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Title: Distribution of total phenols, flavonoids and hypericin in different plant organs of wild-growing St. John's-wort (*Hypericum perforatum* L., Hypericaceae) from North Macedonia



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Distribution of total phenols, flavonoids and hypericin in different plant organs of wild-growing St. John's-wort (*Hypericum perforatum* L., Hypericaceae) from North Macedonia

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Abstract

Hypericum perforatum L. (Hypericaceae) contains secondary metabolites that show noteworthy pharmacological activities. Therefore, the content of total phenols, flavonoids and hypericin from whole herb, flowers and leafs from St. John's-wort collected over four consecutive seasons (2014-2017) from three different locations in western part of North Macedonia (Tetovo, Debar and Mavrovo) were investigated by three different spectrophotometric methods (using Folin-Ciocalteu reagent, NaNO₂-AlCl₃-NaOH and mixture of water/tetrahydrofuran). The quantity of total phenols obtained from different plant organs (leafs, whole herb and flowers) were 35.15-83.08 mg GAE/g, 55.41-98.52 mg GAE/g and 75.44-121.19 mg GAE/g, respectively. Afterward the total flavonoids contents were: 61.64-106.86 mg CE/g, 73.04-117.57 mg CE/g and 108.65-125.35 mg CE/g in leafs, whole herb and flowers, respectively, while the hypericin amounts were: 0.03-0.17 mg/g, 0.04-0.29 mg/g and 0.07-0.60 mg/g in leafs, whole herb, and flowers, respectively. Significant differences were observed in the quantities of total phenols, flavonoids and hypericin between the locations of harvesting, while the collection seasons were found not to be significant. Additionally, it can be seen that the flowers are characterized by higher amounts of total phenols, total flavonoids and hypericin

compared to total herb and leaf in all three locations, therefore harvesting of St. John's-wort should be made in a manner of obtaining larger quantities of flowers as harvesting material which could increase the bioactive compounds in the herbal raw material.

Keywords: *Hypericum perforatum*, plant organs, total phenols, flavonoids, hypericin, statistical analysis

Introduction

Hypericum perforatum L. (St. John's-wort) is an important natural source of secondary metabolites with a wide range of pharmacological attributes including antiviral, antimicrobial, anti-inflammatory, antioxidant, hepatoprotective and anti-tumoral activity (Crocket and Robson, 2011; Linde et al., 1996; Muller, 2005; Saddiqe et al., 2010). Today, this plant is best known for its use in the treatment of mild to moderately severe depressive disorders (Linde and Mulrow, 2003).

The major active constituents are considered to be naphthodianthrones (pseudohypericin, hypericin), phloroglucinol derivatives (mainly hyperforin and adhyperforin), flavonoids, catechin tannins, procyanidines and smaller amounts of essential oil (Bradley, 2006; Nahrstedt and Butterweck, 1997). The antidepressant activity of St John's Wort extracts has been variously attributed to the hypericin and pseudohypericin (Butterweck et al., 2002), hyperforin (Chatterje et al., 1998; Muller et al., 2001; Singer et al., 1999; Wonnemann et al., 2001) and to several flavonoids (Butterweck et al., 2000; Calapai et al., 1999).

From a pharmacological point of view, the hypericins are the most interesting compounds of *H. perforatum*. Massive inhibition of monoamine oxidase A (MAO-A) can be shown with the total extract of *H. perforatum* (Thiede and Walper, 1994). Using pure hypericin, no relevant inhibiting effects can be shown because *H. perforatum* extracts have only weak activity in assays related to the mechanisms of the synthetic antidepressants, that is, inhibition of MAO, catechol O-methyltransferase, or serotonin reuptake. It has been postulated that the clinical efficacy of *H. perforatum* extract could be attributed to the combined contribution of several mechanisms, each one too weak by itself to account for the overall effect (Bennet et al., 1998). Hypericin has shown

antiviral activity against several types of viruses, including herpes simplex virus types 1 and 2 (Weber et al., 1994; Wood et al., 1990) and human immunodeficiency virus (HIV) (Hudson et al., 1991; Lavie et al., 1989; Meruelo et al., 1988) and has been tested as a photosensitizer in the treatment of cancer (Hamilton et al., 1996; Liu et al., 2000; Kamuhabwa et al., 2000).

