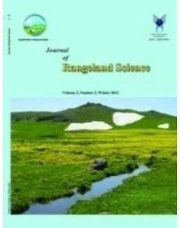
Contents available at ISC and SID

Journal homepage: www.rangeland.ir



Full Length Article:

Investigation of the Changes in the Amount of the Secondary Essential of Hypericin in *Hypericum perforatum* L. in Different Highlands of Golestan National Park, Iran

Parinaz Asghari[A], Seyedeh Khadijeh Mahdavi[B], Seyed Ali Hosseini[C], Masumeh Mazandarani[D]

[A]MSc. Student of Rangeland Management, Islamic Azad University, Noor Branch, Nour, Iran. Email: asghari_parinaz@yahoo.com. (Corresponding author).
[B]Assistant Professor, The Islamic Azad University, Noor Branch, Nour, Iran.
[C]Faculty Member of the Research Center for the Agriculture and Natural Resources of Gorgan, Iran.
[D]Assistant Professor, The Islamic Azad University, Gorgan Branch.

Manuscript Received: 11/11/2011
Manuscript Accepted: 06/02/2012

Abstract. In the present study, the Tangrah region located in the vicinity of Golestan National Park was selected for exploring the changes in the amount of the secondary essential of hypericin in *Hypericum perforatum* in different highlands of the eastern Golestan province. The aerial flowered parts of the herb were collected randomly and in three replications from four altitudes of 300 m, 600 m, 900 m, and 1200 m in June (5-12) 2011, and dried in the open air, with their methanol essential being extracted using cold maceration method. The HPLC method was also used to determine the amount of hypericin samples. The variances of the obtained data were analyzed in F-test by using the SPSS software package. In addition, the means were compared by means of LSD. The results revealed that the amount of hypericin extracted at the altitudes of 300 m, 600 m, 900 m, and 1200 m were 1.903 mg/g, 1.393 mg/g, 1.710 mg/g, and 2.107 m g/g, respectively, so that the altitude of 1200 m recorded the greatest amount (2.107 mg/g) while the altitude of 600 m represented the smallest amount (1.393 mg/g). The statistical analyses showed that there is a significant difference between the altitude of 600 m and the altitudes of 300 m and 1200 m in the amount of observed hypericin ($p>0.1$), whereas no significant difference was observed between the altitude of 300 m and that of 1200 m. Moreover, there was no correlation between soil characteristics percentage despite the increase in the hypericin at different highlands.

Key words: *Hypericum perforatum*, Hypericin, Altitude, Golestan National Park.

Introduction

Over the last century, there has been a growing proclivity in the use of herbal plants as a result of the rise in side effects of taking chemical medicines. The ever-greater desire and demand for the godsend, i.e. herbs, have caused the unique, precious species to reduce in number, or even peter out, in some cases, regardless of their negative effects on the structure and biodiversity of the plant communities in the rangelands and of the decrease in the soil surface vegetation and increase in its erodibility. Thus, the identification of natural sites as well as secondary and pharmaceutical substances of the herbs, and most importantly of all, cultivation and localization of the precious herbal species in a wide area of land, can not only lessen the demands upon the bionetwork, but also pave the ways for mass production of such herbs, and hence fulfill the internal needs and even allow for the exports of pharmaceutical products. Although the production of the herbal substance results from genetic processes, it is significantly affected by the environmental factors so that such factors bring about certain changes in the growth of the herbs and in the quantity and quality of the substance such as alkaloids, glycosides, asteroids, and essentials and the like (Omidbeigi, 1995). In the natural ecosystems, a variety of factors such as climate, soil and geographical locations have great effects on the rise or fall of the quantity and quality of the essence in the herbs (Omidbeigi, 2009; Lebaschi, 2000; Lebaschi *et al*., 2004). Briskin and Gawienowski (2001) demonstrated that the density of hypericin in the samples of *Hypericum perforatum*, which grows in the highlands under the intense sunlight, is heightened. It was later shown that the quality and intensity of light influenced the proportions of hypericin in different species of *Hypericum perforatum*. In a study carried out in Greece concerning the effect of altitude of the ecological site on the amount of hypericin, Marina *et al.*, (2008) revealed that there was obviously an increasing trend in the overall amount of hypericin as the altitude maximized, considering the intensity of sunlight and low temperature as being the factors that could raise the amount of hypericin. In a different study conducted in various sites in the US, the altitude of the site was reported to be the main factor that increases the amount of hypericin in *Hypericum perforatum* (Walker *et al.*, 2001). Campbell (1985) suggested in their investigations that the most appropriate condition for the *Hypericum perforatum* to grow in the natural environment is the altitude of 600 m and the precipitation of over 760 mm. Also, in the study carried out by Storanova and Apostdova (1992), it was demonstrated that the secondary compositions in the aerial flowered parts of the *Hypericum perforatum* depended on the altitude of the sites so that the there was a decline in quantity of phylonoid as the altitude minimized. The results of this research indicated that the given herb had the greatest amount of secondary compositions at the altitude of 1250m. In their investigation of the quantity of hypericin in two regions of the natural sites, i.e. Touskestan and Deraznow, where *Hypericum perforatum* grows, Dorri *et al.,* (2009) concluded that the maximum amount of extracted hypericin proved approximately equal for both sites under study. But this amount was obtained at the altitudes of 450-750 meters in Touskestan (0.26 mg/g) and at the altitudes of 1950-2250 meters in Deraznow (0.25 mg/g). This result shows that there is a degree of altitude in each of the sites that supplied better conditions for the herb to grow, hence yielded the highest amount of hypericin. The herb was taken from *St John's Wort*, and is scientifically known as *Hypericum perforatum*, that belongs to the Hypericaceae or Clusiaceae family, including that it is a Herbaceous and

