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Comparative Study on the Effect of Elevation Variation in North of Iran on *Hypericum perforatum* Essence

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ABSTRACT: *Hypericum perforatum* (HYPE) is from Hypericaceae family that use as medicinal herb. In the present investigation, the effects of elevation variation in north of Iran on HYPE essence have been studied. Experiments were carried with random plan form by 3 replications in 4 different north elevation of Iran (Fereydunkenar, Amol Azad University, Chelav and Reineh) in 2013.To identify essential oil composition and its value, GC and GC/MS and essence split method were used.The result showed that the highest elevation, the highest Nonan, pinene-β, funebrene-β, Cadinene delta and 5- methyl-3-heptanone (5M3HPONE). The highest amount of homolene-α and Caryophyllene was observed in lowest height. Also, Amol Azad University with 135 (m) height difference from sea was appropriate for maximum essence of pinene-α, 2-methyle octane and Cadinene-γ. Also, it found that elevation variation had significant effect in level of 1% on essence quantities.

Keywords: Elevation, *Hypericum perforatum*, essence.

INTRODUCTION

A. Medicinal plants importance

The use of herbs to treat diseases has been coincides with mankind history (Foldes and Szasz, 1984). Today medicinal herb have assigned to their large share of Commercial pharmaceutical products (Arouei *et al*, 2000). Environmental factors, inheritance structure, agronomic factors and living and non-living factorsare some of key parameters for medicinal herb production (Beigi, 1997).

B. The properties of Hypericum perforatum

Hypericum perforatum (HYPE) rarely forms thickets and usually grows in patches or narrow strips along forest edges. In the forest area, this plant also occurs in upland meadows, glades, cutover areas, and open pine or conifer small leaf forests. In the forest-steppe zone, it can be found in oak and birch groves, meadow steppes, and felling areas. In mountain regions, HYPE grows mainly on stony slopes in the foot hills, rarely ascending to the subalpine meadow belt. In addition, it occurs as a weed at roadsides, in crop fields or at their margins, and in fallows (Maevskii, 2006; Rastitel'ny, 1985; Illyustrirovanny, 2004).

The behavior of HYPE changes under certain habitat conditions (Harris, 1967).There are many publications dealing with various aspects of the biology and ecology of HYPE and other medicinal herb. Parkhomenko (2011) analyzed the species composition and ecological structure of such communities as a possible factor having an effect on the state of HYPE populations.

C. Essence production methodology

The result of Jamshidi research (2006) showed that essential oil Thymus Kotschyanus content were between 0.95 - 1.87% and the yield was 1.23% and due to high content of essence was valuable in drugs industry. Generally, 37 different combinations were analysed by GC-MS. In elevation bands which showed Carvacrol (60.82-82.05%) and Thymol (1.56-13.94%) were the most important substances in T. kotschyanus. This study showed that essence of this species has high content of Thymol and Carvacrol which were two medically important.

Shams (2012) evaluated the effect of organic and chemical fertilizers on amount of essence, biological yield and harvest index of matricaria chamomile. This experiment was carried out in 2008-2009 at the field experiment of Hariri scientific foundation, Babol, Iran in two seasons. The ANOVA test was used to determine significant ($p \leq 0.01$ or $p \leq 0.05$) treatment effect and Duncan Multiple Range Test to determine significant difference between individual means.

Saeb (2011) was investigated five essential oils (EOs) of *Hypericum perforatum* including α-pinene, myrcene, cineol, tridecane and phytol in different light situation. Results indicated that there was significant difference between the averages of EOs in five light classes. α-pinene was the main compound, and the maximum average percent of mixtures were detected in light classes of 80 to 100% and then 60 to 80%. Therefore, increase of light caused medicine mixture to increase in this study.

Morshedloo (2012) had studied on the essence composition of tree species of *Hypericum* spp. (*H. perforatum*, *H.* tetrapterum and *H. patulum*) and comparison of them. The result illustrated that in *H. perforatum* species, pinene-α (21.8%), nonane (9.8%), n- octane (9.1%) and in *H. tetrapterum* species, n-decane (30.8%), n-nonane (9.9%) and in *H. patulumapecies*, pinene-β (30.2%), pinene-α (18.3%) were the highest values, respectively.

