

Full Length Research Paper

Study of variation of biochemical components in *Hypericum perforatum* L. grown in North of Iran

Asadian Ghavamaldin¹, Rahnavard Aptin^{1*}, Pourshamsian Khalil², Ghorbanpour Mansour³ and Taghavi Mariamalsadat⁴

¹Department of Medicinal Plants, Faculty of Agriculture, Islamic Azad University Tonekabon Branch, Tonkabon, Iran.

²Department of Chemistry, Islamic Azad University Tonekabon Branch, Tonkabon, Iran.

³Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Iran.

⁴Agricultural Engineering Organization Tonekabon, Iran.

Accepted 18 November, 2011

Hypericum perforatum L. is among important herbs worldwide, growing wild in Iran in various areas and altitudes particularly north of the country. However, according to different regional and genetic reasons, its secondary metabolites differ in its vegetative growth. Therefore, in order to define its best vegetating area, the most important biochemical components in five regions and three altitudes and four samples from each region (60 samples all together) were sampled and analyzed. Experiment revealed that the highest values of hypericin (0.251%) and total phenols (412 mg/g DM) were obtained in Jannat Roodbar region in a height of 1218 m and the highest amounts of flavonoid (21 mg/g DM) and carotenoid (0.67 mg/ml) were observed in Pole zangoole region in a height of 2300 m. Regression equations have been demonstrated to have a positive linear relationship between hypericin and total phenols contents. While among hypericin and flavonoid as well as carotenoid, this relation forms a second degree equation. The relationship of hypericin and total phenols contents was a negative correlation as well.

Key words: *Hypericum perforatum* L., hypericin, total phenol, flavonoid, pigments, provinces, height.

INTRODUCTION

Hypericum perforatum L., also going by the name of St. John's wort, is an important customary medicinal plant native to Europe, but it is grown worldwide for commercial purposes. *H. perforatum* has gained international popularity mainly for the treatment of depression and wound healing (Xi-Hua and Chun-Hua, 2010). Studies on *H. perforatum* were shown to have potential as a source of novel anticancer compounds (Schempp et al., 2002). The active ingredients of this perennial herb are one of the top-selling phytopharmaceuticals in North America (Zobayed and Saxena, 2003). The complex phytochemical profile of *H. perforatum* consists of several groups of phytochemicals including the phenolic acids (chlorogenic acid), flavonoids (rutin, hyperoside, isoquercitrin, quercitrin, quercetin),

naphodianthrones (hypericin, pseudohypericin), and the phloroglucinols (hyperforin, adhyperforin). Pharmacologic activity has been attributed to several phytochemicals within St. John's Wort (Butterweck, 2003; Silva et al., 2005; Susan et al., 2001).

According to the US and the EC Pharmacopoeias, the crude drug consists of the dried flowering tops or aerial parts of the plant, at present coming almost exclusively from field-grown plants. Drug is used as an extract both in monopreparation and in multi-ingredient formulations (Bruni and Sacchetti, 2009). Three drug qualities are known: *Hyperici herba* (cuttings of the entire plant at flowering, including stem); *Hyperici herba* flowering horizon (cuttings of the upper 30 cm of the entire plant at flowering, including stem) and almost pure flowers (Bruni and Sacchetti, 2009). Today's market is supplied with St. John's Wort products from various herb producers as the plant is cosmopolitan. Therefore, available preparations of the *H. perforatum* products may differ significantly in

*Corresponding author. E-mail: rahnavard_aptin@yahoo.com.

Table 1. Sites characteristics of samples collection.

Population	Height/m	Longitude and latitude	Species	Sample names	Main plots No.
Marzanabad	1500-500	N 36 26 971	<i>Hypericum perforatum</i>	Mr1...Mr12	9-5-1
Pole zangoole	2000 to up	N 36 46 123	<i>Hypericum perforatum</i>	PI1...PI12	21-17-13
Tonekabon-Sehezar	1500-0	N 36 47 627	<i>Hypericum perforatum</i>	Ton1,...,Ton12	33-29-25
Gennat Roudbar	2200-0	N 36 48 118	<i>Hypericum perforatum</i>	Jer1,...,Jer12	45-37-41
Ghalegardan	2000-0	E 51 15 50.4	<i>Hypericum perforatum</i>	Ghl1...Ghl12	57-53-49

quality depending on a number of factors such as the different subspecies and varieties used, on the geographic location where the raw material is being grown and harvesting time (different plant development stages), and on the different and poorly controlled analysis conditions (Filippini et al., 2010). Several studies have reported variation of hypericin levels in *H. perforatum* in Australia (Campbell et al., 1997; Southwell and Campbell, 1991; Jensen et al., 1995) and Switzerland (Buter et al., 1998), but there are no reports documenting variation of Biochemical Components in *H. Perforatom* L. in Iran.