Perennial plant which grows in areas ranging from the sunlit forestry roads to the high rangelands. *Hypericum perforatum* originally grows in Europe, West Siberia, Northwest China, Asia Minor, the Mediterranean, North Africa, Canada, and Australia (Campbell, 1985). It can also be found in the north, northwest and northeast of Iran as well as in Fars province and at the feet of Elburz Mountains (Azadi, 2001). The hypericin in *Hypericum perforatum* has been utilized in medication for a long time, and, today, it is used to treat certain illnesses such as depression and migraine by developing drugs from the substance of hypericin (Kireeva *et al.*, 1998). Therefore, the abundance of sites and ecologic areas available for the growth of different species of *Hypericum perforatum* in a variety of regions of Golestan province, and the extensive use that the regional people make of the given herb as an anti-inflammatory, sedative, and a powerful anti-spasm to relieve the headache, nervous tensions and migraine, and also the research studies conducted on the determination of environmental and ecologic factors influencing the herbal substance of *Hypericum perforatum*, have all caused us to carry out a study so as to determine the effect of the altitude on the quantity of the herbae substance of *Hypericum perforatum* in the natural sites as well as identification of the optimum altitude.

Materials and Methods

The Tange-Rah region nearby the western side of the Golestan National Park located in 145 k of the northeast Gorgan was selected to determine the optimum altitude in the synthesis of the greatest amount of the secondary hypericum substance in the *Hypericum perforatum* in the natural sites of the province. The area (Longitude 55°47′E & Latitude 37°25′N) has a semi-arid and moderate climate, covered with silt-loam, and is one of the forestry lands of the province.

Four altitudes of 300 m, 600 m, 900 m and 1200 m were selected for sampling using GPS at the southern feet from the lowest to the highest altitudes where the given herb was seen growing, and then the aerial flowered parts of the herb were collected in three replications from the altitudes of 300 m to 1200 m in June (5-12) 2011, respectively. The samples were dried out in shade after they were cleared up; then they were kept in a paper bag and taken to the Gorgan IAU Laboratory of Herbal Research Center for extracting the essential and determining the substance of the herb. At the same time, the soil samples of the sites were gathered from different highlands and transported to the Soil Laboratory of the Gorgan Research Center for Agriculture and Natural Resources for physical-chemical tests.

Methanol extraction of *Hypericum perforatum*

In order to obtain methanol extract of the *Hypericum perforatum*, the dried samples of the aerial flowered parts of the herb were weighed and ground into powder in a Chinese mortar. Extraction was carried out with 80% methanol using cold maceration method (Hajipour *et al.*, 2009), 10 ml of 80% methanol was added to 1g of powdered sample. Then, it was put into a Shaker for 24 hours, and the essentials were filtered after a while; and after removing the solvent from the essentials (Hajipour *et al.*, 2009), the samples were kept in smoked bottles in the refrigerator before they were separated by the HPLC.