The flavonoid glycosides (rutin, hyperosid, isoquercitrine, quercitrine) and aglycones (quercetin, kaempferol and luteolin) found in *Hypericum* are also considered to be potentially therapeutic compounds due to their anti-inflammatory, antioxidative, antimicrobial and spasmolytic effects (Luo et al., 2004; Morales and Lozoya, 1994; Zou et al., 2004). Besides, total phenolic compounds are usually attributed to antioxidant activity of plant raw materials (Kahkonen et al., 1999) as well as of *Hypericum* species (Zou et al., 2004).

However, many authors found great variations in the content of total phenols, flavonoids and hypericins in *H. perforatum* and other species of *Hypericum* (Cirak et al., 2006; Toker, 2009) that might influence on the biological activity of the plant extracts. On the other side, very little is known about the distribution of total phenols, flavonoids and hypericins in different parts of the *H. perforatum* which could be important in process of collection of plant material from nature. Therefore, the aim of this study was to determine the total phenols, flavonoids and hypericins in different plant organs including herb (dry over-ground part), leaves and flowers of *H. perforatum* collected in western part of North Macedonia, in order to identified seasonal variations and differences in the distribution of the secondary metabolites within the plant.

Material and methods

Plant material

Plant material was collected from three different locations in western part of North Macedonia: Tetovo, Mavrovo and Debar (Table 1) in the period 2014-2017, in full blossom of the plant (June-August). Plant identity was verified and voucher specimens were deposited at the Institute of Pharmacognosy, Faculty of Pharmacy, Skopje.

The plant material (aerial parts 25 cm from the top = herb) was air dried, packed in paper bags and kept in a dark and cool place until analysis. For purpose of analysis, flowers (Fl) and leaves (Fol) of some samples were separated (Table 1). Before analysis plant material was milled and homogenized.

Table 1

Determination of total phenols

Total phenolic content was determined with the Folin-Ciocalteu reagent according to the procedure described by Singleton et al. (1965) with slight modifications made from Karapandzova et al. (2015). Briefly, to 1 mL of test sample (methanol extract), 0.5 mL Folin-Ciocalteu reagent (1:10 v/v diluted with distilled water) was added and stirred for 5 min at room temperature. After 5 min, 0.4 mL of 7.5% sodium carbonate was added and made up to 10 mL with distilled water. These mixtures were incubated at room temperature in the dark for 2 hours. After incubation, absorbance was measured at 765 nm using a UV-VIS spectrophotometer. The total phenolic content was determined as mg of gallic acid equivalents per gram of plant material (mg GAE/g DW) using an equation obtained from standard Gallic acid calibration graph.

Determination of total flavonoids

The total flavonoid content was determined using the aluminum chloride assay described by Talari et al. (2012) with slight modification. To an aliquot of the test sample (1 mL methanol extracts), 4 mL of distilled water and 0.3 mL of 5% sodium nitrite were added and allowed to stand for 5 min. Later, 0.3 mL of 10% aluminum chloride was added and the mixture was incubated for 6 min. 2 mL of 1 M sodium hydroxide was added and the volume was made up to 10 mL with distilled water. After incubation of 15 min, the mixture turned to pink and the absorbance was measured at 510 nm using a UV-VIS spectrophotometer. The total flavonoids were expressed in mg of catechin equivalents per gram of plant material (mg CE/g DW) using an equation obtained from standard (+)-catechin calibration graph.

Determination of total hypericin

The total hypericin was determined by official method described in St. John's Wort monograph (Ph. Eur. 9.0, 2017). Sample solution was prepared by introducing 0.8 g of pulverized drug into a 100 mL round-bottomed flask and 60 mL of a mixture of 20 volumes of water and 80 volumes of tetrahydrofuran. The mixture was put on a magnetic stirrer and then boiled to fall

out in a bain-marie at 70°C and centrifugated (2 minutes at 700 g). The supernatant was decanted into a 250 mL flask. The residue was then taken with 60 mL of a mixture of 20 volumes of water and 80 volumes of tetrahydrophurane. The last was repeated once more and the combined extracts were evaporated to dryness. The residue was taken with 15 mL of methanol using ultrasound bath and transfer to a 25 mL volumetric flask. The 250 mL flask was washed with methanol and diluted with 25 mL with the same solvent. Afterwards the solution was centrifugated (2 minutes at 700 g) and 10 mL of the centrifugated sample was filtered through a syringe filter (0.2 µm, Agilent Captiva Premium Syringe Filters). Finally, 5.0 mL of the filtrate was dilute with 25 mL methanol. The absorbance of the sample solution was measured at 590 nm against the blank (methanol) and the percentage of total hypericins, expressed as hypericin, were calculated with the following expression: $A \times 125/m \times 870$, where: 870 = specific absorbance of hypericin, A = absorbance at 590 nm, m = weight of drug in grams (Longo and Schulz, 2002).

