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COMPARISON OF PHYTOCHEMICALS AND ANTIOXIDANT CAPACITY OF *HYPERICUM PERFORATUM*; WILD PLANT PARTS AND *IN VITRO* SAMPLES

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ABSTRACT

The aim of study is to compare the phytochemicals and free radical scavenging activities in methanol extracts of flower, leaf, stem *in vitro* plantlet, callus of *Hypericum perforatum* L. *In vitro* cultures of *H. perforatum* was established by using MS-B5 medium contained plant growth regulators such as BAP, TDZ and picloram. Total phenolics and flavonoids were analysed by spectrophotometric methods.The stem was the richest in total phenolics (228.9 mg GAE/g extract) and flavonoids (102.4 mg QE/g extract). Quinic acid, gallic acid, (+)-catechin, ferulic acid, vanillic acid, *p*-coumaric acid, caffeic acid, and quercetin were determinated by LC-MS/MS. Free radical scavenging activities (ABTS and DPPH) of all samples were detected as IC_{50} values, and was compared to standards such as trolox and ascorbic acid. As a result, the stem exhibited the stronger antioxidant activities than other samples, and vanillic acid, ferulic acid and gallic acid could be produced by *in vitro* culture.

Keywords: *Hypericum perforatum*, antioxidant activity, *in vitro* plantlets, phytochemicals.

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INTRODUCTION

Hypericum perforatum (St. John's wort), the most well-known member of Hypericum genus belonging to the Hypericaceae (previously Clusiaceae or Guttiferae) family, a rich source for flavonoids, widely consumed for medicinal purpose in all over the world. Particularly, extracts of St. John's wort are widely utilized as a supplement drug in the treatment of depression in Europe and the US (Kasper *et al.,* 2010), moreover it has full marketing authorization in many European countries (Zorzetto *et al.,* 2015).

Hypericum species include a wide variety of secondary metabolites known as mainly napthodianthrones (hypericin, pseudohypericin, protohypericin), flavonoids (campherol, quercetin, rutin, luteolin, hyperin, hyperoside), phenolic acid (chlorogenic acid), phloroglucinols (hyperforin, furohyperforin), xanthones and essential oils (Fobofou *et al.,* 2015). These components exhibit very important biological activities such as antioxidant, antidepressant, antitumor, antibacterial, antimicrobial, anti-inflammatory effects and others (Zorzetto *et al.,* 2015).

Phytochemicals such as flavonoids and phenolic acids is mostly associated with many biological activities, especially antioxidant activity associated with lowering the risk of occurrence of many chronic diseases, including cancer and cardiovascular diseases (Saddiqe *et al.,* 2010). Establishing *in vitro* cultures is a great tool for the production of these secondary metabolites under a controlled environment, independent of seasonal and geographical conditions such as temperature, soil properties, altitude and others. In previous studies, significant secondary metabolite production such as total phenols, flavonoids, hyperoside, hypericin, pseudohypericin and others were obtained specially from shoot, root, hair, callus and suspension culture of *H. perforatum* were reported (Nigutová *et al.,* 2019). These *in vitro* cultures grown under controlled conditions have been emerged as an attractive alternative to field cultivation and wild plants, and provide a stable substance production (Shakya *et al*., 2019). Therefore, the aim of the present study was to investigate *H. perforatum* in terms of (1) production of undifferentiated callus and shoot culture; (2) content of eight secondary metabolites, such as gallic acid, quinic acid, quercetin, vanillic acid, (+)-catechin, *p*-coumaric acid, caffeic acid, ferulic acid, in different parts of the wild-growing plant and *in vitro* samples by LC-MS/MS; (3) characterization and comparison of the callus, *in vitro* plantlets, the plant parts extracts with respect to their to determine total phenolic and flavonoid contents, moreover antioxidant properties using DPPH and ABTS radicals.

MATERIALS AND METHODS

Plant material: The aerial parts of *H. perforatum* representing a total of 40 shoots were collected at full

flowering period between 11:00 a.m. and 13:00 p.m. from Çorum in Turkey (40º41ˈN, 34º7ˈE, 1372 m). The species was identified, and the herbarium specimen was deposited in a herbarium (voucher number: 28281) placed in Selcuk University. The seeds of *H. perforatum* were handpicked from 30 randomly selected *Hypericum* plants and stored at 4 ± 2°C in sealed plastic bags until used for *in vitro* cultures.

Source of explants for *in vitro* culture: The seeds of *H. perforatum* were surface sterilized by treatment by immersion in 20% sodium hypochlorite for 20 min, and followed by 3–4 times rinses in sterile distilled water. After sterilization, the seeds were germinated on the Murashige and Skoog (MS) basal media containing 1.5 mg/L GA_3 (gibberellic acid), 30% (w/v) sucrose and 0.7% (w/v) agar. Twenty five seeds were cultured in each petri dishes and incubated in a growth chamber under photoperiod of 16 h at 24 ± 1°C. Two week-old seedlings served as the source for further explants.