HPLC analysis of hypericins

The HPLC system was Agilent Series 1200, with an Agilent UV detector, which was set on 590 nm wavelength. The column was ZORBAX XDB C18 with 150×4.6 mm dimension and 5μm particle size. The mobile phase was prepared by sodium dihydrogen phosphate (15.6g/L), ethyl acetate and methanol by (41:39:160) ratio. The elusion was isocratic mode and injection volume was (20 μL). The hypericin chromatogram peak was appeared less than 15 min. (Malgorzata *et al.*, 2010).

Standardization

One mg of standardized hypericin was picked up from sigma-Aldrich, its catalogue No. is K56178123.

Determination of hypericin in samples

The standard hypericin (with 100 pp density) was injected into the HPLC and the hypericin peak Area was measured by the HPLC software. Then, the samples from different essentials of the herb were injected into the HPLC, and the amount of hypericin in different essentials was obtained by comparing the hypericin peak Area.

Statistical analyses

Data were analyzed using the statistical software SPSS. At first, the normalization of the variables was defined by employing the Kolmogorov-Smirnov Test, and after we were certain of the normalization, an F-test was administered to analyze the data, and the LSD was used to compare the means of data ($p>0.05$). Finally, the figures were drawn using the Excel software.

Results

The means of the hypericin content (mg/g) of *Hypericum perforatum* are shown in (Fig. 1) and the comparison of their means is indicated in (Table 2). The amount of hypericin were measured according to the herbae substance.

As was shown in (Fig. 1) and Table 2, the amounts of hypericin at the altitudes of 300 m, 600 m, 900 m and 1200 m were 1.903 mg, 1.393 mg, 1.710 mg and 2.107 mg/g, so that the amount of hypericin produced in the given herb at the 1200 m altitude (2.107mg) was the highest; this amount decreased by 1.903 mg/g at the 300 m altitude, which stands after the 1200 m altitude in order, while the amount of hypericin at the 600 m altitude is at the lowest degree, which shows 1.39 mg/g (Table 2). The results indicated that there is a statistically significant difference ($p<0.01$) between the 600 m altitude at and the altitudes of 300 m and 1200 m in terms of obtained hypericin amounts so that no such significant difference was observed between the 300 m altitude and the 1200 m altitude (Table 1. and Fig. 1). The statistical analyses also showed that there is a significant correlation between the amount of hypericin and the altitude (Fig. 2). Regarding (Table 1), the results obtained from the comparison of different characters of soil in four altitudes reveal that in the area under study there is a statistically significant difference between the altitudes of 1200 m and 600 m, on the one hand, and the altitudes of 1200 m and 900 m, on the other, in terms of the amount of potassium, percentage of clay, and soil acidity at $p<0.05$. Moreover, there is a significant difference between the altitudes of 300 m and 600 m, on the one hand, and the altitudes of 300 m and 900 m and also the altitudes of 300 m and 1200 m, on the other, in view of the amount of soil phosphorus as well as the Electrical Conductivity (EC). Regarding the percentage of carbon and that of soil nitrogen, there is a significant difference between the altitudes of 1200 m and 300 m, on the one hand, and the altitudes of 1200 m and 600 m and also the altitudes 1200 m and 900 m, on the other. In addition, the results revealed no such significant difference between the four altitudes in the light of the percentage of silt and that of sand. It is inferred from (Table 4), that there was no correlation between soil characteristics percentage despite the increase in the hypericin at different highlands.