Instruments

The measurements of absorbance for determination of total phenols, flavonoids and hypericin were made on UV-VIS spectrophotometer instrument Agilent 8453, Agilent Technologies, USA.

Statistical analysis

Data obtained from determination of total phenols, flavonoids and hypericin in different plant material (total herb, leaf and flower) are expressed as mean values \pm SD ($n = 3$). Statistical analysis were carried out by employing two factor ANOVA without replication ($p < 0.05$) in order to determine if location and season of harvesting have statistically significant influence on each component investigated. Also, multiple comparison procedure was carried out to determine which means are significantly different from which others using *Fisher's least significant difference (LSD)* procedure ($p < 0.05$). Statistical package STATGRAPHIC Centurion XVI version 16.1 was used for data analysis.

Results and discussion

Total phenols

Total phenols were determined in three different plant organs (whole herb, flowers and leaves) of St. John's-wort (Table 2). The content of total phenols ranged from 35.15-83.08 mg GAE /g DW, 55.41- 98.52 mg GAE/g DW and 75.44-121.19 mg GAE/g DW in leaf, herb and flower, respectively.

Table 2

The highest amount of total phenols (121.19 ± 0.78 mg GAE/g DW) were determined in flowers, particularly in one sample (HP/16 FL-D) collected in Debar in 2016 compared to all other investigated plant material, followed by one sample harvested in Tetovo from 2016 (HP/16 FL-T, 113.49 ± 0.51 mg GAE/g DW) and then one collected in Mavrovo from 2015 (HP/15 FL-M, 94.19 ± 0.46 mg GAE/g DW). On the other hand, the lowest amount of total phenols was obtained in leaf as examined plant organ of *Hypericum*, in sample HP/14 FoL-M (35.15 ± 0.21 mg GAE/g DW) harvested in Mavrovo from 2014. Leaf samples from Tetovo and Debar were characterized by low amount of total phenols in each harvest year.

Total flavonoids

Total flavonoids were determined in whole herb, in separated leaves and flowers (Table 3). The content of total flavonoids ranged from 61.64-106.86 mg CE/g DW, 73.04-117.57 mg CE/g DW and 108.65-125.35 mg CE/g DW in leaf, herb, and flower, respectively.

Table 3

The highest amount of total flavonoids (125.35 ± 0.52 mg CE/g DW) was determined in the sample, HP/16 FL-D collected in Debar in 2016 which also show highest level of total phenols, followed by two samples from Mavrovo: HP/14 FL-M (119.50 ± 0.67 mg CE/g DW) and HP/17 FL-M, 118.38 ± 0.95 mg CE/g DW) collected in 2014 and 2017 respectively. On the other hand, the lowest amount of total flavonoids similar as with total phenols was determined in one sample of leaf, HP/16FoL-T (61.64 ± 0.11 mg CE/g DW) harvested in Tetovo, from 2016. Leaf samples from Debar and Mavrovo also showed low amounts of total flavonoids in each harvest year.

Total hypericin

Total hypericin was also determined in whole herb, in separated leaves and flowers (Table 4). The content of total hypericin ranged from 0.03-0.17mg/g DW, 0.04-0.29 mg/g DW and 0.07-0.60 mg/g DW in leaf, herb and flower, respectively.

Table 4

The highest amount of total hypericin (0.60 ± 0.03 mg/g DW) was determined in the sample from flower (HP/15 FL-D) collected in Debar in 2015, followed by one sample of flower from Tetovo (HP/17 FL-T, 0.45 ± 0.02 mg/g DW) harvested in 2017 and another flower sample from Debar (HP/16 FL-T, 0.45 ± 0.02 mg/g DW) collected in 2016. On the other hand, the lowest amount of total hypericin similar as with total phenols and total flavonoids were determined in leaf samples from all three locations of harvest (Tetovo, Debar and Mavrovo). Sample, HP/14 FoL-D collected in Debar, from 2014 had lowest amount of total hypericin (0.03 ± 0.01 mg/g DW).

According to the monograph of *Hyperici herba* in Ph. Eur. the value obtained for total hypericin must not be below 0.08%. Today it is known that the cultivation methods could provide plants with higher production of these metabolites, obtaining 0.1% of naphthodiantrones or even reaching 1.5% (Longo, R., Schulz, V. 2002).

