Journal of Medicinal Plants Research Vol. 5(17), pp. 4157-4161, 9 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Investigation of the effect of light intensity on five essential oils of *Hypericum perforatum* L: A case study of Ramsar, Mazandaran, Iran

Keivan Saeb[1]*, Ali Askar Kiaee[1], Mahmoud Asadi[1], PourShamsian Khalil[1], Jafari Hajati Razieh[1] and Eslami alireza[2]

[1]Department of Plant Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.
[2]Islamic Azad University, Branch of Rasht, Iran.

Accepted 22 June, 2011

The present study investigated five essential oils (EOs) of *Hypericum perforatum* including α- pinene, myrcene, cineol, tridecane and phytol in different light situation. For this purpose, examinations were done at 700 m heights of the Javaherdeh Forest, Ramsar City, Mazandran Province, Iran. Firstly, light measurement was done using camera equipped with fish eyes lens. Results were tabulated in five light classes: 0 to 20, 20 to 40, 40 to 60, 60 to 80, and 80 to 100%. Flowers of the studied area were sampled, dried, grained, and its essences were extracted three times for each class. Finally, in determining the percent, essence analysis was done by the Clevenger machine and Gs-Ms technique. 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.

Key words: *Hypericum perforatum*, light, essential oils (EOs), light class.

INTRODUCTION

Hypericum perforatum L. (St. John's wort) is one of the most important medicinal plants, used as a medical herb for over 2000 years (Patoĉka, 2003). *Hypericum* is a genus with about 450 species from the Guttiferae family, formerly often treated separately in their own Hypericacea family (Bertoli et al., 2011). The average height is 40 to 60 cm which sometimes grown up to 100 cm. The budding time is at the end of May and the best time for harvest is June (Lebaschi et al., 2002; Maleš et al., 2006). Among the pharmacological activities of H. perforatum extracts, antidepressant, antiviral and antibacterial effects are crucial reasons of its traditional uses (Barnes et al., 2001). In herbal medicine, *H. perforatum* L. was often applied to the treatment of burns, skin injuries, neuralgia, fibrositis, sciatica and depression (Barnes et al., 2001; Patoĉka, 2003). The plants as a part of the environment have an important value in human life.

*Corresponding author. E- mail: keivan.saeb@toniau.ac.ir.

The quantity and quality of effective material of *H. perforatum* species would be changed by geographic characters of growth place such as; locality, weather, and also time. The photo period, the quality, and the intensity would have effect on secondary metabolite biosynthesis and plant growth. Though, the secondary metabolite level depends on genes (Buter et al., 1998) but, the amount is significantly related to the environment situation (Yanive and Palevitch, 1982). Several authors indicate that St. John's wort requires abundant light for best development. Occurrence in open grasslands and open or disturbed forest sites, but not within undisturbed, dense forests, dense brush fields, or under the shade of trees in open forests are the clear evidence for this claim. Typically, where St. John's wort occurs in forested areas, it is often observed along roads and/or forest edges (MacQuarrie and Lacroix, 2003; Parendes and Jones, 2000; Walker, 2000). Many plito-chemical studies was done on *H. perforatum* L. whereas investigating the effect of light intensity, as an important ecological factors on the essential oils (EOs) constituents, was ignored somewhat.