There has been only some investigation attempting to dissect the chemical composition of field-grown clonal accessions of *Hypericum* as influenced by environment or as a result of genetic variation (Buter et al., 1998). The green parts and flowers of St. John's wort contain a number of substances, including flavonoids, hypericins and other UV-B-absorbing secondary metabolites with biological effects (Erken et al., 2001). There have been several studies of the effect of environmental factors on hypericin concentrations in St. John's wort (Zobayed et al., 2007; Zou and Wei, 2004). Hypericin is a component of the inducible plant defence response of *H. perforatum* against fungal pathogens (çirak et al., 2005). Umek et al. (1999) reported a positive correlation between the concentrations of some flavonoids in *H. perforatum* and the altitude of their growing sites. It is reported that some environmental factors such as light intensity and CO₂ concentration can significantly change the secondary metabolite synthesis and production in plants.

Light is known to adjust not only plant growth and development, but also the biosynthesis of primary and secondary metabolites (Kurata et al., 1997; Zhong et al., 1991). The synthesis of medicinal components in herbs is affected by light intensity with changes in plant morphology and physiology characteristics (Kurata et al., 1997; Jaafar and Rahmat, 2008; Briskin and Gawienowski, 2001). Briskin et al. (2001) concluded that hypericin synthesis increased significantly in *H. perforatum* when grown under high light intensity (400 $\mu\text{mol m}^{-2}\text{s}^{-1}$). It seems that a high photosynthetic rate under high light intensity resulted in an increased amount of carbon assimilation and enhanced the secondary metabolites in the leaf tissues. Phenolic biosynthesis requires light or is enhanced by light, whereas flavonoids formation is absolutely light-dependent, and its

biosynthetic rate is related to light intensity and density (Xie and Wang, 2006). Previous studies showed that changes in light intensity are capable of changing the production of flavonoids and total phenols in herbs (Graham, 1998).

Michel and Klaus (2001) reported TF production related to plant pigments (chlorophyll and carotenoids). In contrast with flavonoids, the xanthophyll cycle seems to be mainly relevant to the protection of photosynthesis against sudden increase in light intensity.

This study focused on analyzing the chemical composition of hypericins, total phenolss, flavonoid and carotenoid from wild populations of *H. perforatum* collected in the north of Iran. Genetic, physiological, or environmental influences may affect hypericin yields, but such studies correlating hypericin concentrations to specific influences have not been well documented. We here in report our investigations of *H. perforatum* populations sampled from a total of five sites and three heights. It is possible that a relationship exists between Hypericin, flavonoids and total phenolss production and photosynthesis rate in this plant.

MATERIALS AND METHODS

Plants and growth conditions

Plant Materials of *H. perforatum* were collected between August and September of 2010 from five sites and three heights with four samples (totally 60 samples) in west of Mazandaran province (North of IRAN) (Table 1) including: Marzanabad(Mr1...Mr12), Polezangooleh(PI13...PI24), Tonekabon-Sehezar (Ton25,...,Ton36), Gennat Roudbar(Jer37,...,Jer48) and Ghalehgardan (Ghl49...Ghl60).

The top 1/3 of the plants crown were harvested between 9:00 AM and 1:00 PM. Conditions on the day of collection were clear and sunny at all sites. Temperatures ranged from 25 to 30°C. Samples were placed on ice during transport to the laboratory of Islamic Azad University Tonekabon Branch (IAUTB), where they were dissected into tissue parts and dried overnight (or until constant weight) at 65°C, the current temperature used by laboratory employee. Reference specimens were placed in the (IAUTB) herbarium.

Determination of hypericin, total phenolic and flavonoid contentes

Hypericin determination

Hypericin content was determined by a modified method of the

European Pharmacopoeia (2008) as follows: 80 mg of powdered sample were extracted by 6 ml of 80% tetrahydrofuran in water at 65°C for 30 min. The samples were centrifuged at 2236 g for 10 min, the supernatant was transferred to a fresh test tube and the sediment was extracted once more. After centrifugation, the supernatant was combined and 250 µl of the combined extract were transferred to a plastic microcentrifuge tube and evaporated under vacuum. The sediment was dissolved with 500 µl of methanol in an ultrasonic bath and then centrifuged at 11,269 g for 10 min. Then, 300 µl of the supernatant were transferred to a microtitre plate vial and the absorbance was measured at 590 nm. The concentration of hypericin was calculated by comparison with a hypericin standard (Roth). For each treatment, six plants were analysed, with one extraction per plant.

Total phenolic determination

Total phenols were determined by Folin Ciocalteu reagent (McDonald et al., 2001). A dilute extract of each plant extract (0.5 ml of 1:10 g ml⁻¹) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na₂CO₃ (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L⁻¹ solutions of gallic acid in methanol: water (50:50, v/v). Total phenolss values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound.

