

## VARIATION OF HYPERICIN IN ST. JOHN'S WORT (*HYPERICUM PERFORATUM*) FROM WILD POPULATIONS OF NORTHERN TURKEY

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*Hypericum perforatum* L. (St. John's wort) is a well-known traditional medicinal plant that has been used for centuries for the treatment of several diseases and this feature is obviously attributed to the presence of a broad spectrum of secondary metabolites including hypericins. In the present study, the aim was to determine total hypericin content of this plant from wild population of Northern Turkey. Thus, *Hypericum perforatum* plants representing a total of 45 wild populations were collected from 15 sites in each of 3 regions, during flowering and subsequently assayed for total hypericin. Hypericin content in these wild populations varied from 0.28 to 4.46 mg/g dry matter. According to the results of analysis of variance, there was a significant difference in hypericin content between sites as well as regions. One of the problems associated with medicinal plant preparations is the extreme variability in the phytochemical and marker compounds. Products containing *H. perforatum* have varied greatly in content of secondary metabolites since it is currently supplied by cultivated and wild-harvested materials. Therefore the variation of phytochemicals like hypericin in *H. perforatum*, especially from wild populations has a great importance in term of health care and herbal industry.

Key words: hypericin, *Hypericum perforatum*, Turkey, variation, wild population

### INTRODUCTION

*Hypericum perforatum* L. has become a widely popular herbal remedy. It, commonly known as St. John's wort, is native to relatively dry temperature zones of Europe and North America (Deltito and Bayer 1998). *Hypericum perforatum* contains namely naphthodianthrone and phloroglucinols, biologically active secondary metabolites belonging to at least ten different classes

(Greeson *et al.* 2001). It was reported that its extract exhibited antiviral properties (Meruelo *et al.* 1988, Lavie *et al.* 1989). That is why, it is suggested that *Hypericum perforatum* has a potential in Acquired Immuno Deficiency Syndrome (AIDS) treatment (Schinazi *et al.* 1990). Use of the crude extract of this plant as an antidepressant is very popular today (Biffignandi and Bilia 2000). This is evidenced by the fact that the market for *Hypericum perforatum* has exceeded 210 million USD in the USA and 570 million USD worldwide annually (Sirvent *et al.* 2002). Hypericin, a photoactive dianthone, has a great importance in economical and health care point of view (Patocka 2003). It belongs to group of compound known as naphthodianthrones (Gerassim and Kitanov 2001). Total hypericin content of products has been used for standardisation purposes within the botanical industry (Nahrstedt and Butterweck 1997). The analytical method utilised for *Hypericum* identification depends on the presence of the naphthodianthrones, as marker compounds (Constantine and Karchesy 1998). Thus, it has importance from quality control point of view. Initially, hypericin was considered to be the antidepressant principle of *Hypericum perforatum*, but according to the latest studies hyperforin has emerged as antidepressant principle of the plant (Cervo *et al.* 2002, Roz and Rehavi 2003). Hypericin, however, also may contribute to the antidepressive action (Cott 1997, Raffa 1998) in addition to its well-documented in vitro antimicrobial, antiviral (Sakar and Tamer 1990, Diwu 1995) and antineoplastic activities (Kubin *et al.* 1996, Agostinis *et al.* 2002).

Several studies have reported variation of hypericin levels in *Hypericum perforatum* in Australia (Southwell and Campbell 1991, Campbell *et al.* 1997, Southwell and Bourke 2001), Nova Scotia, Canada (Jensen *et al.* 1995), Switzerland (Büter *et al.* 1998), USA (Constantine and Karchesy 1998, Walker *et al.* 2001, Sirvent *et al.* 2002) and Armenia (Melikian *et al.* 1998, Kirakosyan *et al.* 2002), but there are no reports documenting variation of hypericin among Turkish populations so far. The present study focused on determining the variation of hypericin from wild populations of *Hypericum perforatum* collected in Northern Turkey. Because, some companies have collected plant materials for export to herbal industry, especially in this part of Turkey (Çırak *et al.* 2004). Genetic, physiologic or environmental factors may affect hypericin yield, but such studies correlating hypericin concentrations to specific influences have not been well documented (Sirvent *et al.* 2002). In the present study we report our investigations of *Hypericum perforatum* populations sampled from a total of 45 sites within 3 regions of Northern Turkey.