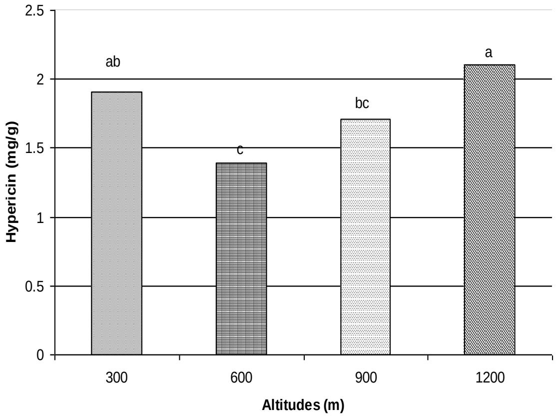


Fig. 1. Means of Hypericin amount extracted in *Hypericum perforatum* collected from different highlands of Golestan National Park

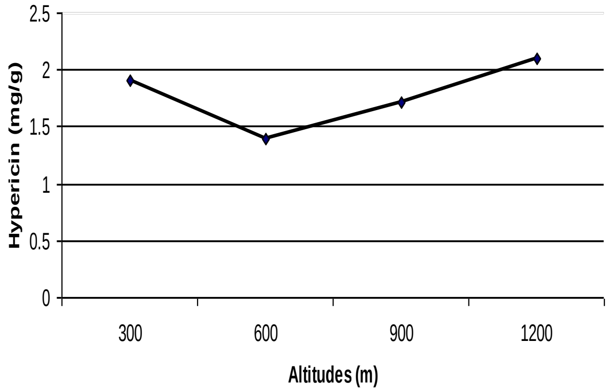


Fig. 2. Consistency of altitude and Hypericin in *Hypericum perforatum* collected from Golestan National Park

Table 1. Variance of the effect of height on the amount of Hypericin and soil parameters – Golestan National Park

Source Changes	D.F	Hypericin	pH	EC	N	P	K	Clay	Silt	Sand	O.C
Repeat	2	0.033	0.023	0.038	0.008	3.466	3096.333	1.333	63.000	75.250	0.774*
Treatment	3	0.276**	0.025*	0.149*	0.046**	9.304*	10813.639*	20.444*	25.222	80.750	4.738**
Test error	6	0.031	0.004	0.037	0.001	1.684	1520.889	4.444	18.556	39.250	0.117
Coefficient of variation%		9.874	0.890	35.112	19.010	42.311	20.163	22.588	7.120	21.059	20.303
٪5= P	LSD	0.351	0.133	0.385	0.064	2.592	77.915	4.212	8.606	12.517	0.685

**Significance in 0.01 level, *Significance in 0.05 level

Table 2. Mean comparison of Hypericin amounts in *Hypericum perforatum* collected from Golestan National Park

Altitudes (m)	Amounts of Hypericin (mg/g)
300	1.903ab
600	1.393c
900	1.710bc
1200	2.107a

*Comparable letters represent no significant difference.

Table 3. Mean comparison of soil parameters in four altitudes – Golestan National Park

Soil Parameters	Organic Carbon (%)	Sand (%)	Silt (%)	Clay (%)	Absorbable Potassium p.p.m	Absorbable Phosphor p.p.m	Total Nitrogen (%)	EC	pH
Altitude 300 m	1.090b	32.000a	58a	8.667ab	201ab	5.633a	0.107b	0.880a	7.433bc
Altitude 600 m	1.207b	26a	63.333a	10.667a	181.67bc	1.633b	0.120b	0.457b	7.600a
Altitude 900 m	0.893b	25a	62.667a	12a	122.67c	2.400b	0.090b	0.393b	7.533ab
Altitude 1200 m	3.563a	36a	58.000a	6b	268.33a	2.600b	0.353a	0.467b	7.400c

Table 4. Pearsonian correlation between the physical- chemical properties of soil and herbae substance of *Hypericum perforatum* - Golestan National Park

The Physical-chemical Properties of Soil	Hypericin
Electrical Conductivity(EC)	0.07
Acidity(pH)	-0.44
Potassium	0.30
Phosphor	0.18
Nitrogen	0.50
Organic Carbon	0.50
Sand	0.29
Clay	-0.48
Silt	-0.21