Statistical analysis

Two-factor ANOVA ($p < 0.05$) indicated that seasonal variations do not have any significant influence on the content of total phenols, flavonoids and hypericin in St. John's-wort. However, there was statistically significant difference observed in amount (mg/g) of total phenols, total flavonoids and hypericin in different part of the plant (flower, total herb and leaf) as well as locations of harvest (Debar, Mavrovo and Tetovo). From Fig.1, Box-and-Whisker plot, it can be seen that the flowers are characterized by higher amount of total phenols, total flavonoids and hypericin compared to total herb and leaf in all three locations.

Fig. 1

Detailed analysis of amount of total phenols in total herb, flower and leaf from three locations (Debar, Mavrovo and Tetovo) was conducted by multiple comparison procedure, *LSD* ($p < 0.05$). It indicated that in the harvesting period 2014-2017 significant difference was observed in case of flower compared to leaf from Debar (94.57 ± 18.29 to 68.94 ± 10.09 mg/g in average per year) and leaf to total herb (68.94 ± 10.09 to 85.76 ± 8.58 mg/g in average per year), accordingly. No difference was detected between flower and total herb harvested in Debar. In location of Mavrovo following statistical significance was observed: flower to leaf and total herb (83.49 ± 7.9 to 40.38 ± 5.58 and 63.50 ± 8.77 mg/g in average per year, consequently) as well as leaf to total herb. Tetovo location was characterized by significant difference between flower to leaf and total herb (98.395 ± 15.74 to 61.71 ± 4.97 and 70.13 ± 6.36 mg/g in average per year, accordingly). No difference was observed in case of leaf and total herb in this location.

In the case of total flavonoids, *LSD* ($p < 0.05$), pointed that amount in flower from Debar was significantly higher than that in leaf (115.05 ± 7.26 to 81.59 ± 14.71 mg/g in average per year) and amount in total herb compared to leaf (104.8 ± 9.62 to 81.59 ± 14.71 mg/g in average per year). No significant difference was observed between flower and total herb in Debar location. Results from Mavrovo location pointed that flower had significantly higher amount of total flavonoids compared to leaf (118.05 ± 1.17 to 94.69 ± 6.46 mg/g in average per year, accordingly), but no difference was determined between flower and total herb (106.77 ± 11.66 mg/g) as well as leaf and total herb. Similar were observation for plant material harvested in Tetovo location where significance was determined in case of flower and leaf (112.22 ± 3.17 and 85.81 ± 22.8 mg/g in average per year), but no difference was indicated between flower and total herb (94.20 ± 17.88 mg/g in average per year) as well as leaf and total herb.

LSD ($p < 0.05$) showed that hypericin amount was significantly higher in flower compared to leaf and total herb in Debar location (0.328 ± 0.24 to 0.113 ± 0.07 and 0.158 ± 0.10 mg/g in average per year, consequently), but no difference was determined between flower and total herb. In Mavrovo location no significant difference was determined when amount of hypericin in flower, total herb and leaf was compared (0.18 ± 0.06 , 0.123 ± 0.05 and 0.068 ± 0.02 mg/g in average per year, accordingly). Flower harvested in Tetovo location was characterized by significantly higher amount of hypericin compared to leaf (0.283 ± 0.13 to 0.10 ± 0.04 mg/g in average per year).

However, no difference was determined between amount in flower and total herb (0.138 ± 0.07) as well as total herb and leaf.

According to literature data total phenols and flavonoids were found in amounts of 17.6 mg/g dry acetone extract, for phenols and 16.85 mg/g dry acetone extract, for flavonoids (Maskovic et al., 2011). The quantity of total flavonoids was ranged from 0.1 to 1.6% in different species of *Hypericum*, with 1.5% in *H. perforatum* (Pilepic et al., 2013). The content of total phenolic acids in the investigated species of *Hypericum* was found to be between 1.1-10.4% and the highest quantity (10.4%) were found in *H. perforatum* (Pilepic et al., 2013). The examined taxa of *Hypericum* contained flavonoids and phenolic acids in different quantities, although the differences between species and years of harvest were not found to be significant (Pilepic et al., 2013) which also correlate with our findings and results.