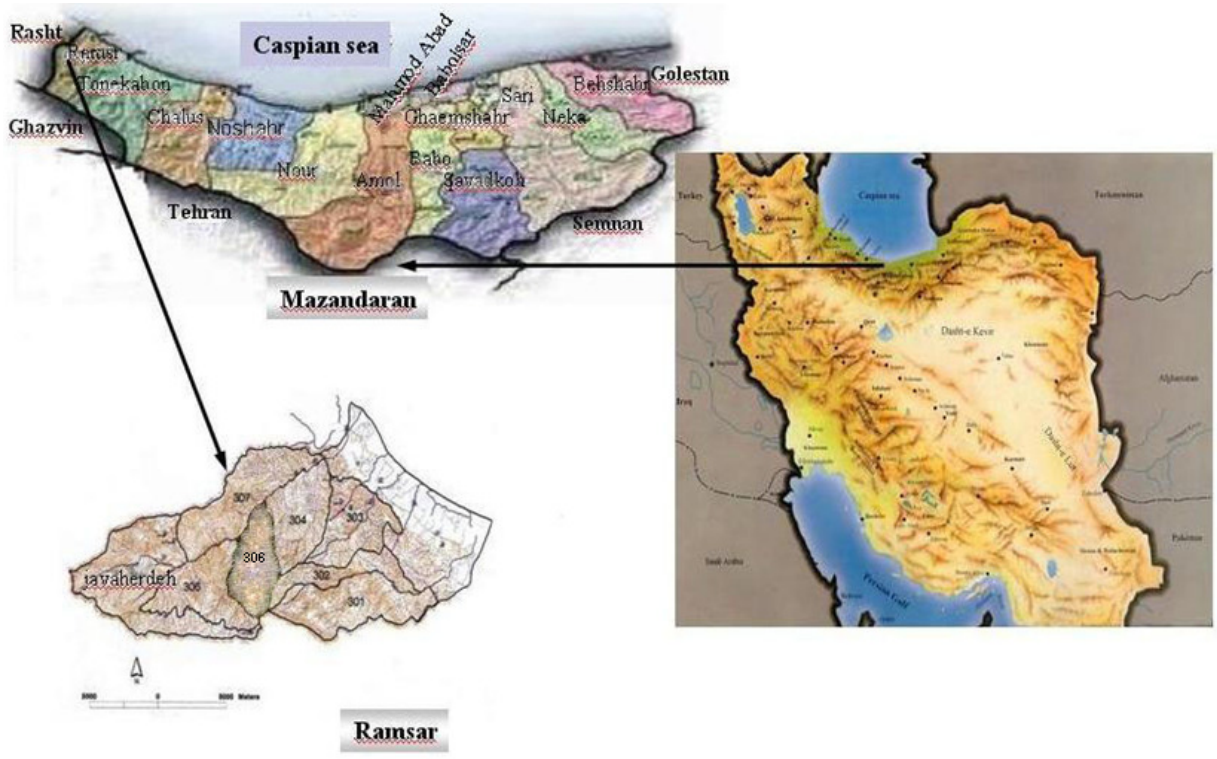

Figure 1. Map of the study area located in Javaherdeh, Ramsar, Mazandaran, Iran.

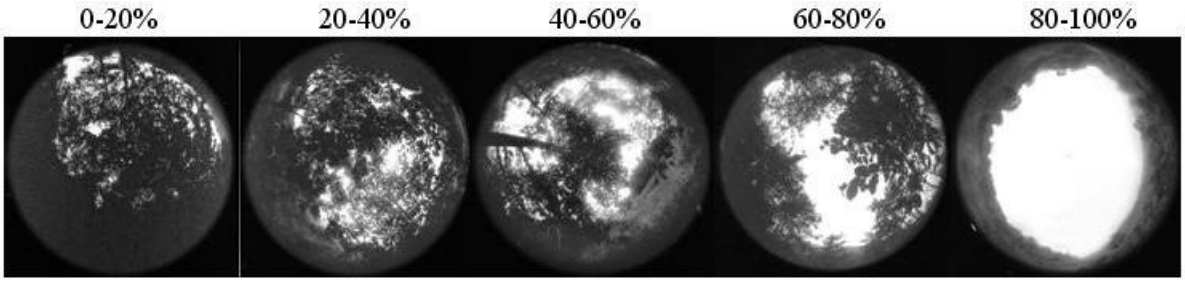

Figure 2. Sample picture of studied area in five light classes.

With regard to practical use of this plant in medicine industry and commercial value of it in compare with other genus of this family (Fegert et al., 2006; Canning et al., 2010; Linde, 2010; Bertoli et al., 2011) the present study is of high importance.

MATERIALS AND METHODS

The studied area located in 700 m heights of Javaherdeh Forest, Ramsar City, Mazandran Province, Iran, stretched between N 36°53′11.68″ and E 50°34′11.68″ (Figure 1). 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 were 32.6 and -3°C, and the average yearly rainfall was 1107 ml. After recognition of the study area with good density of St. John's wort, the operation light measurement was done in June, 2009. The location was splited to different parts from relative measure of light. Field stage of light measure operation was carried out using camera canon, AE1 Model fish eyes lens with the focal space of 8 mm black and white film, mark of forte with sensitivity of 200, photography tripod and balance. After choosing the regeneration pit of this plant, first the pit area was measured, then the pit center was determined and the photography tripod settled. The fish eye lens has the ability to make the all surface like a cane and transfer it on film surface like a circle. Referring to this ability, the arriving light to the pit is measurable. Finally, the pictures are edited with high contrast.