Total flavonoid determination

Total flavonoid contents were determined spectrophotometrically using AICl₃ and vanillin-HCl reagents, respectively, as previously reported (Kreft et al., 2002). In short, 20 mg of powdered St. John's wort sample was extracted in 10 ml of 60% ethanol overnight on a shaker. For the determination of flavonoids, the sample was diluted 1:6 with 60% ethanol. Two aliquots of 180 µl of diluted sample were prepared in the wells of a microtitre plate and 20 µl of 5% AICl₃ in methanol were added to the first aliquot and 20 µl of methanol were added to the second aliquot. After 30 min, the absorbance at 420 nm was measured in both solutions. The concentration was calculated from the differences in the measurements and compared with a rutin standard (Fluka, Sigma-Aldrich Corporation, St. Louis, USA).

Chlorophyll a,b and carotenoid determination

Chlorophyll was extracted in 80% acetone and the absorption at 663 nm (Ca: Chlorophyll a), 645 nm (Cb : Chlorophyll b) and 470 nm (Cx+c : Carotenoid) was read in an UV-160 spectrophotometer. Chlorophyll and carotenoid contents were calculated using the absorption coefficients (Arnon, 1949; Witham et al., 1971).

Ca = 12.25 A 663.2 to 2.79 A 645.2; Cb = 21.5 A 645.8 to 5.1 A 663.2; Total chlorophyll = Ca + Cb;
Cx+c = (1000 A470-1.82 Ca -85.25 Cb) /198

Total protein extraction

A method suitable for high-quality protein extraction from *H. perforatum* tissues was optimized during the present study. The qualitatively and quantitatively optimized protocol based on sodium borate extraction and phenol/methanolic ammonium acetate. The protein concentration of the extracted samples was measured according to a study by Bradford (1976). Analysis of proteins in

their native form is carried out in polyacrylamide buffer gel. The classical disc electrophoresis using cylindrical gels has been described by Davis (1964).

Statistical analyses

The data were analyzed by Correlation bivariate and Regression (SPSS version 13.0- SPSS Inc., Chicago, IL, USA) and significance was accepted at P < 0.05.

RESULTS AND DISCUSSION

The correlation of studied traits showed a high positive correlation between the amounts of hypericin and total phenols (Table 2) which would be stated by linear relation ($Y = 0.07234 + 0.000444 X$) (Figure 1). While hypericin increases, total phenols increases as well, and vice versa. This may be associated with hypericin and phenol contents. There is also a significant correlation between hypericin and flavonoid (Table 2).

However, their relationship is as a second degree equation ($Y = -76.58 + 974.7X - 2468X^2$) in which at first, the mean value of flavonoid increases with increase in hypericin amount and after a peak, it decreases with decrease in hypericin content (Figure 2). This is may be ascribed to investing priority in order to produce antrons and flavonoids according to ecologic situation. Whereas, there is a negative correlation between hypericin and carotenoid contents as well as total chlorophyll (Table 2), since secondary metabolites production while the plant needs them in any challenge means consuming substrates in order to produce secondary metabolites instead of pigment production, which is the result of different pathways like respiratory ones (Figure 3).

Measuring total phenols demonstrated its positive correlation with flavonoid and following the second degree equation of ($Y = -25.39 + 0.3258X - 0.00058X^2$) (Figure 4). It should be noted that regression coefficients for equations of hypericin and total phenols with flavonoid is less than 90%. This can be interpreted as hypericin itself is composed of components such as pseudohypericin, protohypericin, etc. and total phenols itself is composed of other components such as caffeic, chlorogenic, paracoumaric, ferulic, parahydroxibenzoic, and vanillic acids (Sirvent et al., 2002). Flavonoid, on the other hand, is composed of flavonol (such as kaempferol, quercetin), flavones (such as hyperoside, isoquercetin, quercetin, rutin), biflavonoids as biapigenin, amentoflavone (a derivative of biapigenin) and catechines (flavonoids often together with tannens) (Barnes et al., 2001). Therefore, it is better to deal with relations ruling their components in order to distinguish their qualitative relations. Results have shown that there is a negative correlation between total phenols and total chlorophyll (Table 2) and the result is the same as hypericin reaction. Measuring carotenoid reveals its positive relationship with chlorophyll-a and total

Table 2. Corellation coefficients among various traits.

Variatons	Total phenoles	Carotenoid	Flavonoid	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Hypericin	0.992**	-0.185 ^{n.s}	0.992**	-0.322**	0.229 ^{n.s}	-0.343**
Total chlorophyll	-0.361**	0.56**	0.23 ^{n.s}	0.707**	0.844**	-
Chlorophyll b	-0.263*	0.223 ^{n.s}	0.114 ^{n.s}	0.217 ^{n.s}	-	-
Chlorophyll a	-0.31*	0.713**	0.268*	-	-	-
Flavonoid	-0.903*	0.091 ^{n.s}	-	-	-	-
Carotenoid	-0.185 ^{n.s}	-	-	-	-	-

