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FIRST CULTIVATION OF *HYPERICUM PERFORATUM* L. UNDER LOCAL EGYPTIAN CONDITIONS

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Abstract

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Several efforts have been paid for growing *Hypericum* in Egypt adopting the usual agricultural practices in the open field however, none of these trials succeeded. All the trials led to just creeping plants without erect stems or flowering. Therefore, in the present study we tried to grow the plant under artificial conditions adopting a light regime aiming to get normal plants as a source for producing a standardized *hypericum* extracts for the first time in Egypt. Two types of St. John's Wort (*Hypericum perforatum* L.), i.e. *Hypericum perforatum* L. and *Hypericum perforatum* var. Topas were used in the present investigation. The vegetative growth and flowering of the plants were compared in the green house as well as in the open field. Lack of artificial lighting just led to creeping plants without main stems or flowering. In this case, plants of either *H. perforatum* (HP) or *H. perforatum* var. Topas (HT) failed to continue their growth cycle and started to dry. On the other hand, supplying the plants with light regime favored the *H. perforatum* var. Topas (HT) plants to continue growth and flowering, giving two harvests during the growth season. The same light regime failed to force (HP) to continue growth after the first harvest. The light regime led to significant increments in the growth and flowering of both types of *Hypericum*. In addition, the light treatment led to significant increment in the total hypericins content in both HT and HP. The whole herb of HP processed higher content of hypericins than HT due to the higher content of hypericins in flowers of HP. Plants grown in the green house were significantly superior in their growth parameters in comparison with those cultivated in the open field and also supplied with light regime.

*Key words***:** *Hypericum perforatum***,** light regime, cultivation, hypericins

Introduction

St. John's Wort (*Hypericum perforatum* L.) is an herbaceous perennial plant belongs to Fam. *Hypericaceae*. It is widely distributed in warm and temperate regions of Europe, Asia, North Africa, North America and Australia (Bombardelli and Morazzoni, 1995 and Briese, 1997). This species has been found to be effective in treating mild to moderate depression, as well as anxiety and insomnia. Extracts of the inflorescences and upper stem leaves have been prescribed for many years in Europe, and are available as dietary supplement in the United States, to treat mild to moderate depression (Muller, 2005). Depression affects an estimated 121 million people worldwide and the antidepressant drug market reached sales of almost $11 billion in 2008, (World Health Organization, 2008). *Hypericum perforatum* extract in sales in 2007 reached 2.1 billion US dollars, showing an increase of 11% in comparison with 2002 (www.articleintelligence.com/Art/14835).

The effectiveness of *H. perforatum* extracts as an antidepressant has been clinically proved through several clinical investigations (Linde et al., 1996). After comparison with some standard antidepressants, Barnes et al, 2001) concluded that St. John's Wort does appear to have a more favorable short –term safety profile than do standard antidepressants.

Hydro-alcoholic extracts are produced from the aerial parts of the flowering plants (Upton, 1997). While these extracts may contain several chemical constituents (Nahrstedt and Butterweck, 1997) however, recent studies have referred the anti-depressant action of this plant to its contents of hyperforin, hypericin and pseudohypericin (Chatterjee et al., 1998; Laakmann et al., 1998). The commercial preparations of St.

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Gohn's Wort are recently standardized on bases of their content of "hypericins" equal to the amount of hypericin, pseudohypericin and other minor naphthodianthrones (Upton, 1997).

Hypericin and pseudohypericin are thought to be localized in dark glands on the margins of leaves and flower petals (Fomasiero et al., 1998). There is evidence that the size, number and chemical content of such glandular structures in the plant can be influenced by environmental factors such as nutrition, light quality and light intensity as well as field conditions (Buter et al., 1998, Denke et al., 1999).

It has been concluded that increasing the light intensity resulted in parallel increase in the number of dark glands on the leaves (Donald and Margaret, 2001). In addition, Radusiene et al. (2010) reported that increase in temperature determinate continuous increases in contents of bioactive compounds. Controlling the light quality can be an important technique for enhancing production of St. John's Wort (Nishimura et al., 2006).

Although *Hypericum* has been intensively investigated from the chemical and pharmaceutical points of view however, very little could be traced in the literature about the cultivation of this plant.

In Egypt, three species of *Hypericum* are grown wild in the sandy soils and rock crevices in Sinai Peninsula namely *H. trequatrifolium* (syn. *H. crispum*), *H. sinaicum* and *H. lanuginosum* (Boulos, 1999). However, none of these species was cultivated or used in pharmaceutical preparations. Pharmaceutical preparations of *Hypericum* in the local market still depend mainly on imported standardized extracts.