Besides mentioned reviews, Radusienea, *et al.*, (2005); Halahan, (2000) and Zhang, *et al.*, (2009) have published papers in HYPE essence percentage identification and the effective factors on them.

MATERIAL AND METHODS

To perform this research, it was used 4 randomized case studies or treatments in north of Iran with different elevation around of Amol in 2013 (Table 1). The GPS system was used to determine the regions height and geographic location. All of these places, aspect of Reineh being in maintenance area, are located in Temperate and humid Positions (Fig. 1).The essence extraction and identifying process with three replications has done in university of agricultural sciences located in Amol (Fig. 2).

Table 1: The treatments attribute.

Number of treatment	Name of place	Elevation from the sea(m)	Place general vegetation
(1)	Fereydunkenar	-16	Paddy field
(2)	Amol Azad University	135	Rice and rape
(3)	Chelav	1005	Forest pasture
(4)	Reineh	2003	Pasture and Thyme

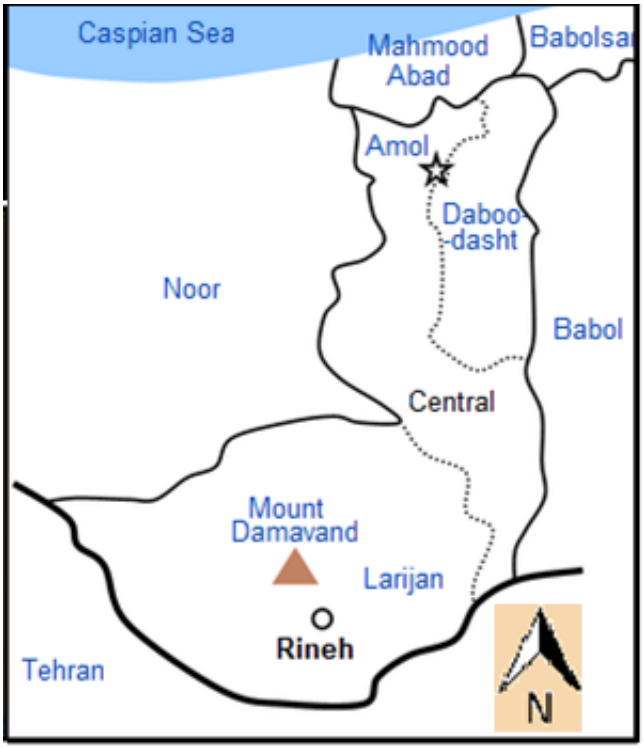

Fig. 1. Restrict of Reineh.