Second step progresses by an arachnidan grille transferred to software called PF and the scanned photos. By identifying the center and one point around photo, light measurement operation was done in digital form.

The sampling area is classified into five classes (Figure 2). Then *H. perforatum* flowers in light measuring area and the same height

Table 1. Result of variance analysis among essential oils content in *Hypericum perforatum* in five light classes.

EOs	Source changes	Sum of squares (ss)	Mean square (m.s)	Degrees of freedom (d.f)	F
α -pinene	Light class	34.503	8.6259	4	130.39**
	Error	0.6615	0.0662	10	
Myrcene	Light class	2.747	0.6867	4	239.01**
	Error	0.0287	0.0028	10	
Cinoel	Light class	0.382	0.0956	4	124.73**
	Error	0.0076	0.00077	10	
Tridecane	Light class	0.318	0.0795	4	71.48**
	Error	0.011	0.00111	10	
Phytol	Light class	1.008	0.252	4	444.8**
	Error	0.006	0.00056	10	

** Significance in 0.01 level.

Table 2. Result of average comparison among essential oils content in *Hypericum perforatum* in five light classes.

EOs	Light classes				
	0-20%	20-40%	40-60%	60-80%	80-100%
α -pinene	1.42^{cb}	1.25^{c}	1.79^{b}	1.60^{b}	5.28^{a}
Myrcene	1.04^{c}	1.05^{c}	1.62^{b}	1.97^{a}	2.02^{a}
Cinoel	1.68^{b}	1.71^{b}	2.01^{a}	2.07^{a}	1.97^{a}
Tridecane	1.69^{b}	1.67^{b}	1.90^{a}	2.00^{a}	1.96^{a}
Phytol	0.20^{b}	0.17^{b}	0.18^{b}	0.20^{b}	0.84^{a}

The averages that have one common letter by Turkey exam do not have different in probability level of 0.05.

and weather gathered in three repeat of each lightening class, the samples are transferred to laboratory. After being sure of samples' purity and removing all pollution, they were dried by pressing operation for better essence extraction and then ground. Since the main essence of St. John's wort is in the secretion sack of its leaf, the budding branch of the sample was gathered and powdered in the clean grinding instrument. The operation of essence extraction was done by hydro distillation system, Clivenger apparatus, which include 2 L balloon, distiller, and warmer set. For essence extracting, 100 g of dried plant powder mixed with 300 ml distilled water into a balloon and the essence extraction operation was carried out by Clivenger apparatus. In this research, for qualitative determining and analyzing EOs of samples, the Ge-Ms technique was used. Inventory analyzing was done using Minitab 13 and Excel with Randomized Complete Design.

RESULTS

With regard to the photos taken from canopy cover of trees, the sampling area was classified into five light classes (Figure 2). As shown in Figure 2, light intensity would gradually increase from one class to another. The maximum and minimum lights, which come to the floor of forest, settled in 80 to 100%, and 0 to 20% classes of light, respectively.

By the results of extracting essence, the percent of EOs in St. John's wort was estimated as 0.5%. Interpreting Gc-Ms spectrum of mixtures of this plant, 20 main EOs determined after analyzing, 5 of which were chosen according to their importance in medical and perfume industry. These EOs includes cinoel, phytol, α -myrcen, α -pinen, tridecane.

Analysis of variance of percent in five light classes show a completely significance difference between the percent of EOs in studied light classes in level of 99% ($p<0.01$) probability (Table 1). Overall, it means that the average percent of EOs of this plant showed significant changes from one class of light to another one.

According to Table 2, comparing the average of ever mixture in the level probability of five percent ($P< 0.05$), while probability the maximal average percent of EOs are α –pinene (5.28%), myrcene (2.02%), phytol (0.84%) were observed in 80 to 100% of light class. The average percent of α -pinene's EOs in 80 to 100% of light classes show a higher measure compared to other mixtures. The

average percent of phytol has just significant difference in 80 to 100% light class compared to other classes. Therefore, the EO of phytol did not decreased from 0 to 20% class of light to 60 to 80%. The maximal averages of cineol (2.07) and tridecane (2.00) are in 60 to 80% of light class.

There is no significant difference between EOs average percent in all studied mixtures in 0 to 20 and 20 to 40% of light classes. It means that there is no significant increase or decrease in EOs of studied plant from 0 to 20% to 20 to 40% of light classes. But in comparing to other classes, this different is meaningful and the amount of EOs gradually increased by increasing of light.