Few companies for preparation of certain pharmaceutics import Hypericum extracts. Since imports of these extracts could be faced by any unforeseen complications, trials for cultivation of this plant in Egypt became interested. We as well as others tried to grow *hypericum* in Egypt adopting the usual agricultural practices in the open field however, none of these trials succeeded. All the trials led to just creeping plants without erect stems or flowering. Therefore, in the present study we tried to grow the plant under artificial conditions adopting a light regime aiming to get normal plants as a source for producing a standardized *hypericum* extracts in Egypt. This study was partially funded through STDF (Science and Technology Developing Fund), Egypt.

Material and Methods

Two types of St. John's Wort (*Hypericum perforatum* L.) were used in the present investigation. Seeds of *Hypericum perforatum* L. (code No. 66) and *Hypericum perforatum* var. Topas (code No. 67) were obtained from Jelitto Company, Germany. The last one is marketed as a hypericin rich variety. The seeds were sown in the nursery on the 8th of October 2008, in a green house in the experimental farm of SEKEM Academy for Scientific Research and Technology, Cairo, Egypt. Seed germination took place after 10 days. Seedlings of 10cm height were ready for transplanting on the 23rd of January 2009. The obtained seedlings were used as the planting material in the two experiments constituting this study:

Experiment I:
Response of *hypericum* plants to light regime

Seedlings of *Hypericum perforatum* L. and *Hypericum perforatum* var. Topas were transplanted into two green houses covered with polyethyelene sheet, each of 300 m^2 equipped with drip irrigation system. Two months prior to transplantation the soil was enriched with mature compost at the rate of 30 m^3/acre (=4000 m^2), while another dose of 4 m^3/ acre was added at the beginning of April. The seedlings were planted at 30 cm distances on rows 1m in-between. Chemical analysis of the used soil is presented in Table 1, while the chemical analysis of the used compost is presented in Table 2.

Plants in one of the two greenhouses were grown under the natural light conditions of the green house. The other green house was supplied with artificial lighting. Incandescent bulb lamps of 200 W were fixed at 3x3 m distances and a height of 1.5 m above the ground. The lamps were switched on for four hours after sun set every day providing a light intensity of 1500 to 2000 lux, measured at 1m height above the ground.

The plants were irrigated every week for the first 30 days, then every 3-4 days from the first of March until June. Weeding was carried out manually as required.

Plants of *Hypericum perforatum* var. Topas under light regime started flowering at the third week of March 2009 while those of *Hypericum perforatum* flowered two weeks later. The plants reached full flowering stage at med of April while harvested at the end of April.

In both green houses, plants of *Hypericum perforatum* started to dry after the first cut and did not continue growth. On the other hand, plants of *Hypericum perforatum* var. Topas were harvested twice, the first at the end of April while the second was at the second week of June. At harvest time, three groups of the plants (as three replicates) each of 10 plants from each greenhouse were assessed for their growth parameters namely; plant height, number of branches, number of flowers, fresh weight and dry weight. The plants were divided into their plant parts namely; leaves, flowers and flowering tops. The samples were immediately sent to an artificial air-drying unit at 40°C for 6 hours. The dried samples were kept in paper bags in a desiccator over calcium chloride in a dark place until chemical analysis in duplicate.

Experiment II:
Comparison between cultivation in the open field and in a green house

On the 23rd of January, seedlings of *Hypericum perforatum* var. Topas were transplanted to an experimental area in the open field under drip irrigation system. On the other hand, other seedlings were transplanted into a green house equipped with lighting system as mentioned in the first experiment. Temperatures in and out the green house were recorded. The daily mean temperature in the open field gradually increased from 14 to 27°C during the period from January till June. In the green house the temperature exceeded the recorded temperature in the open field by about 8°C in average. In addition, during this period the daily sunlight hours increased from 7 to 12 hours. The seedlings were planted at 30 cm distances on rows 1m in-between. All the usual agricultural practices; irrigation, fertilization and weeding were applied in both the green house and the open field. At harvest time, three groups of the plants (as three replicates) each of 10 plants from both the open field and the greenhouse were assessed for their growth parameters namely; plant height, number of branches, number of flowers, fresh weight and dry weight/plant. Samples of the whole plant of the first cut were taken for chemical assessment in duplicate as previously mentioned in the first experiment.