Shoot production: The nodal segments were cultured on MS-B5 medium supplemented with 1.0 mg/L BAP (6-benzylaminopurine) (Nigutová *et al.,* 2017). The explants were subculture at the end of the two week, and after two month of culture, secondary metabolite contents and antioxidant activities consisting of shoot were determined.

Callus production: Nodal segments of *H. perforatum* were placed on MS-B5 medium supplemented with 1.0 mg/L TDZ (thidiazuron) (Yamaner and Erdag, 2020) and 0.25 mg/L picloram. Callus formation was observed one week later. The explants were subculture every two weeks, and 45 day old callus cultures were analyzed.

Extraction: The aerial parts (flower, leaf and stem) of the wild-growing *H. perforatum* and *in vitro* samples (callus and *in vitro* plantlet) were used for the extraction. The aerial parts were dried under shade and mechanically ground with a blender while callus was ground with a mortar and pastle in a liquid nitrogen. 4 g (three replicate) of each grounded plant materials were extracted individually in 40 ml of 100% methanol at 40°C for 24 h (Yaman *et al.,* 2020). The resulting solutions were filtered through whatman paper and the solvent was removed on a rotary evaporator at temperature bellow 40°C. Extract yields of flower, leaf, stem, *in vitro* plantlet and callus were found 18.6%, 14.5%, 2.5%, 6.4% and 2.1%, respectively.

Total phenolic content (TPC): The total phenolic content was analyzed according to the Folin-Ciocalteu reagent (FCR) by the methodology of Singleton *et al.* (1999) with slight modification. The total phenol content was calculated at 760 nm after incubated in dark at room temperature for 2 h. The results were expressed as mg equivalents of gallic acid (GAE) per gram of extract according to the equation obtained from the standard gallic acid graph (y = 0.0089x − 0.0003, R2 = 0.999).

Total flavonoid content (TF): Total flavonoid contents were determined according to Arvouet-Grand *et al.* (1994). Absorbance measurements were recorded at 417 nm after 40 min incubation at room temperature in dark. Total flavonoid contents were expressed as mg equivalents of quercetin (QE) per gram of extract according to the equation obtained from the standard quercetin graph (y = 0.0122x + 0.065, R2 = 0.998).

LC-MS/MS analysis and identification of phytochemicals*:* Phytochemicals analyzed such as gallic acid, quinic acid, quercetin, vanillic acid, (+)-catechin, *p*-coumaric acid, caffeic acid, ferulic acid were purchased from Sigma–Aldrich. Quantitative analysis of compounds was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), Thermo Scientific - Dionex Ultimate 3000 - TSQ Quantum with Thermo ODS Hypersil 250 × 4.6 mm, 5 µm column. The injection volume was 10 µL. The mobile phase included eluent water with 0.1 % formic acid (A) and methanol (B). The flow rate was 0.7 Ml/min, and the column temperature was set to 40°C. The gradient programme was fixed as follow: 0–5 min, 100% A, 5–20 min, 95% A, 20–20.1 min, 20% A, 20.1–26 min, 5% A, 26–30 min 100% B. Total process time was 30 min. The injection volume was 10 µL. The analytical method was validated to determine the linearity, limits of detection (LODs), limits of quantification (LOQs) and precision (Table 1). The relationship between peak area and concentration was linear from 50 to 100 µg/mL (ppb) for each compound. Linearity was assessed using linear regression analysis of six points for each compound. Linear plot consists of triplicate per point. The correlation coefficients (R^2 values) were found to be ≥ 0.99. LOD and LOQ were determined by using measurements of reagent blanks spiked with low concentrations of analyte according to Eurachem Guide (Laboratory Guide to Method Validation and Related Topics, 2014). The blanks were spiked to 5 ppb standard. Calculate LOD and LOQ as LOD = 3 × S_0 and LOQ = 10 × S_0. The repeatability in the intra-day values (relative standard deviation, RSD %) for compounds, using the corresponding peak areas of three replicate analyses at all the concentration levels. The trueness was examined as recovery of each compound from mixed stock standard solutions in spiked plant extracts. The recovery was evaluated by means of three replicate measurements in a day. The average recovery data of the compounds were determined using the following formula:

$$\text{Recovery } (100\%) = \left(\frac{M \quad c}{S \quad c}\right) \times 100 \quad (1)$$

The concentration of compounds in samples of the plant was obtained from either one of the corresponding calibration curves. Finally, each bioactive amount for

each sample were calculated µg/g extract (Shrivastav *et al.,* 2011).