**Significant at the 0.01 level. *Significant at the 0.05 level .

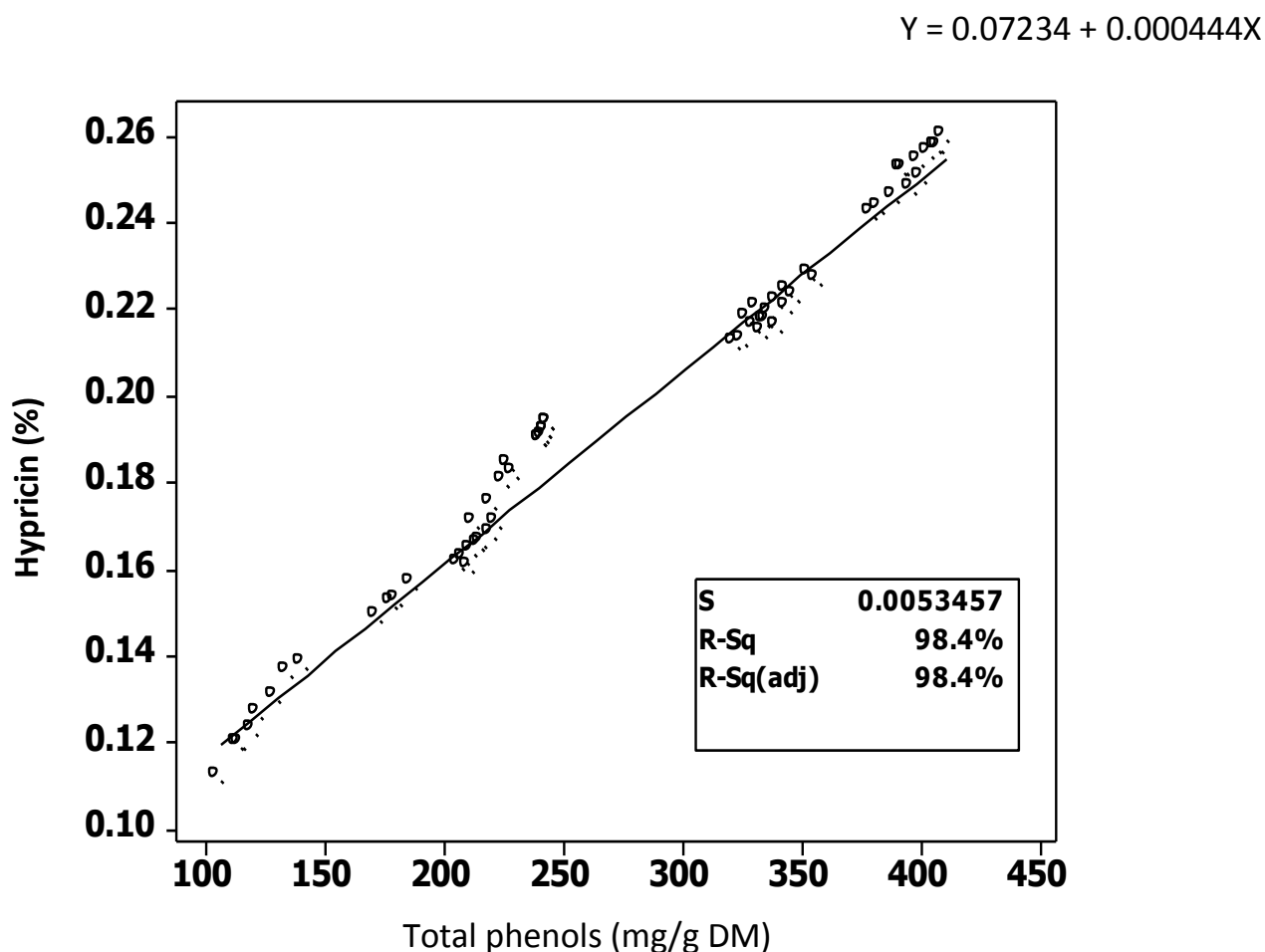


Figure 1. Variations in amount of Hypericin and total phenols contents in collected samples.

chlorophyll (Table 2) indicating stronger relation between chlorophyll a and minor pigments. On the other hand, chlorophyll a indicated positive correlation with chlorophyll a:b proportion, so that we could say chlorophyll a is a parameter having correlation—whether positive or negative—with most measured parameters that is, chlorophyll-a has a role in synthesis rate of most plant metabolites according to its critical role(Figure 5). Such results were also in accordance with previous report by Khan et al. (2000). Chlorophyll-b variations indicated that

this photosynthesizing pigment had positive correlation with total amount of chlorophyll and had negative correlation with chlorophyll a:b proportion (Table 2). Current study demonstrated that the highest amount of hypericin (Figure 6) and total phenols contents (Figure 7) is related to Jannat region of Roodbar with an altitude of 1218 higher than sea level and the highest amount of flavonoid, carotenoid and total chlorophyll contents collected samples was measured at 2300 m altitude of Pole Zangoole (Figures 8, 9 and 10). Protein bands