Determination of the hypericines content

The total hypericins content in the fresh and dried herb material was determined adopting the spectrophotometer technique as described in the European Pharmacopeia (3rd ed., 2002, pp1438) using a UV mini 1240 Spectrophotometer (Schimadzu, Japan). The total hypericins content expressed as hypericin was calculated as follows:

A sample of 0.8 g of the dried herb was refluxed in a mixture of H_2O and tetrahydrofuran (20:80) at 70ºC for 30 min then filtered. The same was repeated three times. The three filtrates were combined and concentrate till dryness in vacuum oven at 50°C. The residue was taken up with 70% methanol and the volume was adjusted to 25 ml in a measuring flask. The percentage of total hypericins content was calculated based on the following equation:

$$\% \text{ of hypericinins} = (A \times 125) / (M \times 870),$$

where: A = absorbance at 590 nm,
M = mass of the substance to be examined in grams, considering the specific absorbance of hypericin to be 870.

Statistical analysis

The vegetative growth data in the present study were analyzed with the Analysis of Variance (ANOVA test) (MS DOS/ Costat Exe Program). Duncan's multiple range test was used to compare the means of treatments according to Waller and Duncan (1969) at probability 5%.

Data of the chemical investigations were analyzed by one-way ANOVA test for comparison among means ±S.E (standers error) according to the method described by Snedecor and Cochran (1967). The differences were considered significant if the probability was associated with $p<0.001$ and $p<0.05$, (SPSS 9.05).

Table 1
Chemical analysis of the planting soil

Clay	Silt	Sand	$C_{org.}$	$M_{org.}$[1]	pH	EC	N[2]	P[3]	K[3]
%						Sm^{-1}	ppm		
14.94	29.75	55	0.16	0.23	8.23	2.8	480	37.8	35.7

1= Organic matter; 2= total; 3=available.

Table 2
Chemical Analysis of the used compost

Constituent	Value	Constituent	Value	Constituent	Value
Density, kg/m^3	510	C/N Ratio	18.22	Mn, ppm	320
Moisture, %	18.2	Total P, %	1.6	Cu	140
EC, dS/m	9.65	Avail. P, mg/kg	410	NH_4-N, mg/kg	880
pH	7.6	Total K, %	2.3	NO_3-N, mg/kg	450
Organic C, %	24.6	Avail. K, mg/kg	620	Parasites	nil
Org. Matter, %	42.41	Fe, ppm	960	Pathogens	nil
Total N, %	1.35	Zn, ppm	280		

Results and Discussion

The first experiment aimed to alter the growth habit of hypericum plants, and to force them towards erect growth and flowering through supplying them with artificial lighting (Figure 1). Lack of artificial lighting just led to creeping plants without main stems or flowering as shown in Figure 2. In this case, plants of either *H. perforatum* (HP) or *H. perforatum* var. Topas (HT) failed to continue their growth after the first cut and started to dry.

On the other hand, supplying the plants with light regime favored the *H. perforatum* var. Topas (HT) plants to continue growth and flowering, giving two harvests during the growth season. The same light regime failed to force (HP) to continue growth after the first harvest (Figure 3).

Results of the growth parameters recorded in the two seasons of the first experiment are presented in Table 3.

Data in Table 3 reveal that the light regime led to significant increments in the growth of both types of *Hypericum*. In the first cut, the light treatment increased the height of HP from 19.9 to 49.8cm, the number of branches from 4.5 to 6.2, the fresh weight from 40.7 to 78.1 g/plant and the dry weight from 8.7 to 32.1 g/plant. The same took place in case of HT however, plants of the second cut were shorter but having more branches in comparison with the plants of the first cut. Plants of the second cut showed lower growth in terms of number of flowers (28.9) and fresh (84.9 g) and dry weight (30.8 g) than those of the first cut; 49.0, 106.4 g and 32.1 g, respectively. It is obvious that plants of HT were superior in growth parameters compared with those of HP.

Results of the second season almost followed the same trends and confirmed the results of the first one.

Previous studies have reported the production of *hypericum* under controlled environments with artificial light as a promising technique to standardize and enhance the growth and medicinal content (Nishimura et al., 2006). These improvements are due to the amount of light actually absorbed by the leaves.