DISCUSSION

Relative access to light is very important for utmost grassy and medicinal plant. Several investigations have been done about the effect of environmental condition on the plant's growth, and also the effect of different geographical locations on the plant's effective materials (Pietta et al., 2001; Walker et al., 2001; Zobayed et al; 2005). Despite the large number of species, only *H. perforatum* has been studied in depth by the pharmaceutical industry to control the content of its well known bioactive constituents: hypericins, hyperforins and flavoniods in the flowering aerial parts (Bertoli et al., 2011).

According to Campbell et al. (1995), at the heights more than 1500 m, there is no sign of this plant, because of the absence of plant's permanence and seed germination in very low temperature. The measure of effective materials of this plant depends on height. So, when the height increases, the level of total flavonoid will decrease.

Therefore, the studied plant gathered of 700 m height with dominant temperature and suitable density. The researches done by Dorri et al. (2008) support this sentence. Investigation of Hypericin content of (St. John's wort) in natural habitats of Golestan Province showed that the highest content of hypericin was obtained from Tusakaestan sample (0.26 mg/g) in class 450 to 750 m. Observing the chemical mixture table from St. John's wort in five different light classes, five mixtures were evaluate and showed different levels in different light classes. By increasing light, the percent of these mixtures were increased (Table 2). This result is in accordance with the experiences results of appendix researchers. Briskin and Gawienowski (2001) indicated that by increasing the light intensity up to triple of witness treat in the laboratory culture of this plant, the amount of hypersin increased. Makovetskaya (1992) studied the tanon level changes in this plant, through two different light ware lengths. He showed that in light intensity of 5000 lox, compared to light intensity of 18000 lox, the level of tanon will decrease. In other words, shadows would cause decrease in the gathering of tanon in this plant.

One of the special characteristic of *H. perforatum* is the presence of colorful glands in form of distributed small spots on the plant organs. The number of these glands will increase by the photosynthesis light intensity (Zobayed et al., 2005, 2006). In this research the samples extracted from the flowering top branches.

Walker et al. (2001), by studying the measure of effective material in *H. perforatum* L. find out that the effective material achieved from flowering top branches and leaves are more than the effective material in stem. Akhbari and Batooli (2009) perceived that EOs are 55 compounds, while for leaves flower and fruit are 26 compounds. The maximum of effective materials and essential oil are when the plant spend it's polintion and the inflorescences have mainly open flowers (Pietta et al., 2001; Walker et al., 2001; Zobayed et al., 2005). So, the end of June is the best time for harvest of this medicine plant because the plant in the studied area passed the natal step and has arrived to growth step.

In this research, ل-pinene is the main component which is in close relation with the result of Akhbari and Batooli (2009) and Sharopov et al. (2010). *H. perforatum* is light demander, thus increasing the amount of light would have effect on this plant by increasing the operation of essences. However, the main effective material would be active by increasing the amount of light. Significance difference between lighting classes (Table 1) indicated the effective role of light on changing the effective material of this plant.

The knowledge of changes in essences level in nature would help in recognition of the needs of this plant in agricultural situation (Lebaschi et al., 2002). The amount of light over 60% will result to maximum production of EOs, whereas, the present study aims to harvest high percent of essences. In addition, the results can be applied to sampling and selection of the seeds for improvement the generation of this plant by more EOs. Natural ecosystem as the inheritance resources, and the production area which has high effect on effective material production should be known. Also with cultivating in different areas and applying agricultural treated, the area's ability would distinguished.

REFERENCES

Akhbari M, Batooli H (2009). Composition of *Hypericum perforatum* L. Volatile Oil from kashan Oil Composition of *Hypericum perforatum* L. America-Eurasia. J. Sustain. Agric., 3(1): 107-110.

Barnes J, Anderson LA, Phillipson JD (2001). St John's wort (*Hypericum perforatum* L.): A review of its chemistry, pharmacology and clinical properties. Pharm. Pharmacol., 53(5): 583-600.

Bertoli A, Cirak C, Teixeira DSJA (2011). Hypericum Species as Sources of Valuable Essential Oil. Med. Aromat. Plant Sci. Biotechnol., 5(1): 29-47.

Briskin DP, Gawienowski MC (2001). Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. Plant Physiol. Biochem., 39(12): 1075-1081.