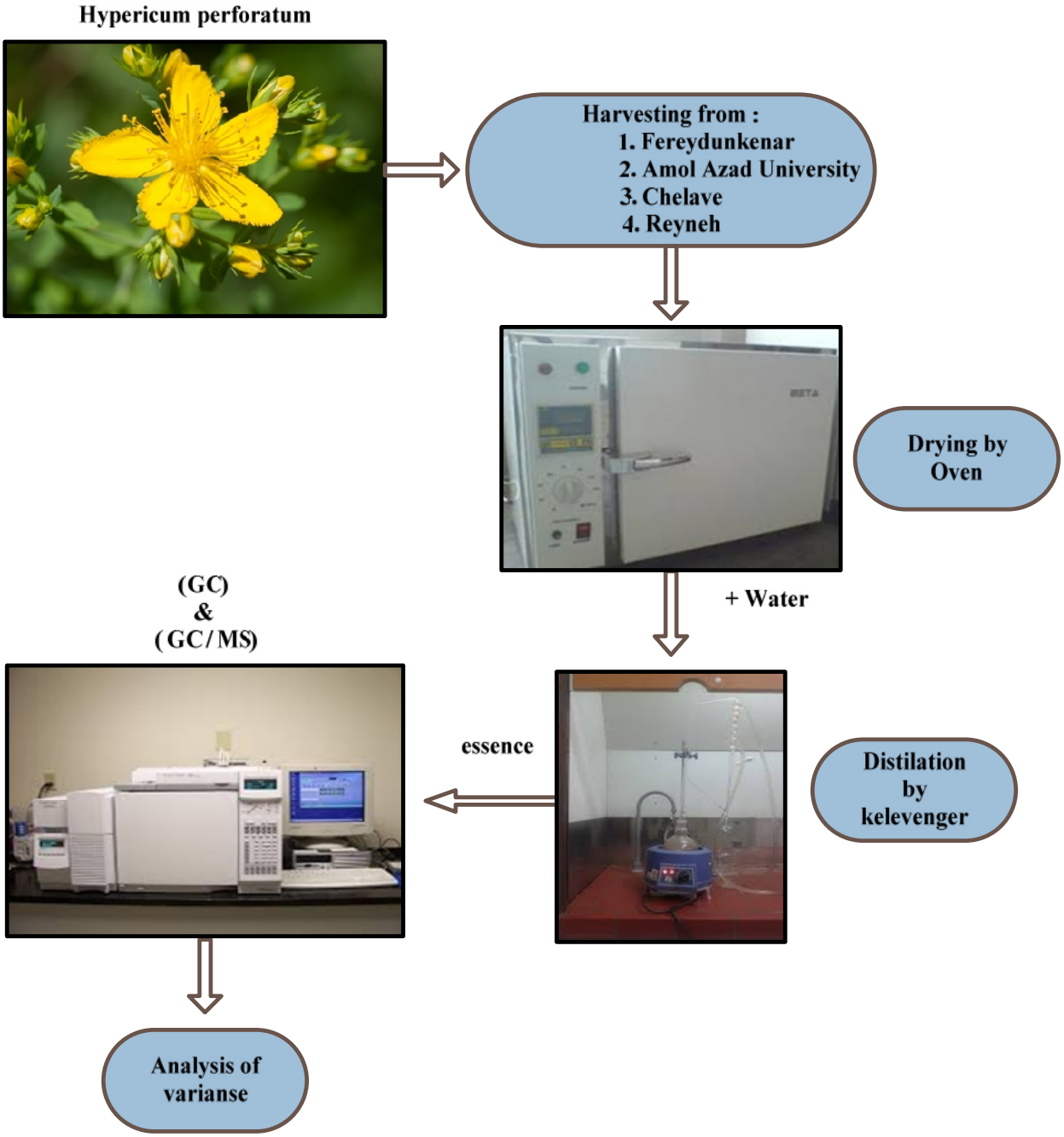

Fig. 2. The process of research.

A. Characteristics of chromatography and Mass Spectrometry devices

To identify essential oil composition, it was applied GC (Mdl Agilent 7890)equipped by DB5 column, with a length of 30m length, inside diameter of 0.25mm and thickness of 0.25mm. The initial temperature of column was set in 60°C per minute and the final temperature in 240°C. The total time was considered 5min and rate of temperature rise was adjusted in 3°C.

Also, the injection chamber temperature and detector chamber temperature were 230°C and 250°C respectively. The GC/MS (MdlAlgilent C5937) with 70eV ionization voltage, He gas as Carrier gas, column and temperature program similar to GC system was used for investigation of essence value. The injection temperature was set in 10°C higher than final temperature of column. The split method with volume of 0.2ml was used for this operation.

B. The procedure of operation

The samples choose by considering flowering appearance accumulation of this plant in mentioned treatments. The oven was applied for HYPE drying. Then in every treatment 25gr of dried HYPE combined with 300ml of water for nourish to kelavenger for 3 hours. The extracted essence was dehydrated by sodium sulfate and Maintenance by vial and parafilm paper in 40C. The essential oil composition and value was determined by GC and GC/MS. SPSS and Excel were used for evaluation of values.

RESULT AND DISCUSSION

According to evaluation of value results, different treatments have a significant effect on all composition in level of 1% aspect Pinene-α which was in 5% (Table 2 & Table 3).

Table 2: Analysis of variance of essence percentage in different treatments.

S.O.V	Df	2-methyl octane	Nonane	Pinene-α	5M3HPONE	Pinene-β
Repetition	2	12.30	0.20	1.83	1.83	0.03
Treatment	3	71.79**	42.76**	75.88**	22.48**	0.55**
Error	6	1.84	3.87	19.57	0.97	0.19
CV		10.55	26.26	29.49	17.97	31.98

** and **: significant in level of 5% and 1% , ns: no significant*

Table 3: Analysis of variance of essence percentage in different treatments.