Regarding the effect of the light treatment on the total hypericins content in the plant parts, the data in Table 4 show that the light treatment led to significant increment in the total hypericins content in both HT and HP. In the first season, the content in the completely fresh plant of HT increased from 0.062 to 0.130% as well, it increased from 0.116 to 0.325% in the dry herb. In case of HP, similar increments were recorded. It could be noticed that the whole herb of HP processed higher content of hypericins than HT. That was due to the higher content of hypericins in flowers of HP. The hypericins content in the dry flowers of HP reached 0.411% compared with 0.383% in HT. On the contrary, leaves of HT

Fig. 1. The installed lighting

Fig. 2. *Hypericum* plants without lighting

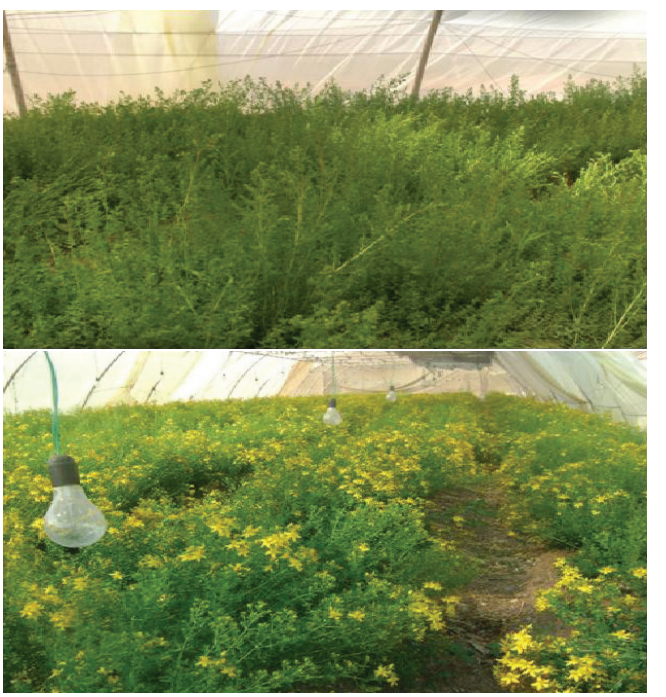

Fig. 3. Plants of *Hypericum perforatum var.* Topas at vegetative stage under lighting regime

were richer in hypericins (0.141%) than those of HP (0.128%). Results of the second season followed the same trend of the first one. Several investigators emphasized the significant role of light in increasing the total hypericins content in hypericum plants, Fomasiero et al. (1998), Buter *et al.*, (1998), Denke et al. (1999), Donald and Margaret (2001), Nishimura et al. (2006), Brechner et al. (2007). Poutaraud et al (2001) reported that protopseudohypericin and protohypericin (protopigments) are converted into pseudohypericin and hypericin (pigments) under the action of light.

Table 3
Effect of light treatment on growth parameters of H. *perforatum* and *H. Perforatum* var. Topas

Light treats.	Varieties	Plant height		No. of branches		No. of flowers		Fresh wt., g/plant		Dry wt., g/plant	
		1st cut	2nd Cut	1st cut	2nd Cut	1st cut	2nd Cut	1st cut	2nd Cut	1st cut	2nd Cut
First season											
Without lighting	*H. Perforat.*	19.9	---	4.5	---	---	---	40.7	---	8.7	---
	H.Topas	22.3	---	6	---	---	---	42.9	---	9.2	---
With lighting	*H. Perforat.*	49.8	---	6.2	---	32	---	78.1	---	32.1	---
	H. Topas	55.9	40.3	9	22.5	49	28.9	106.4	84.9	32.1	30.8
Mean of	*H. Perforat.*	34.9	---	5.4	---	16	---	59.4	---	20.4	---
	H. Topas	39.8	20.2	7.5	11.2	24.5	14.5	74.7	42.5	20.7	15.4
LSD at 5%	Lighting	2.3	2.2	0.5	1.7	4.5	1.6	3.2	1.9	0.2	0.6
	Varieties	1.7	2.3	0.6	1.6	2.8	1.7	3.7	2.1	2.3	0.7
	Interaction	3.2	3.1	0.7	2.4	2.1	3	4.6	2.8	0.3	0.9
Second season											
Without lighting	*H. Perforat.*	20.8	---	4.8	---	---	---	42	---	8.4	---
	H. Topas	23.7	---	6.3	---	---	---	45.8	---	10.2	---
With lighting	*H. Perforat.*	52.7	---	5.9	---	28	---	75.4	---	30.1	---
	H. Topas	57.8	36.4	12	26.7	53.7	33.2	122.3	92.1	36.2	32.1
Mean of	*H. Perforat.*	36.8	---	5.3	---	14	---	58.7	---	19.3	---
	H. Topas	40.8	18.2	9.2	13.4	26.9	16.6	84.1	46.1	23.2	16.5
LSD at 5 %	Lighting	1.9	2.1	0.5	1.5	2	1.42	3.1	2	0.2	0.5
	Varieties	1.9	2.1	0.7	1.6	2.6	1.21	3.8	2.2	1.1	0.6
	Interaction	2.8	3	0.7	2.2	2.1	2.9	4.2	2.5	0.3	0.8