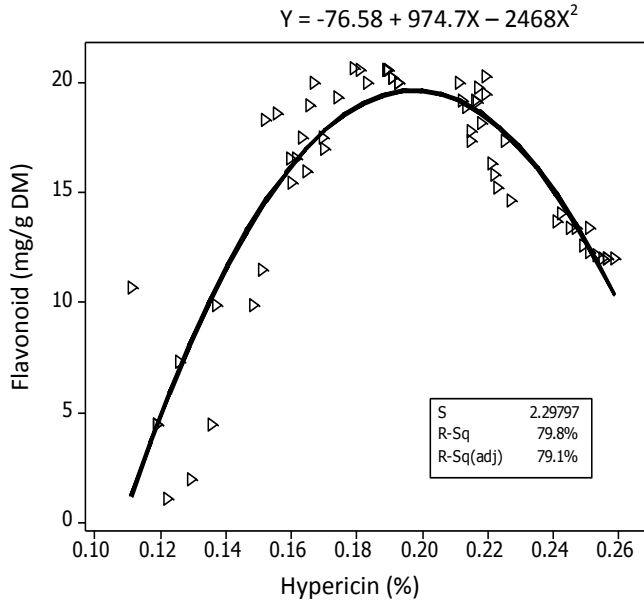


Figure 2. Variations in amount of Hypericin and Flavonoid contents in collected samples.

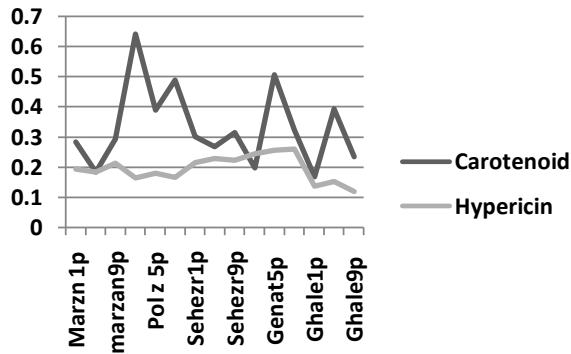


Figure 3. Variations in amount of Hypericin (%) and Carotenoid (mg/ml) contents in samples of main plots.

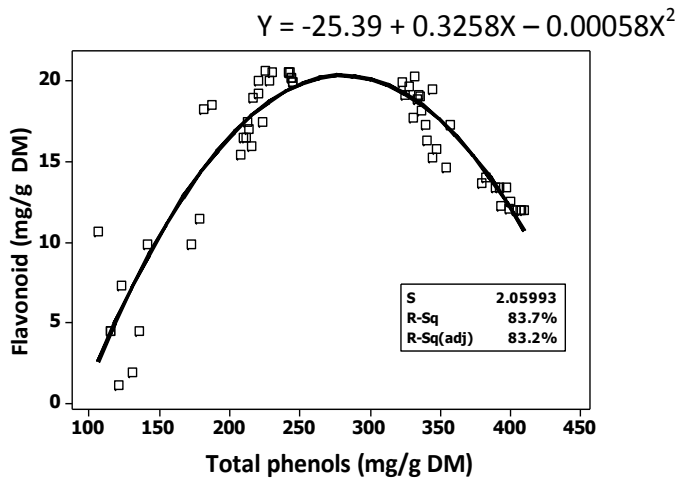


Figure 4. Variations in amount of total phenols and Flavonoid contents in collected samples.

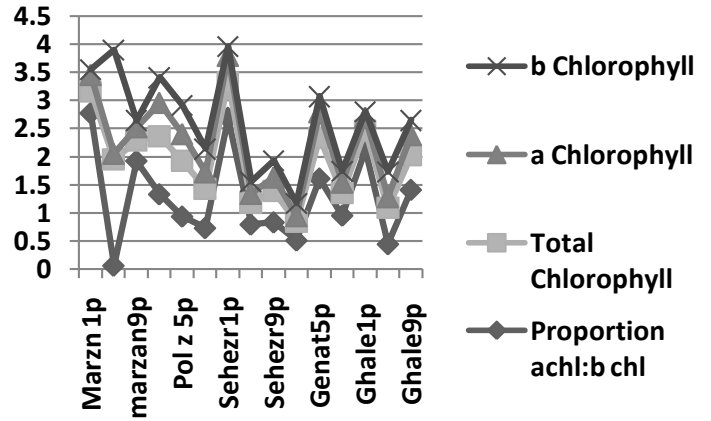


Figure 5. Variations in amounts of Pigments in samples of main plots.

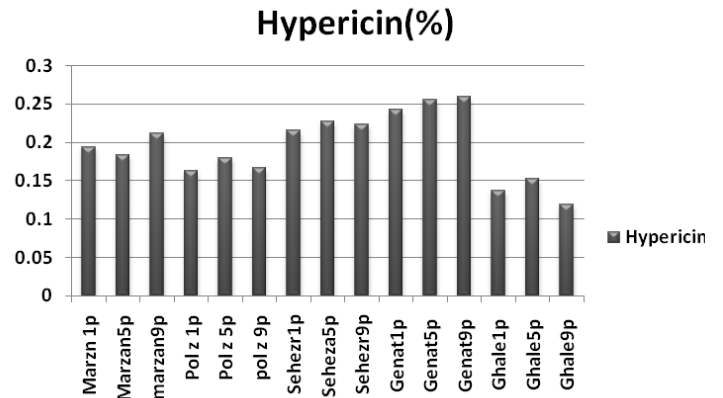


Figure 6. Variations in amount of Hypericin content in samples of main plots.