Buter H, Hemmelder MH, Navis G, De JPE, De ZD (1998). The blunting of the antiproteinuric efficacy of ACE inhibition by high sodium intake can be restored by hydrochlorothiazide. Netherlands. J. Nephrol.

Dial. Transplant, 13(7): 1682-1685.
Campbell RB, Sinclair DAR, Couling M, Brock HW (1995). Genetic interactions and dosage effects of Polycomb group genes of Drosophila, Mol. Gen. Genet., 246(3): 291-300.
Canning S, Waterman M, Orsi N, Ayres J, Simpson N, Dye L (2010). The efficacy of *Hypericum perforatum* (St. John's wort) for the treatment of Premenstrual Syndrome: A randomized, double-blind, placebo-contolled trial. CNS Drugs, 24: 207-225.
Dorri MA, Hoseini HSA, Lebaschy MH (2008). Investigation of Hypericin content of *Hypericum perforatum* L. in two natural habitats of Golestan province. Iran. J. Med. Aromat. Plants, 24(2(40)): 117-125.
Fegert JM, Kolch M, Magno Zito J, Glaeske G, Janhsen K (2006). Antidepressant Use in Children and Adolescents in Germany. Child Adolesc. Psychopharmacol., 16(1-2): 197-206.
Lebaschi M, Matin A, Sharifi AAA (2002). Comparing agricultural and natural ecosystems in production of hyperisin. Iran. J. Res. Prod., 7(1): 48-54.
Linde K (2010). *Hypericum* extract worse than placebo in a trial in irritable bowel syndrome patients. Focus Altern. Complement. Ther., 15(3): 242-243.
MacQuarrie K, Lacroix C (2003). The upland hardwood component of Prince Edward Island's remnant Acadian forest: determination of depth of edge and patterns of exotic invasion. Can. J. Bot., 81: 1113-1128.
Makovetskaya EY (1992). Comparative study of the growth and productivity of some Hypericum L. species cultivated in Ukraine during the first year of vegetation. Rastitel'nye Resursy, Cent. Repub. Bot. Gard., Acad. Sci. Ukr., Kiev, Ukraine, 28(3): 59-67.
Maleš Ž, Brantner AH, Sović K, Pilepić KH, Plazibat M (2006). Comparative phytochemical and antimicrobial investigations of *Hypericum perforatum* L. *subsp. perforatum* and *H. perforatum subsp. angustifolium* (DC) Gaudin. Acta Pharm., 56: 359-367.
Parendes L, Jones J (2000). Role of light availability and dispersal in exotic plant invasion along roads and streams in the H. J. Andrews Experimental Forest Oregon: Ecological effects of roads. Conserv. Biol., 14(1): 64-75.
Patočka J (2003). The chemistry, pharmacology and toxicology of the biologically active constituents of the herb *Hypericum perforatum* L. Appl. Biomed., 1: 61-70.
Pietta P, Gardana C, Pietta A (2001). Comparative evaluation of St.John's Wort from different Italian regions. Farmacol., 56(5-7): 491-496.
Sharopov FS, Gulmurodov IS, Setzer WN (2010). Essential oil composition of Hypericum perforatum L. and *Hypericum scabrum* L. growing wild in Tajikistan. J. Chem. Pharm. Res., 2(6): 284-290
Walker L (2000). St. John's wort (*Hypericum perforatum* L. Clusiaceae): biochemical, morphological, and genetic variation within and among wild populations of the northwestern United States. Portland, OR: Portland State University. Thesis, p. 162.
Walker L, Sirvent GD, Vance N (2001). Regional differences in hypericin and pseudohypericin concentrations and five morphological traits among *Hypericum perforatum* plants in the north western United States. Can. J. Bot., 79: 1248-1255.
Yanive Z, Palevitch D (1982). Effects of drought on the secondary metabolite of medicinal and aromatic plants, In: Atal, C.K. and B.M. Kapur (Eds.). Cultivation and Utilization of Medicinal Plants. CSIR Jammu-Tawi, India, pp. 1-23.
Zobayed SM, Afreen F, Kozai T (2005). Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. Plant Physiol. Biochem., 43(10-11): 977-984.
Zobayed SM, Afreen F, Goto E, Kozai T (2006). Plant–Environment Interactions: Accumulation of Hypericin in Dark Glands of *Hypericum perforatum*. Oxf. J. Ann. Bot., 98(4): 793-804.