Discussion and Conclusion

This study showed that hypericin content of *Hypericum perforatum* varies in different altitudes. The results are in agreement with the numerous studies including Walker *et al.*, (2001) and Dorri *et al.*, (2009), which reported the effect of environmental conditions in the sites of herbs on the quantity and quality of the herbal substances. In addition, the results are similar to the achievements reported by Marina *et al.*, (2008), providing evidence that there are differences in the amounts of hypericin in different altitudes. Regarding (Fig. 1 and Table 2), more amounts of hypericum substances were extracted from the sampling areas pertinent to the altitudes of 300 m and 1200 m compared to the other sampling areas. Since the study area was a forestry one, there was seen to be a reduction in the mass of forestry trees, and this provided a wider space for the *Hypericum perforatum* to grow well, including the fact that more areas were found to be in the lack trees. Hence, all this caused plenty of sunlight to come up to the given herb during the growth period. The decreasing period of shading due to the increasing amount of sunlight in the aforementioned areas brought about greater temperature throughout the day, a factor having influence on the amount of hypericin. This result is consistent with the study carried out by Zobayed *et al.*, (2005), on the rise of temperature as one of the factors resulting in the increase of secondary metabolites and hypericin in the *Hypericum perforatum*. According to Oshima *et al.*, (1997), Provision of more sunlight in the forestry environments is significant for the herbs to grow better. In addition, as described by Zobayed *et al.*, (2006) and Briskin and Gawienowski (2001) provision of further amount of sunlight in such areas, could be effective for the colored glands to maximize in the parts of the herb, and hence giving rise to the higher production of hypericin. The results are also in agreement with those obtained in the study conducted by Dorri *et al.*, (2009) on the amount of hypericin in two different sites. Poutaraud *et al.*, (2001) revealed in their investigation that the light quality and its intensity tend to change the hypericin concentration in *Hypericum perforatum* L. The results indicate that there is a degree of altitude in each study area that provided more appropriate conditions for the herb to grow and brought a greatest amount of hypericin production. Generally speaking, the results that have been achieved from the determination of the amount of hypericin in different highlands of the Tange-Rah region in the vicinity of Golestan National Park reveal that as the altitudes become higher, the amount of hypericin decreases due to the mass of forestry trees, but the trend undergoes a rise in the amount of hypericin as of the 600 m altitude up as a result of the drop in the mass of trees. It can be concluded that the most suitable area for the given herb to grow in order to achieve the most effective performance of the hypericin substance is the altitude of 1200 m to 1250 m. These results are consistent with the findings obtained by Storanova and Apostdova (1992), who demonstrated that the *Hypericum perforatum* growing at the altitude of 1250 produces the greatest amount of secondary compositions. The results from the analyses of the site soil parameters in the Golestan national park of as indicated in Table 4 revealed that there was no correlation between soil characteristics and the amount of hypericin. Also, according to Table 3 the organic carbon, the nitrogen and potassium percentages increased at the altitude of 1200 m and added up to the soil sand, which the hypericin surge at the altitude of 1200 m can eventually be attributed to the drop in soil acidity, the clay percentage, absorbable potassium increase, nitrogen percentage, as well as sand percentage. In their study of certain aspects of monoecology of *H. perforatum* L. in Khorasan province, Ejtehadi *et al.*, (2004) concluded that *H. perforatum* L. grows suitably in light non-salty calcic soils containing pH of 7.5 to 8. Moreover, in an investigation of the ecophysiological needs of *Hypericum perforatum* in three different sites, Yesaghi (2006) introduced the optimum conditions for *H. perforatum* L. to grow as soil-induced and full of carbon, which is in agreement with the results achieved in the present study. Based on the findings of this study, the conditions for growth in the altitude of 1200 of the Golestan national park can be introduced as the optimal harvest zone with the greatest amount of hypericin substance of the study herb. It is highly recommended another study, like the present one, be conducted on all the areas where the *Hypericum perforatum* grows to identify the most effect place for the herb to grow for the purpose of achieving the most indexical performance of hypericin assumed to be the most significant substance of *Hypericum perforatum* and to have high herbae quality (Kaveh, 2000; Najafi, 1997).

References

Azadi, R., 2001. The Flore of Iran: the holotype of Hypericaceae. Research Institute of Forest and Rangeland. No. **1:** p. 12.

Briskin, D. P. and Gawienowski, M. C., 2001. Differential effects of light and nitrogen on Production of hypericins and leaf glands in *Hypericum Perforatum*. *Plant Physiology and Biochemistry,* **39(12):** 1075-1081.

Campbell, M. H., 1985. Germination,emergence and seedling growth of *Hypericum perforatum*. *Weed Research*, **25:** 259-266.