DPPH$^{\bullet}$ scavenging activity: Radical scavenging activity of the extracts was determined using the stable 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) (Blois, 1958). Absorbance of all samples was measured at 517 nm. Ascorbic acid (AA) and trolox as standard were used. The experiment was carried out in four replicates using a UV–visible spectrophotometer. The results were determinate half maximal inhibitory concentration (IC_{50}).

ABTS$^{\bullet+}$ scavenging activity: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid was used for evaluation of radical cation scavenging activity according to the method described by Re *et al.* (1999). Absorbance of all samples was recorded at 734 nm. The results were determinate half maximal inhibitory concentration (IC_{50}).

Table 1. Linear regression equation and correlation coefficient, precision of each detected compounds by LC-MS/MS analysis on *H. perforatum*.

Composition (µg/g)	Linear regression equation	R^2	LOD (µg/L)	LOQ (µg/L)	RSD (%)	Recovery (%)
Quinic acid	y=483.60×-10563	0.999	5.52	6.92	4.07	97.75
Gallic acid	y=464.58×–10423	0.999	5.38	6.78	4.45	96.45
Vanillic acid	y=603.27×-19881	0.997	4.99	6.11	4.12	101.25
Caffeic acid	y=1219.67×-7914	0.999	5.45	7.13	2.74	98.55
p- coumaric acid	y=4773.03×+86775	0.996	7.33	11.11	3.14	99.85
Ferulic acid	y=322.29×-4272	0.998	6.62	9.00	4.90	100.15
(+)-catechin	y=598.80×+882	0.997	5.12	6.31	2.85	99.14
Quercetin	y=18760×-131657	0.998	6.62	11.59	2.21	99.90

R^2 – regression coefficient, LOD – limit of detection, LOQ – limit of quantification, RSD -relative standard deviation

Statistical analysis. All data were statistically analyzed using one-way ANOVA, and comparison of the means was carried out by Duncan's multiple range tests at P≤ 0.01 and the data were given as the mean ± standard error. Correlation coefficients between phytochemicals and antioxidant activities were evaluated using the Pearson's correlation. *H. perforatum* data were analyzed by PCA to visualize relationships among antioxidant activities, compounds and the plant samples (flower, stem, leaf, *in vitro* plantlet and callus). All statistical calculations were made using IBM SPSS statistics 20.

RESULTS AND DISCUSSION

Callus and Shoot Induction: Callus formation was determinate in the MS-B5 medium supplemented with 1.0 mg/L TDZ and 0.25 mg/L picloram. The callusproduced from *Hypericum perforatum* was yellowish in color and fragile (Fig. 1). Also, callus development was observed to be slow and poor. Many researchers notified that induction and growth of callus of *Hypericum* species were slow and compact (Khan *et al.,* 2018). But, BAP was found favourable for multiplication of some *Hypericum* species *in vitro* shoots (Swain *et al*., 2016). We found that BAP was effective on shoot induction from nodal segments of *H. perforatum.*

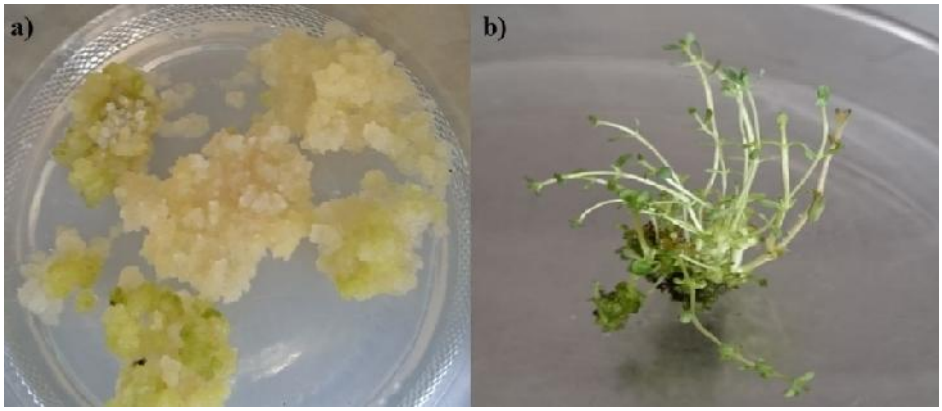

Figure 1. *In vitro* samples of *H. perforatum*. a) Callus formation from nodal segments cultivated on MS-B5 + 1.0 mg/L TDZ + 0.25 mg/L picloram (45. day). b) Shoot induction from nodal segments cultivated on MS + 1.0 mg/L BAP (60. day)

Phytochemical Analysis: In this study, the amount of total bioactive, namely TPC (18.9 - 228.9 mg GAE/g extract and TFC (9.3 - 102 mg QE/g extract) in methanol extracts of stem, flower, leaf, callus and *in vitro* plantlet of *Hypericum perforatum* is presented in Fig. 2. The total quantity of TPC and TFC showed statistically significant differences in all samples at the level of 0.01.