The distribution of total phenols and total flavonoids were investigated previously and the highest total phenolic content was found in ethanol extracts of leaf (182.93 mg GAE/g DW), while the highest total flavonoid content was found in ethanol extract of flower (20.50 mg QE/g DW) (Sekeroglu et al., 2017). In our investigation, the highest amount of total phenols and total flavonoids were determined in sample HP/16 FL-D (sample of flower harvested in Debar in 2016) where they rich the value of 121.19 mg GAE/g DW and 125.35 mg CE/g DW (Table 2 and 3), respectively.

In order to find the optimization of harvest stage to increase bioactive compounds production, the levels of hypericin, flavonoids and polyphenols in *Hypericum perforatum* as well as antioxidant capacity were previously evaluated by the assays of high performance liquid chromatography (HPLC), $\text{NaNO}_2\text{-AlCl}_3\text{-NaOH}$ and Folin Ciocalteu as well as 1,1-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), respectively. It was found that the levels of hypericin, flavonoids and polyphenols in aerial parts decreased during harvest stages, even if bioactive compounds contents in flower reached the highest at blooming stage on a dry weight basis. These findings indicated that the optimization of harvest stage of *H. perforatum* should be at floral budding stage (Sun et al., 2018) which correlate with the results that we obtained for St. John's-wort harvested from three different locations in North Macedonia.

Total hypericins content in stems, leaves, and flowers of some *Hypericum* species growing in Turkey, was determined by HPLC. The content observed in the study varied greatly depending

on species and plant tissues. The lowest levels of hypericin and pseudohypericin were detected in leaves of *H. hyssopifolium* (0.030 and 0.051 mg/g DW, respectively) whereas flowers of *H. montbretii* produced the highest levels of both hypericin forms (2.52 mg/g DW hypericin and 3.58 mg/g DW pseudohypericin) (Ayan and Cirak, 2008). The results that we have obtained for distribution of hypericin in different plant organs from *H. perforatum* correlate with the findings of Ayan and Cirak, 2008 and with other available literature data (Repcak and Martonfi, 1997), which can be attributed to the specific defence role of the particular compound. This also could be considered as a natural source of hypericin.

Conclusion

The content of total phenols, flavonoids and hypericin was determined in whole herb, flowers and leaves of *Hypericum perforatum* L. (Hypericaceae). Samples were collected during 2014-2017, from three different locations in western part of North Macedonia (Tetovo, Debar and Mavrovo). The content of total phenols ranged from 35.15-83.08 mg GAE/g DW, 55.41-98.52 mg GAE/g DW and 75.44-121.19 mg GAE/g DW, in leaf, whole herb and flower, respectively. On the other hand, the amount of total flavonoids was 61.64-106.86 mg CE/g DW, 73.04-117.57 mg CE/g DW and 108.65-125.35 mg CE/g DW in leaf, whole herb and flower, respectively. Finally, the hypericin values were: 0.03-0.17 mg/g, 0.04-0.29 mg/g, and 0.07-0.60 mg/g, in leaf, whole herb and flower, respectively. The statistical analysis pointed that season of harvesting did not have any significance, opposite to the location which was identified as significant variable that influenced the amount of total phenols and flavonoids, as well as hypericin, in whole herb, flower and leaf.

From the results it can be anticipated that the flower of *H. perforatum* was most abundant with all three groups of secondary metabolites and most likely it should be advised that harvesting of St. John's-wort should be made in a manner which provides larger amounts of flowers as plant material for extraction and other purposes.

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Резиме

Дистрибуција на вкупни феноли, флавоноиди и хиперицин во различни растителни органи на диворастечки кантарион (*Hypericum perforatum* L., Hypericaceae) од Северна Македонија

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Клучни зборови: *Hypericum perforatum*, растителни органи, вкупни феноли, флавоноиди, хиперицин, статистичка анализа

Hypericum perforatum L. (Hypericaceae) содржи секундарни метаболити кои покажуваат значајни фармаколошки активности. Затоа, содржината на вкупните феноли, флавоноиди и хиперицин од херба, од цветовите и од листовите од кантарион собрани во текот на четири последователни сезони (2014-2017) од три различни локации во западниот дел на Северна Македонија (Тетово, Дебар и Маврово) беа испитани со три различни спектрофотометриски методи (со Folin-Ciocalteu реагенс, со $\text{NaNO}_2\text{-AlCl}_3\text{-NaOH}$ и со мешавина од вода/тетрахидрофуран). Содржината на вкупните феноли од различни растителни органи (листови, херба и цветови) изнесува 35,15-83,08 mg GAE/g, 55,41-98,52