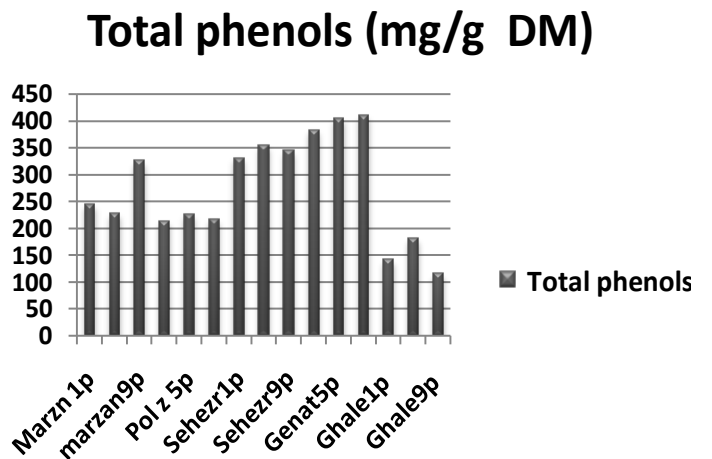


Figure 7. Variations in amount of total phenols content in samples of main plots.

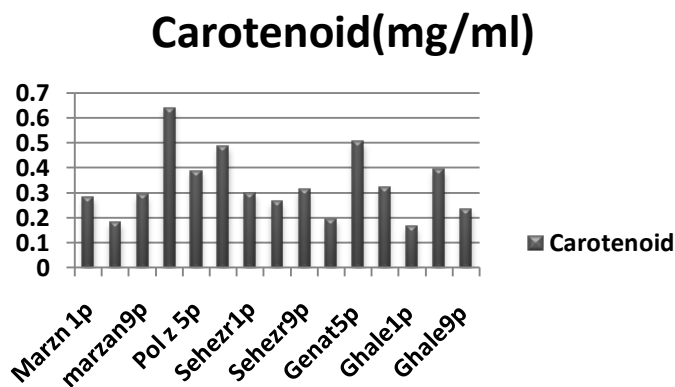


Figure 8. Variations in amount of Carotenoid content in samples of main plots.

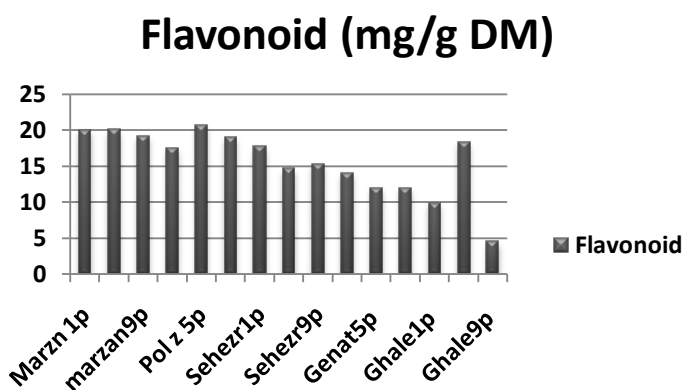


Figure 9. Variations in amount of Flavonoid content in samples of main plots.

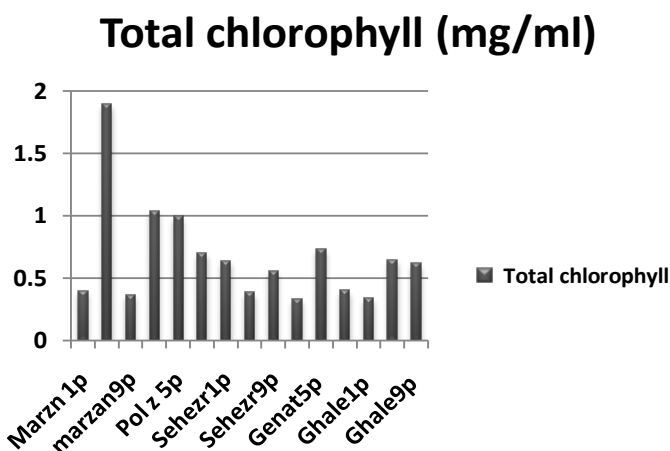


Figure 10. variations in amount of total chlorophyll content in samples of main plots.

Indicated the highest amount of protein accumulation in

major plot number 13 (Jannat Roodbar region with an altitude of 1218 higher than sea level) which could be stated that protein accumulation is increased due to environmental challenges which most probably relates to reductase type enzymes and increase in hypericin and total phenols amount indicates high activity of anti-challenge mechanisms in this place (Figure 11, band number 13).