S.O.V	Df	Funebrene-β	Homolene-α	Caryophyllene	Cadinene-ϒ	Cadinene delta
Repetition	2	0.016	2.16	0.56	0.04	0.36
Treatment	3	1.04**	33.52**	27.26**	2.44**	81.04**
Error	6	0.028	0.33	0.30	0.05	1.80
CV		28.52	17.73	17.72	27.41	19.46

Table 4: Mean comparison of characters.

Number of treatment	(1)	(2)	(3)	(4)
2-methyl octane	8.18c	18.40a	9.33c	15.46b
Nonane	5.06b	4.66b	7.36b	12.86a
Pinene-α	8.63c	20.90a	14.76b	15.69ab
5M3HPONE	2.13c	7.20a	4.46b	8.18a
Pinene-β	0.02c	0.15c	0.60b	0.95a
Funebrene-β	0.07b	0.10b	1.00a	1.40a
Homolene-α	8.23a	0.93c	1.71bc	2.26b
Caryophyllene	7.90a	1.06b	2.04b	2.90b
Cadinene-γ	0.04b	1.70a	0.04b	1.50a
Cadinene delta	17.73a	2.86c	4.90bc	8.39b

In each row, means with the similar letters are not significantly different at 5% level of probability.

The result illustrated that the highest elevation, the highest Nonan, pinene-β, funebrene-β, Cadinene delta and 5M3HPONE. The highest amount of homolene-α and Caryophyllene was observed in lowest height. Also, Amol Azad University with 135 (m) height difference from sea was appropriate for maximum essence of pinene-α, 2-methyle octane and Cadinene-γ. Also, the result indicated that Pinene-α percentage was the highest value compared to other components percentage, generally (Fig. 3).

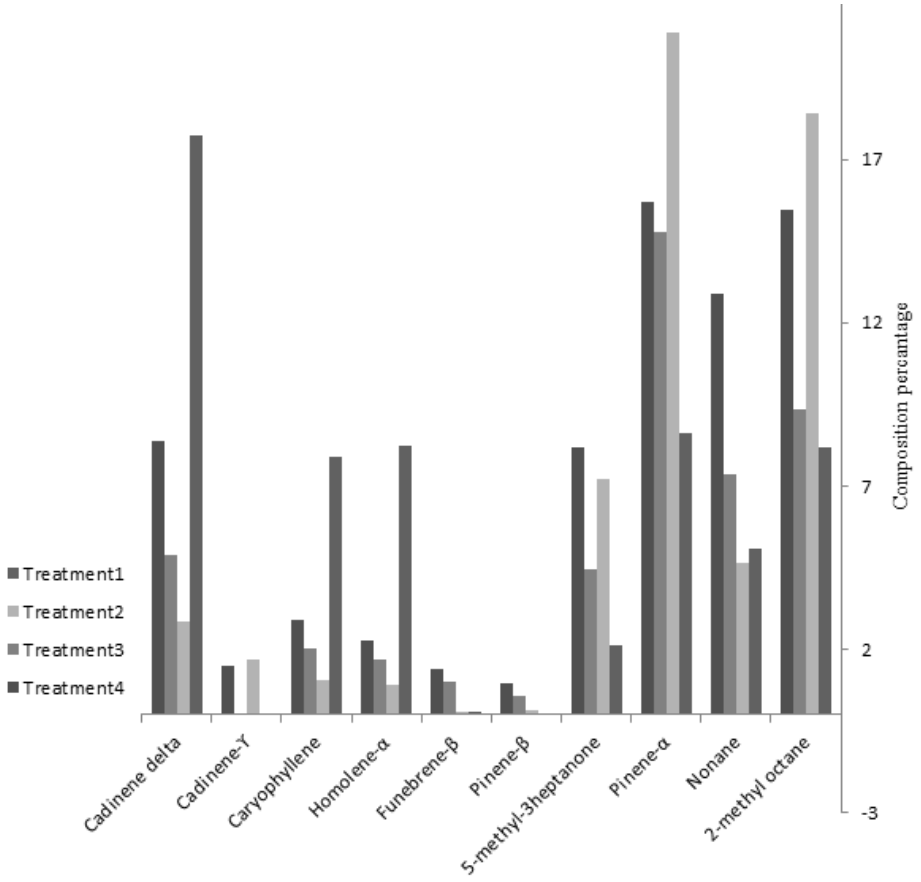

Fig. 3. The Essential oil composition percentage.

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