mg GAE/g и 75,44-121,19 mg GAE/g, соодветно. Потоа, вкупната содржина на флавоноиди беше: 61,64-106,86 mg CE/g, 73,04-117,57 mg CE/g и 108,65-125,35 mg CE/g во листови, херба и цветови, соодветно, додека количините на хиперицин беа: 0,03-0,17 mg/g, 0,04-0,29 mg/g и 0,07-0,60 mg/g во листови, херба и цветови, соодветно. Значајни разлики беа забележани во количините на вкупните феноли, флавоноиди и хиперицин помеѓу локацијата на бербата, додека сезоните за собирање се покажаа како не значајни.

Дополнително, може да се види дека цветовите се карактеризираат со поголеми количини на вкупни феноли, вкупни флавоноиди и хиперицин во однос на хербата и листовите на сите три локации, затоа бербата на кантарион треба да се направи на начин за добивање поголема количини на цветови како материјал за собирање што може да ги зголеми биоактивните соединенија во собраната херба.

Table 1. Plant samples of *H. perforatum*

No.	Sample	Part of the plant	Locality	Year
1	HP/14 FoL-T	leaf, dry	Tetovo	2014
2	HP/14 FL-T	flower, dry	Tetovo	2014
3	HP/14 HB-T	herb, dry	Tetovo	2014
4	HP/14 FoL-D	leaf, dry	Debar	2014
5	HP/14 FL-D	flower, dry	Debar	2014
6	HP/14 HB-D	herb, dry	Debar	2014
7	HP/14 FoL-M	leaf, dry	Mavrovo	2014
8	HP/14 FL-M	flower, dry	Mavrovo	2014
9	HP/14 HB-M	herb, dry	Mavrovo	2014
10	HP/15 FoL-T	leaf, dry	Tetovo	2015
11	HP/15 FL-T	flower, dry	Tetovo	2015
12	HP/15 HB-T	herb, dry	Tetovo	2015
13	HP/15 FoL-D	leaf, dry	Debar	2015
14	HP/15 FL-D	flower, dry	Debar	2015
15	HP/15 HB-D	herb, dry	Debar	2015
16	HP/15 FoL-M	leaf, dry	Mavrovo	2015
17	HP/15 FL-M	flower, dry	Mavrovo	2015
18	HP/15 HB-M	herb, dry	Mavrovo	2015
19	HP/16 FoL-T	leaf, dry	Tetovo	2016
20	HP/16 FL-T	flower, dry	Tetovo	2016
21	HP/16 HB-T	herb, dry	Tetovo	2016
22	HP/16 FoL-D	leaf, dry	Debar	2016
23	HP/16 FL-D	flower, dry	Debar	2016
24	HP/16 HB-D	herb, dry	Debar	2016
25	HP/16 FoL-M	leaf, dry	Mavrovo	2016
26	HP/16 FL-M	flower, dry	Mavrovo	2016
27	HP/16 HB-M	herb, dry	Mavrovo	2016
28	HP/17 FoL-T	leaf, dry	Tetovo	2017
29	HP/17 FL-T	flower, dry	Tetovo	2017
30	HP/17 Hb -T	herb, dry	Tetovo	2017
31	HP/17 FoL – M	leaf, dry	Mavrovo	2017
32	HP/17 FL –M	flower, dry	Mavrovo	2017
33	HP/17 Hb – M	herb, dry	Mavrovo	2017
34	HP/17 FoL –D	leaf, dry	Debar	2017

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35	HP/17 FL-D	flower, dry	Debar	2017
36	HP/17 Hb -D	herb, dry	Debar	2017

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Table 2. The content of total phenols in herb, flower and leaf of *Hypericum perforatum* (mg GAE/g DW)

Year	Tetovo			Debar			Mavrovo		
	herb	flower	leaf	herb	flower	leaf	herb	flower	leaf
2017	77.82±0.27	79.66±0.35	68.67±0.89	82.75±0.52	85.83±0.58	64.90±0.59	60.16±0.19	75.44±0.11	42.68±0.66
2016	63.79±0.26	113.49±0.51	60.92±0.90	98.52±0.58	121.19±0.78	83.08±0.45	55.41±0.93	80.67±1.00	36.54±0.76
2015	72.72±0.81	91.34±0.14	56.91±0.22	81.63±0.23	91.05±0.76	68.26±0.71	62.56±0.061	94.19±0.46	47.16±0.22
2014	66.2±0.30	109.09±0.43	60.34±0.47	80.12±0.14	80.20±0.30	59.52±0.57	75.88±0.79	83.65±0.86	35.15±0.21