## MATERIALS AND METHODS

### *Plant material*

*Hypericum perforatum* plants representing a total of 45 samples were collected between July and August of 2003 from 3 regions, each having 15 sites and located in Black Sea region of Turkey: Çamlıhemşin, Çayeli, Pazar, Fındıklı, Ardeşen, Arsin, Tonya, Akçabat, Uzungöl, Maçka, Trabzon, Kadirga, Soğanlı, Aydıntepe and Bayburt in the vicinity of Çayeli, Trabzon, Bayburt (Region 1); Bolaman, Ünye, Ordu, Fatsa, Bulancak, Espiye, Eynesil, Piraziz, Asarcık, Samsun, Pazar, Niksar, Hamamözü, Gümüşhacıköy and Gümüş in the vicinity of Ordu, Samsun, Niksar (Region 2); and Daday, Ağlı, Taşköprü, Azdavay, Çatalzeytin, İnebolu, Abana, Seydiler, Küre, Ballıdağ, Durağan, Ayancık, Türkeli, Bafra and Vezirköprü in the vicinity of Bafra, Daday and İnebolu (Region 3) (Fig. 1). All plants were growing wild and no herbarium materials were used. Sampling was randomised from plant crowns that had at least three stems. Care was taken to harvest the plants at approximately the same maturity level to minimise the differences originating from plant growth stages. The top 1/3 of the crown was harvested between 10:00 am and 2:00 pm. Conditions on the day of collection were clear and sunny at all sites. Temperatures ranged from 24 to 35 °C. Samples were dried overnight (or until constant weight) at 65 °C, the current temperature used by wildcrafters in USA for industry production (Sirvent *et al.* 2002).

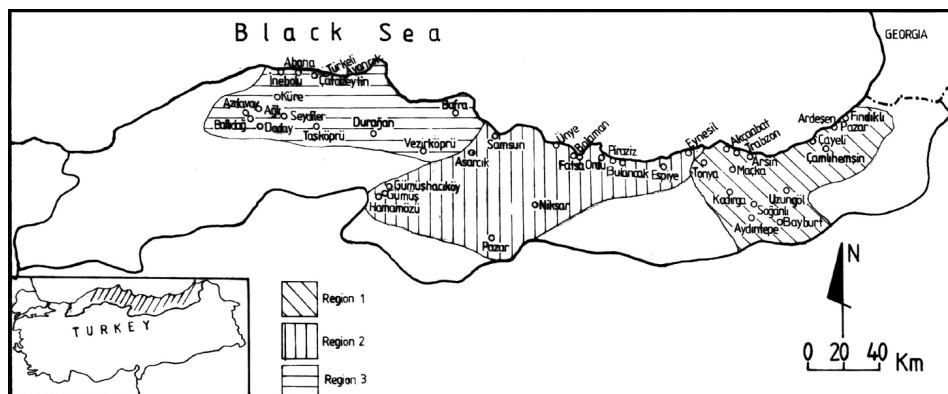


Fig. 1. The map of Black Sea region located in Northern Turkey showing the regions and sites where *Hypericum perforatum* L. plants were collected

*Extraction and spectrophotometry of hypericin*

The method of Cellarova *et al.* (1994) was used to determine the “total hypericin” content of plant materials. Briefly, dried and homogenised plant materials were extracted with chloroform in a sonicator to remove their chlorophyll contents until samples seen colourless and filtered. A repeated and prolonged extraction of the samples with chloroform was used to remove the chlorophyll content, as the secondary band of chlorophyll interferes with that of hypericins. The dried powder was then extracted with methanol and the filtrate allowed to evaporate in a water bath. This was followed by an addition of chloroform, and the mixture was shaken. The supernatants were then discarded and the solid phase, containing hypericin and its derivatives, was dissolved in methanol and filtered. The extractions were performed under light in order to enhance the conversion of the protohypericin and protopseudo-hypericin to hypericin and pseudohypericin, respectively.

Total hypericin content was determined in methanol extracts by a spectrophotometer (Shimadzu UV 3000) at 592 nm. Three determinations were done for each sample and the mean value was calculated.

*Statistical analysis*

The data were objected to ANOVA and differences among treatments were tested with the Duncan Multiple Range Test (level of significance  $p < 0.01$ ).

