temperature and vegetation and area takes the form of pasture so, Influenced by these factors height over 800 m can be reduce the percentage of EOs studied in this research.

The presence of this plant in high-altitude shows that Nettle is tolerant to cold and difficult conditions in heights and is able to produce some pharmaceutical compounds as desired that is consistent with the Baradarn research in 2009. he said, Nettle is a hardy and compatible plant, It have very wide range of compatibility. It grows well in clay soil with a layer of organic material and rich in nitrogen. Thus, depending on the type of EOs available, especially Phytol combined, can be taken plant samples in the desired height.

CONCLUSION

Recognition of the status of medicinal compounds in nature helps to understand the needs of this plant in field conditions. Profile of natural habitats with high production capability can be suitable model for providing culture conditions in farm and increased production and quality. According to the results if the goal is to harvest a high percentage of EOs used in this study, It should be noted high altitudes and very cold regions is not suitable for optimum production and plants nettle. It is best done planting and harvesting in altitudes less than 800 meters. In addition, The results of this study can be used to select and collect seeds for improved varieties with the higher EOs.

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