Dorri, M. H., Hosseini, S. A., and Lebaschi, M. H., 2009. Investigating the amount of hypericin in two natural sites of *Hypericum perforatum* in Golestan province. *Iranian Scientific-Research Quarterly Jour. Aromatic Herbs*, **24(2):** pp.117-125.

Ejtehadi, h., Kianmehr, H., Notghimoghaddam, R., 2004. Study of certain aspects of monoecology of *Hypericum perforatum* L. in the regions Khorasan province. *Iranian Jour. Biology*, **14(1-2):** 47-53.

Hajipour, H., Khanavi, M., Shekarchi, M., Adedi, Z., and Pir-ali, M., 2009. Investigating the best method of extracting fennal composites in *Echinacea purpurea Quarterly Jour. Herbs*. **22(4):** 145-152.

Kaveh, Sh., 2000. Investigating the pharmagonozy of *Hypericum perforatum*. Unpublished Doctoral Dissertation of Pharmaceutics. Islamic Azad University, Tehran Branch. 163.

Kireeva, T. B., Sharanov, V. L. and Letchamo, W., 1998. A comparative biochemical and Ecophysiological study on *Hypericum* spp.

Lebaschi, M. H., 2000. Investigating the ecophysiologic aspects of *Hypericum perforatum* in the agricultural and natural ecosystems. Unpublished Doctoral Dissertation of Agriculture. Tarbiat Modarres University.

Lebaschi, M. H., Matin, A., and Sharifi-Ashurabadi, A., 2004. Comparing the agricultural and natural ecosystems in the production of hypericin. *Research and Construction in Natural Resources*, **16(2):** 48-54.

Malgorzata, A., Annakowal, C., Annalozak-Renata, J., Zynska-Zbigniew, F., 2010. Determination of total Hypericins in St. Johns and herbal medicinal products,Acta Poloniae pharmaceutica – *Drug Research*, Vol. 67 No. 6: 587-593.

Marina, X., Ilias, S., Emmanuel, A., Eleni, N., Dieter, D., Kiriakos, K., 2008. Influence of the Habitat Altitude on the (proto) Hypericin and (proto)Pseudohypericin Levels of *Hypericum* Plants from Crete. *Planta Medica*, **74:** 1496-1503.

Najafi, F., 1997. Separation and identification of essentials in *Hypericum perforatum.*
Unpublished Doctoral Dissertation of Pharmaceutics, Faculty of Pharmaceutics, University of Tehran.

Omidbeigi, R., 1995. Production and productivity of herbal plants. Fekre Rooz Press. p. 283.

Omidbeigi, R., 2009. *Production and productivity of herbal plants*. Asten Ghods Razavi Press, Vol. **1:** 33-165.

Oshima, K., Tang, Y. and Washitani, I., 1997. Spatial and Seasonal Patterns of microsite light availability in a remnant fragment of deciduous riparian forest and their implication in the conservation of *Arisaema heterophyllum*, a threatened plant species. *Jour. Plant Research*, **110(3):** pp. 321-327.

Poutaraud A., Di Gregorio F., Fook Tin VC., Girardin P., 2001. Effect of light on hypericins contents in fresh flowering top parts and in an extract of St.Johns wort (*Hypericum perforatum*). Planta Med. **67:** 254-9.

Storanova, M. and Apostdova, B., 1992. The influence of altitude upon the biochemical composition of tutsan (*Hypericum perforatum*). *Naukazagorata*, **29:** 7-82.

Walker, L., Sirvent, T., Gibson, D. and Vance, N., 2001. Regional differences in hypericin and pseudohypericin concentrations and five morphological traits among *Hypericum perforatum* plants in the northwestern United States. *Canadian Jour. Botany*, **79(10):** 1248-1255.

Yesaghi, S., 2006. An investigation of ecophysiological, phonological, ethnological, and antibacterial needs of two widely-grown species of the herbs, *Hypericum perforatum* in Golestan province. MS Thesis, Islamic Azad University.

Zobayed, SM., Afreen, F. and Kozai, T., 2005. Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. Johns wort. *Plant physiology and Biochemistry*, **43(10-11):** 977-984.

Zobayed, SM., Afreen, F., Goto, E. and Kozai, T., 2006. Plant–Environment Interactions, Accumulation of Hypericin in Dark Glands of *Hypericum perforatum. Annals of Botany*, **98(4):** 793-804.