**RESULTS**

The hypericin contents of *H. perforatum* plants collected from three regions of Northern Turkey were shown in Table 1. According to the results of variance analysis, there was a significant difference in both inter-regional and intra-regional hypericin contents ( $p < 0.01$ ). Average hypericin content for sites was 1.39, 1.22 and 1.60 mg/g dry matter in regions 1, 2 and 3, respectively. In general, higher contents of hypericin were found in samples collected in Region 3 when compared to other regions. In the case of within-region variation of hypericin, the highest content was observed in samples collected from the site Maçka in Region 1, while the site Soğanlı in the same region gave the lowest content (4.46 and 0.28 mg/g dry matter, respectively).

Table 1

Hypericin content of *H. perforatum* plants collected from three regions of Northern Turkey

| Region 1    | Hypericin content (mg/g dry matter) | Region 2     | Hypericin content (mg/g dry matter) | Region 3    | Hypericin content (mg/g dry matter) |
|-------------|-------------------------------------|--------------|-------------------------------------|-------------|-------------------------------------|
| Çamlıhemşin | 1.49 e <sup>1</sup>                 | Bolaman      | 0.51 g                              | Daday       | 1.13 fg                             |
| Çayeli      | 0.76 gh                             | Ünye         | 0.96 ef                             | Ağlı        | 1.56 de                             |
| Pazar       | 0.69 hi                             | Ordu         | 0.93 ef                             | Taşköprü    | 0.97 gh                             |
| Fındıklı    | 0.46 ij                             | Fatsa        | 0.38 g                              | Azdavay     | 1.33 ef                             |
| Ardeşen     | 1.03 fg                             | Bulancak     | 1.50 d                              | Çatalzeytin | 1.04 gh                             |
| Arsin       | 1.77 d                              | Espiye       | 0.48 g                              | İnebolu     | 1.63 d                              |
| Çayırıcı    | 1.52 de                             | Eynesil      | 0.32 g                              | Abana       | 0.57 i                              |
| Akçabat     | 2.89 b                              | Piraziz      | 0.46 g                              | Seydiler    | 3.50 a                              |
| Uzungöl     | 1.12 f                              | Asarcık      | 1.14 e                              | Küre        | 2.19 c                              |
| Maçka       | 4.46 a                              | Samsun       | 3.23 a                              | Ballıdağ    | 2.82 b                              |
| Trabzon     | 0.78 gh                             | Pazar        | 2.00 c                              | Durağan     | 1.97 c                              |
| Kadırga     | 2.14 c                              | Niksar       | 1.57 d                              | Ayancık     | 1.40 def                            |
| Soğanlı     | 0.28 j                              | Hamamözü     | 1.50 d                              | Türkeli     | 1.50 de                             |
| Aydıntepe   | 0.76 gh                             | Gümüşhacıköy | 0.80 f                              | Bafra       | 1.55 de                             |
| Bayburt     | 0.41 j                              | Gümüş        | 2.54 b                              | Vezirköprü  | 0.80 hi                             |
| Mean        | 1.39 B <sup>2</sup>                 | Mean         | 1.22 C                              | Mean        | 1.60 A                              |

<sup>1</sup>Values followed by different small letters in columns and rows.<sup>2</sup>Capital letters in bottom row are significantly different ( $p < 0.01$ ) according to Duncan Multiple Range test.

## DISCUSSION

Methods available for determination of hypericin usually involve solvent extraction followed by either spectrophotometric assay for total hypericins (Cellarova *et al.* 1994, Southwell and Campbell 1991, Southwell and Bourke 2001) or HPLC determination of individual constituents (Kartnig *et al.* 1996, Büter *et al.* 1998, Sirvent and Gibson 2000) including HPLC-MS systems (Brolis *et al.* 1998, Mauri and Pietta 2000) using electrospray and diode array detection. In the present study, the  $\lambda = 592$  nm absorptions of the methanolic solutions were measured in order to obtain estimates of the contents of hypericins. Each estimate included hypericin and the similarly absorbing pseudohypericin, protohypericin and protopseudohypericin (Banks *et al.* 1976, Mauri and Pietta 2000). The absorption of co-occurring phloroglucinols and flavonoids (Mabry *et al.* 1970, Mauri and Pietta 2000) was sufficiently distant from  $\lambda = 592$  nm not to interfere.

Hypericin is contained in glands visible on the epidermal surface of leaves, flowers and to some extent the stems of the plant (Liu and Hu 1999, Lu *et al.* 2001). The glands are visible to the naked eye and seem to be more numerous on the floral parts of the plant and along leaf margins (Ciccarelli *et al.* 2001). In all earlier reports, the floral parts have the highest hypericin concentrations (Sirvent *et al.* 2002, Büter *et al.* 1998, Schütt 1996). Therefore plant materials were not separated into tissue parts for hypericin determination.