Table 4
Total hypericins content (%) in *Hypericum perforatum* and *Hypericum perforatum* var. *Topas* as influenced by lighting, (in the two seasons)

Light treatments	Samples description	Total hyperiicins content (%)			
		Season 2008-2009		Season 2009-2010	
		H. perfor. var. *topas*	*H. perforatum*	*H. perfor.* var. *topas*	*H. perforatum*
Without lighting	Whole plant/ fresh	0.062 ± 0.0018^{ce}	0.082 ± 0.0010^{a}	0.073 ± 0.0001^{d}	0.093 ± 0.0002^{a}
	Whole plant/ dry	0.116 ± 0.0010^{ai}	0.125 ± 0.0002^{ab}	0.127 ± 0.0010^{a}	0.157 ± 0.0002^{g}
With lighting	Whole plant/ fresh	0.130 ± 0.0012^{ji}	0.178 ± 0.0002^{a}	0.154 ± 0.0001^{f}	0.164 ± 0.0002^{g}
	Whole plant/ dry	0.325 ± 0.0015^{le}	0.338 ± 0.0004^{a}	0.337 ± 0.0003^{a}	0.359 ± 0.0002^{a}
	Leaves/ fresh	0.074 ± 0.0012^{c}	0.034 ± 0.0001^{a}	0.086 ± 0.0010^{d}	0.039 ± 0.0010^{a}
	Leaves/ dry	0.141 ± 0.0014^{j}	0.128 ± 0.0001^{ab}	0.165 ± 0.0010^{f}	0.137 ± 0.0020^{a}
	Flowers/ fresh	0.197 ± 0.0012^{a}	0.208 ± 0.0002^{a}	0.185 ± 0.0002^{c}	0.198 ± 0.0002^{a}
	Flowers/ dry	0.383 ± 0.0010^{l}	0.411 ± 0.0005^{a}	0.376 ± 0.0010^{a}	0.422 ± 0.0002^{a}

Data are presented as mean ± S.E. a $P<0.001$ c $P< 0.05$
Groups have the same letter means no significant difference between them

Table 5
Growth parameters of *H. perforatum* var. *Topas*, as affected by cultivation either in the open field or the green house

Growth parameters	1st Cut			2nd Cut		
	Open field	Green house	LSD at 5%	Open field	Green house	LSD at 5%
First Season						
Plant height, cm	35	55.65	5.54	33.25	40.3	N.S.
No. of branches	6.5	14.58	1.76	12.1	22.45	3.57
No. of flowers	25.17	32.1	5.62	22.36	30.8	3.5
Fresh weight, g/plant	80.5	106.4	17.07	73.7	84.9	N.S.
Dry weight, g/plant	37.53	49	6.44	28.9	38.7	4.22
Second season						
Plant height, cm	N.S.	42.8	31.8	5.84	48	39
No. of branches	2.88	21.1	9.7	1.36	11.8	7.6
No. of flowers	3.11	33.6	25.4	5.23	54.6	38.1
Fresh weight, g/plant	8.2	73.1	64.2	14.7	98	75
Dry weight, g/plant	3.11	28.9	19.5	5	29.16	22.32

Results of the second experiment to compare the performance of HT in either the open field or the green house are tabulated in Table 5.

Data in this table clearly show that HT grew well in the open field attaining the flowering stage and gave two harvests because of the light supply. However, the plants grown in the green house were significantly superior in their growth parameters in comparison with those cultivated in the open field. In the first cut, the plants in the green house reached 55.65 cm, 14.58, 32.10, 106.40 g/plant and 49.00 g/plant for the plant height, number of branches, number of flowers, fresh weight and dry weight, compared to 35.00 cm, 6.50, 25.17, 80.50 g/plant and 37.53 g/plant, respectively for the plants grown in the open field. The same took place in the second cut as well as in the second season. Although both the plants in the open field and the green house received the same light treatment however, the higher growth parameters recorded in case of the green house could be attributed to the higher temperatures (about 8°C) in the atmosphere of the green house. Results of the second season followed the same trend of the first one.