(n=3), DW – dry weight

Table 3. The content of flavonoids in herb, flower and leaf of *Hypericum perforatum* (mg CE/g DW)

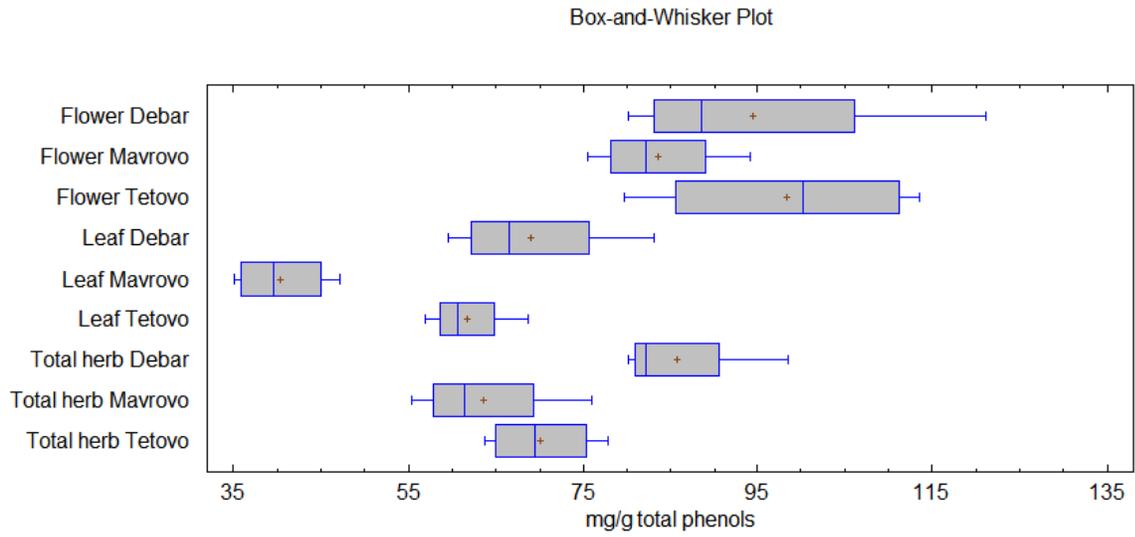
Year	Tetovo			Debar			Mavrovo		
	herb	flower	leaf	herb	flower	leaf	herb	flower	leaf
2017	109.33±0.83	116.42±1.04	103.61±0.45	102.55±0.27	108.65±0.34	72.79±0.89	112.68±0.29	118.38±0.95	98.31±0.34
2016	73.04±0.54	110.62±0.43	61.64±0.11	117.57±0.26	125.35±0.52	102.32±0.90	89.81±0.81	116.78±0.16	86.34±0.82
2015	85.72±0.99	109.12±0.88	71.12±0.72	104.75±0.81	114.38±0.13	81.58±0.22	108.84±0.04	117.53±0.43	101.01±0.69
2014	108.72±0.31	112.74±0.65	106.86±0.21	94.33±0.30	111.81±0.43	69.66±0.47	115.77±0.40	119.50±0.67	93.11±0.13

(n=3), DW – dry weight

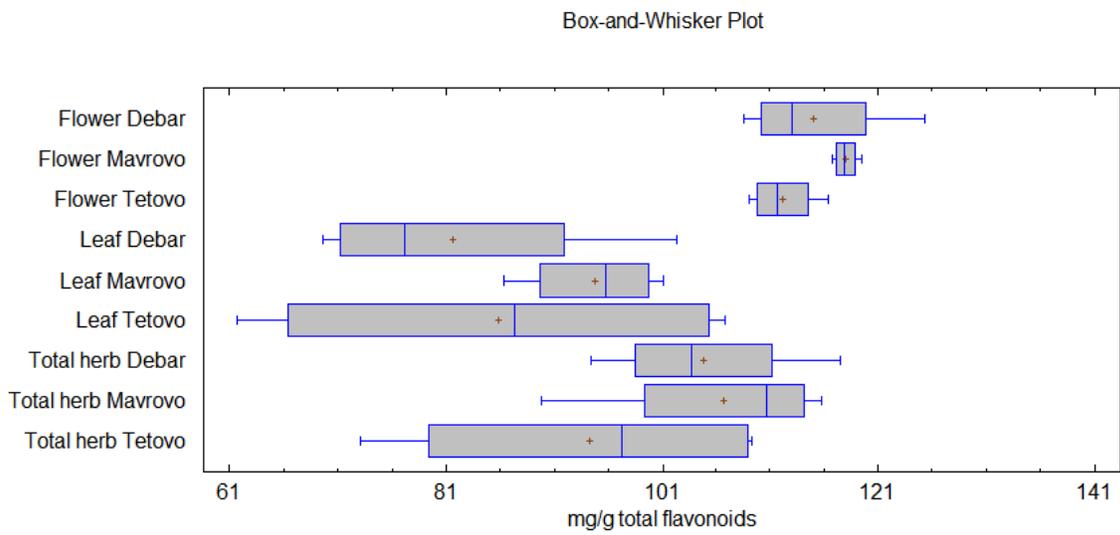
Table 4. The content of hypericin in herb, flower and leaf of *Hypericum perforatum* (mg/g DW)