The study determined that change in environmental factors could change hypericin, total phenols, flavonoids and total protein synthesis in *H. perforatum L.* (Southwell and Bourke, 2001). In this relation, differences in longitude and latitude would be an important factor in producing secondary metabolites since it is so much impressive in the subject of determining production position and potential in establishing a new pattern of herb cultivation. In addition to other environmental factors (humidity, soil and temperature) in the subject of altitude and effective materials, the most impressive factor is light and its quality, which affects the amount of aforementioned metabolites (Kefeli et al., 2003). Changes in amounts of chlorophyll a and b and dominant relations among them itself indicates variability of photosynthesis system by environmental situations which is followed by affecting production cycles of secondary metabolites (Khan et al., 2000). Briskin and Gawienowski (2001) demonstrated that hypericin synthesis significantly increased under intensified light. Photosynthesis speed seems to increase in intensified light which consequently increases carbon assimilation and in turn, increases secondary metabolites in vegetative body. Phenol compound biosynthesis has also direct relation with amount of light while flavonoids formation relates to light wave length particularly ultraviolet light (Xie and Wang, 2006). The amount of chlorophyll a and b differs with change in altitude and light which affects the amount of photosynthesis and consequently metabolite amounts and quantitative relations among them.

According to performed studies, flavonoid changes could change electron transport speed of photosynthesis photosystem which in turn affects photosynthesis speed (Fan et al., 1998). Any reason decreasing photosynthesis speed causes carbon to divert from photosynthesis cycle to shikimic acid and therefore increases flavonoid amount. The reaction indicates that it is possible for phenol compounds and flavonoids to regulate plant growth and improve photosynthesis efficiency and affect dividing photosynthesis materials through affecting resource-reservoir relation.

ACKNOWLEDGEMENT

We are grateful to thank for the financial support granted by the research section of Islamic Azad University Tonekabon Branch, Tonekabon, Iran.

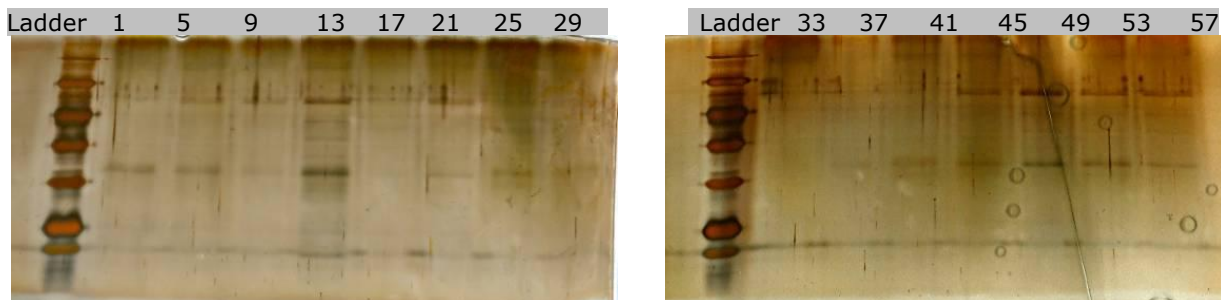


Figure 11. Banding of total proteins extracted from the main plots (plot numbers are listed in Table 1).