The biosynthesis of secondary metabolites like hypericin may be influenced by genetic, metabolic and environmental parameters. Environmental factors including climate, topography, stage of plant development, epigenetic factors, and the methods by which the plant material is harvested and processed, including differences in extraction and analysis techniques are thought especially to affect or modulate the production or yield of hypericins (Bombardelli and Morazzoni 1995, Upton *et al.* 1997, Kurth and Spreemann 1998, Sirvent 2001). The *Hypericum perforatum* plants collected in this study were located in different parts of the Black Sea region of Turkey and isolated from each other by climatic and geographical factors. For example, annual precipitation is approximately 2,000 mm for Region 1, 700–800 mm for Region 2 and 900–1,000 mm for Region 3. The same variation is true for temperatures. Similarly, Regions 1 and 3 are mountainous, especially their rural parts, but Region 2 consists of plains to a great degree and, as a result, generally has a very low altitude (Zeybek 1998). As a result of these climatic and geographic differences, each region evaluated in this study has ecological conditions peculiar to itself. Variation of inter-regional hypericin contents observed may arise from these differences. Likewise, evident differences in environmental factors become more important along with genetics in determining the levels of hypericin (Sirvent *et al.* 2002).

Apart from inter-regional variation for hypericin, an intra-regional variation in hypericin contents was found to be significant. For example, two sites in Region 1, Fındıklı and Kadirga, are separated by 80 km and Eynesil and Hamamözü in Region 2 are separated by 174 km. They should represent distinct populations and hypericin contents were significantly different among these sites (0.46 and 2.14 mg/g dry matter for Fındıklı and Kadirga; 0.32 and 1.50 mg/g dry matter for Eynesil and Hamamözü, respectively). But even within a short distance (< 12 km), hypericin contents at Arsin and Maçka in Region 1 and at Gümüşhacıköy and Gümüş in Region 2 were significantly different as well (1.77 and 4.46 mg/g dry matter for Arsin and Maçka; 0.80 and 2.54 mg/g dry matter for Gümüşhacıköy and Gümüş, respectively). Genetic variation may play more of a role within populations if there is little environmental variation within a site. Intra-regional variation of hypericin content observed in the present study may have been caused by genetic influence in contrast to

inter-regional variation of hypericin content probably arising from environmental variation. Also, it is interesting to note that the highest and lowest hypericin contents were observed at the sites Maçka (4.46 mg/g dry matter) and Soğanlı (0.28 mg/g dry matter) found in the same region supplying moderate hypericin contents when compared to other two regions. This finding suggests that genetic factors may have more dominant effects than environmental factors on hypericin contents of Northern Turkish population of *Hypericum perforatum*.

Previous literature reports cite hypericin concentrations ranging from 0.95 to 3.87 mg/g dry matter from Oregon, USA (Constantine and Karchesy 1998); 0.6–1.9 mg/g dry matter and 0.01–0.46 mg/g dry matter from North-western USA (Walker *et al.* 2001, Sirvent *et al.* 2002), 0.12–0.29 mg/g dry matter from Nova Scotia, Canada (Jensen *et al.* 1995) and 0.04–2.15 mg/g dry matter from Australia (Southwell and Campbell 1991). Our values for hypericin contents ranged from 0.28–4.46 mg/g dry matter. In general, Northern Turkey populations reported in here have greater range of variation than the populations mentioned above. This might suggest that the differences in the Northern Turkey could be due to genetic, environmental, or physiological differences, or a combination of all three.

To conclude, *Hypericum perforatum* has become one of the leading plant-based dietary supplements worldwide and is currently supplied by cultivated and wild-harvested materials. Products containing *H. perforatum* have varied greatly in content of secondary metabolites like hypericin, and as a result, issues of efficacy and safety of these products have risen (Kirakosyan *et al.* 2004). Also, this plant has traditionally been considered a noxious weed, whose consumption by cattle in pasture forage or fodder causes photosensitivity in light-skinned animals and death to many sensitive animals due to the presence of hypericin (Tokur 1988, Vandenbogaerde *et al.* 1998). Therefore the presence and variation of hypericin in *H. perforatum*, especially from wild populations has a great importance in health care and agricultural point of view. Further studies are needed to delineate what environmental and genetic factors affect hypericin production.

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