As a result of several investigations, authors emphasized that temperature and light are important environmental factors to optimize the raw material production of *Hypericum* (Radusiene et al., 2010).

References

Barnes, J., L. A. Anderson and J. D. Phillipson, 2001. St. John's Wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. *Journal of Pharmacy and Pharmacology*, **53** (5): 583-600.

Bombardelli, E. and P. Morazzoni, 1995. *Hypericum perforatum*. *Fitoterapia,* **66**: 43-68.

Boulus, L., 1999. Flora of Egypt. *Al Hadara Publishing,* Cairo, Egypt, **1:** 156-159.

Brechner, M. L., L. D. Albright and L. A. Weston, 2007. Impact of a variable light intensity at a constant light integral: effects on biomass and production of secondary metabolites by *Hypericum perforatum*. American Society of Agricultural and Biological Engineers (ASAE) Annual Meeting, 2007.

Briese, D. T., 1997. Population dynamics of St. John's Wort in Southeastern Australia. *Plant Prot. Quart*., **12:** 59-64.

Buter, B., C. Orlacchio, A. Soldati and K. Berger, 1998. Significance of genetic and environmental aspects in the field cultivation of *Hypericum perforatum*. *Planta-Med*., **64** (5): 431-437.

Chatterjee, S. S., M. Nöldner, E. Koch and C. Erdelmeier, 1998. Antidepressant activity of hypericum perforatum and hyperforin: the neglected possibility. *Pharmacopsychiatry*, **31** (1): 7-15.

Denke, A., H. Schempp, E. Mann, W. Schneider and E. F. Elstner, 1999. Biochemical activities of extracts from *Hypericum perforatum* L., 4th communication: influence of different cultivation methods. *Arzneimittel- Forschung*, **49** (2): 120-125.

Donald, P. and C. Margaret, 2001. Differential effects of light and nitrogen on production of hypericins and leaf glands in *Hypericum perforatum*. *Plant Physiol. Biochem*., **39**: 1075-1081.

European Pharmacopeia (3rd ed., 2002, pp. 1438.

Formasiero, R. B., A. Bianchi and A. Pinetti, 1998. Anatomical and ultrastructural observations in *Hypericum perforatum* L. leaves. *J. Herbs Spices and Med. Plants*, **5:** 12-33.

Lakmann, G., C. Schule, T. Baghai and M. Kieser, 1998. St. John's Wort in mild to moderate depression: the relevance of hyperforin for the clinical efficacy. *Pharmacopsychiatry,* **31**: 54-59.

Linde, K., G. Ramirez, C. D. Mulrow, A. Pauls, W. Weidenhammer and D. Melchart, 1996. St. John's Wort for depression, an overview and meta analysis of randomized clinical trials. *Br. J. Med*., **313:** 253-258.

Muller, W. E., (ed.) 2005. St. John's Wort and its Active Principles in: Depression and Anxiety, Biekhauser Verlag, Basel, Switzerland, 188 pp.

Nahrstedt, A. and V. Butterweck, 1997. Biologically active and other chemical constituents of the herb of *Hypericum perforatum* L. *Pharmacopsychiatry,* 30 (2): 129-34.

Nishimura, T., S. M. Zobayed, T. Kozai and E. Goto, 2006. Effect of light quality of blue and red fluorescent lamps on growth of St. John's Wort (*Hypericum perforatum* L.). *J. Shita*, **18** (3): 225- 229.

Poutaraud, A., F. D. Gregorio, C. Tin and P. Girardin, 2001. Effect of light on hypericins contents in fresh flowering top parts and in an extract of St. John,s Wort (*Hypericum perforatum*). *Planta Medica*, **67** (3): 254- 259.

Radusiene, J., Z. Stanius, C. Cirak, and M. S. Odabas, 2010. Quantitative Effects of Temperature and Light Intensity on Accumulation of Bioactive Compounds in St. John's Worth. 28th International Horticultural Congress, Lisboa – Portugal.

Snedecor, G. W. and W. G. Cochran, 1980. Statistical Methods.7th Ed., *Iowa State Univ, Press*, Ames., Iowa, USA.

Upton, R., 1997. St. John's Wort *Hypericum perforatum*, quality control, analytical and therapeutic monograph. American Herbal Pharmacopoeia and Therapeutic Compendium, 1997.

World Health Organization, 2008. www.**hypericum**.com/hyp02.htm; www.articleintelligence.com/Art/14835

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