Year	Tetovo			Debar			Mavrovo		
	herb	flower	leaf	herb	flower	leaf	herb	flower	leaf
2017	0.22±0.03	0.45±0.02	0.14±0.02	0.13±0.02	0.20±0.02	0.09±0.02	0.17±0.01	0.24±0.01	0.10±0.03
2016	0.16±0.02	0.23±0.02	0.12±0.01	0.17±0.00	0.44±0.02	0.16±0.02	0.12±0.05	0.16±0.03	0.07±0.02
2015	0.11±0.01	0.30±0.04	0.08±0.03	0.29±0.02	0.60±0.03	0.17±0.01	0.06±0.01	0.10±0.02	0.05±0.01
2014	0.06±0.01	0.15±0.02	0.06±0.01	0.04±0.01	0.07±0.01	0.03±0.01	0.14±0.02	0.22±0.02	0.05±0.01

(n=3), DW – dry weight



a)



b)

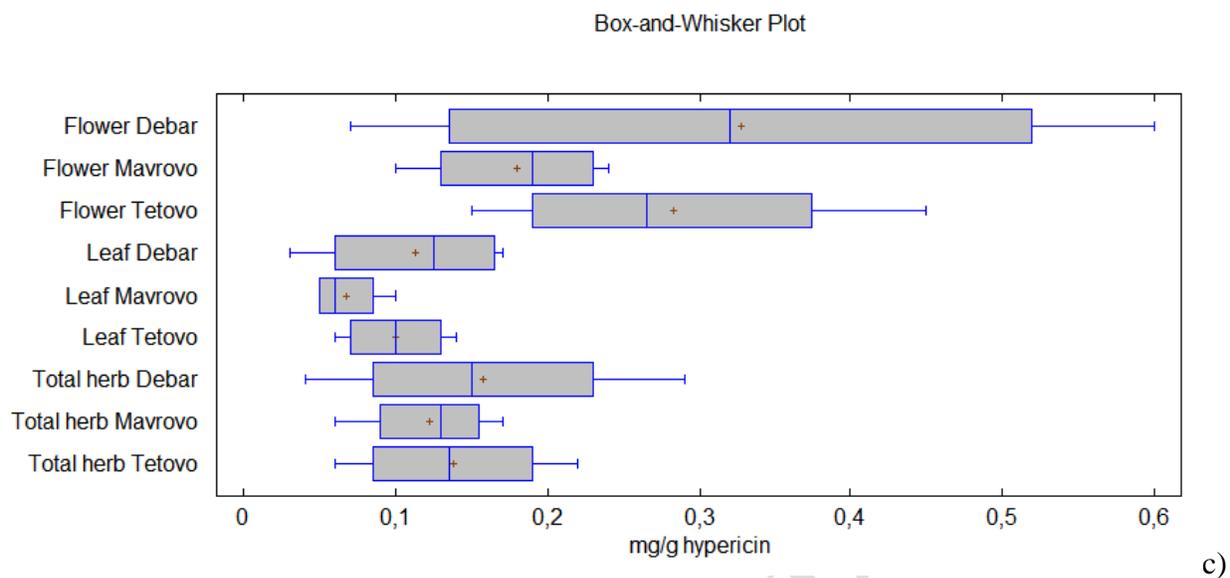


Fig. 1. Box-and-Whisker plot for: a) total phenols, b) flavonoids and c) hypericin in whole herb, flower and leaf of St. John's-wort.

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