REFERENCES

- Arnon DI (1949). Antioxidants and free radical oxidation as regulators of ATP biosynthesis in chloroplasts. *Plant Physiology*, p. 241.
- Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*, 72: 248-254.
- Briskin DP, Gawienowski MC (2001). Differential effects of light and nitrogen on hypericins and leaf glands in *Hypericum perforatum*. *Plant Physiol.*, 39: 1075-1081.
- Bruni R, Sacchetti B (2009). Factors affecting polyphenol biosynthesis in wild and field grown St. John's wort (*Hypericum perforatum* L. Hypericaceae/Guttiferae). *Molecules*, 14: 682-725; doi: 10.3390/molecules14020682.
- Butterweck V (2003). Mechanism of action of St. John's wort in depression: What is known? *CNS Drugs*, 17: 539-562.
- Buter BC, Orlacchio A, Soidati B, Berger K (1998). Significance of genetic and environmental aspects in the field cultivation of *Hypericum perforatum* L. *Planta Medica*. 64: 431-437.
- Campbell MH, May CE, Southwell LA, Tomlinson JD, Michael PW (1997). Variation in *Hypericum perforatum* L. (St. John's wort) in New South Wales. *Plant Prot. Q.*, 12: 64-66.
- çirak C, Aksoy HM, Ayan AK, Saglam B, Kevseroglu K (2005). Enhanced hypericin production in *Hypericum perforatum* and *Hypericum pruinatum* in response to inoculation with two fungal pathogens. *Plant Prot. Sci.*, 41: 109-114.
- Davis BJ (1964). Thin-layer acrylamide gel electrophoresis. *Ann. N. Y. Acad. Sci.*, 121: 404.
- Baser KHC (2001). Chemical investigations on some *Hypericum* species growing in Turkey – I. *Chemistry of Natural Compounds*, 37: 434-438.
- European Pharmacopoeia (2008). St. John's wort, In: *European Pharmacopoeia* (6th ed.). Strasbourg, France: Council of Europe, 2958-2959.
- Fan Y, Wang Y, Tan R, Zhang Z (1998). Seasonal and sexual variety of ginkgo flavonol glycosides in the leaves of *Ginkgo biloba* L. *J. Trad. Chin. Med.*, 23: 267-269.
- Filippini R, Piovan A, Borsarini A, Caniato R (2010). Study of dynamic accumulation of secondary metabolites in three subspecies of *Hypericum perforatum*. *Fitoterapia*, 81: 115-119.
- Graham TL (1998). Flavonoid and flavonol glycoside metabolism in *Arabidopsis*. *Plant Physiol. Biochem.* 36: 135-144.
- Jaafar H, Rahmat A (2008). Accumulation of partitioning of total phenols in two varieties of *Labisia pumila* benth under manipulation of greenhouse irradiance. *Acta Hort.* 797: 387-392.
- Jensen KIN, Gaul SO, Specht EG, Doohan DJ (1995). Hypericin content of Nova Scotia biotypes of *Hypericum perforatum* L. *Canadian J. Plant Sci.*, 75: 923-926.
- Kefeli VI, Kalevitc MV, Borsari B (2003). Phenolic cycle in plants and environment. *J. Cell Mol. Biol.*, 2: 13-18.
- Khan SR, Rose R, Haase DL, Sabin T (2000). Effects of shade on morphology, chlorophyll concentration, and chlorophyll fluorescence of four Pacific Northwest conifer species. *New For.*, 19: 171-186.
- Kreft S, Strukelj B, Gaberščik A, Kreft I (2002). Rutin in buckwheat herbs grown at different UV-B radiation levels: Comparison of two UV spectrophotometric and an HPLC method. *J. Exper. Bot.*, 53: 1801-1804.
- Kurata H, Matsumura S, Furusaki S (1997). Light irradiation causes physiological and metabolic changes for purine alkaloid production by a *Coffea Arabica* cell suspension culture. *Plant Sci.*, 123: 197-203.
- McDonald S, Prenzler PD, Autolovich M, Robards K (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chem.*, 73: 73-84.
- Michel H, Klaus K (2001). The protective functions of carotenoids and flavonoids pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis*. *Planta*, 213: 953-966.
- Schempp C, Kirkin M, Simon-Haarhaus V, Kersten A, Kiss J, Termeer C, Gilb C, Kaufmann B, Borner T, Sleeman JPC, Simon JC (2002). Inhibition of tumour cell growth by hyperforin, a novel anticancer drug from St John's wort that acts by induction of apoptosis. *Oncogene*. 21: 1242-1250.
- Silva BA, Ferreres F, Malva JO, Dias ACP (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem.*, 90: 157-167.
- Southwell IA, Campbell MH (1991). Hypericin content variation in *Hypericum perforatum* in Australia. *Phytochemistry*, 30: 475-478.
- Southwell IA, Bourke AC (2001). Seasonal variation in hypericin content of *Hypericum perforatum* L. (St. John's wort). *Photochemistry*, 56: 437-441.
- Susan H, Kopleman A, Larry L, William S, Fran X (2001). Selected physical and chemical properties of commercial *Hypericum perforatum* extracts relevant for formulated product quality and performance. *AAPS PharmSci*: 3 (4) article 26.
- Umek A, Kreft S, Kartnig T, Heydel B (1999). Quantitative phytochemical analysis of six *Hypericum* species growing in Slovenia. *Planta Medica*, 65: 388-390.
- Witham FH, Blaydes DF, Devlin RM (1971). *Experiments in plant physiology van nostrand* New York, p. 245.
- Xie BD, Wang HT (2006). Effects of light spectrum and photoperiod on contents of flavonoid and terpene in leaves of *Ginkgo biloba* L. *J. Nanjing For. Univ.*, 30: 51-54.
- Xi-Hua C, Chun HW (2010). Adventitious root suspension cultures of *Hypericum perforatum*: Effect of nitrogen source on production of biomass and secondary metabolites. *In vitro Cell. Dev. Biol. -Plant*.
- Zobayed SMA, Saxena PK (2003). *In vitro*-grown roots: A superior explants for prolific shoot regeneration of St. John's wort (*Hypericum perforatum* L. cv 'New Stem') in a temporary immersion bioreactor. *Plant. Sci.* 165: 463-470.
- Zobayed SMA, Afreen F, Kozai T (2007). Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition. *Environ. Exper. Bot.*, 59: 109-116.
- Zhong JJ, Seki T, Kinoshita S, Yoshida T (1991). Effect of light irradiation on anthocyanin production by suspended culture of *Perilla frutescens*. *Biotechnol. Bioeng.*, 38: 653-658.
- Zou Y, Lu Y, Wei D (2004). Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. *J. Agric. Food Chem.*, 52: 5032-5039.