The *in vitro* samples, the *in vitro* plantlet and the callus showed the lowest TPC (27.1 and 18.9 mg GAE/g extract, respectively) and TFC (18.5 and 9.3 mg QE/g extract, respectively) among all samples, and were in the different group statistically. Kwiecień *et al.* (2018) recorded that amount of TPC in shoots cultivated on LS and MS media variants with 1.0 mg/L of BAP was the lower than the present result. Kumar *et al.* (2015) reported that combination effect of picrolam (different concentrations) and 1.0 mg/L TDZ on TPC in callus of *Pelargonium sidoides* exhibited a very strong negative effect compared to control and other concentration of TDZ. Also, Porto *et al.* (2014) notified that picloram on TPC in callus of Barbatimão exhibited a very strong negative effect. On the contrary, the highest TPC and TFC were observed in the stem followed by the leaf and flower. Ozturk *et al.* (2009) were recorded those total bioactive contents in leaf of *H. perforatum* were the higher than that in flower.

The amounts of the identified phytochemicals (quinic acid, gallic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, (+)- catechin, quercetin) by LC-MS/MS in methanolic extracts of flower, leaf, stem, *in vitro* plantlets and callus of *H. perforatum* is presented in Table 2. These phytochemicals are important to both commercially and antioxidant activity.

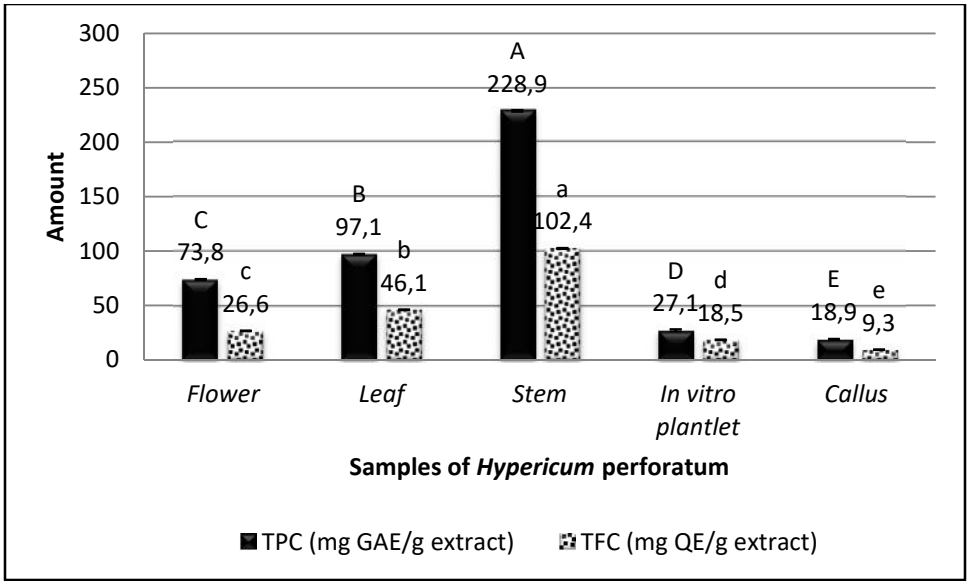

Figure 2. Total phenolic and flavonoid contents in flower, leaf, stem, *in vitro* plantlet, callus of *H. perforatum*. TPC shown in big letters and TFC in small letters indicate significant difference ($p < 0.01$).

Table 2. Phytochemicals in flower, leaf, stem, *in vitro* plantlet, callus of *H. perforatum* (µg/g extract).

Compounds	Flower		Leaf		Stem		*In vitro* plantlet		Callus	
Quinic acid	2003.52	b	1357.02	c	4954.79	a	23.62	e	58.49	d
Gallic acid	7.08	c	3.55	d	8.91	b	10.93	a	8.58	b
Vanillic acid	35.81	b	7.28	d	9.82	c	55.77	a	4.19	e
Caffeic acid	5.23	a	2.23	c	4.41	b	0.84	d	1.30	d
p-coumaric acid	21.35	a	0.05	d	2.55	b	1.34	c	2.24	b
Ferulic acid	10.80	a	6.11	c	4.92	d	8.34	b	9.09	b
(+)- catechin	1193.36	b	460.65	c	1628.20	a	0.45	d	0.10	d
Quercetin	354.61	a	80.77	b	77.00	c	17.90	d	1.48	e

Statistically, each column was evaluated separately and indicated in small letters ($P<0.01$)

As shown in Table 2, the level of quinic acid was found in aerial parts of the plant as a major product among other the compounds. The maximum quinic acid (4954.79 µg/g extract) was recorded in stem whereas the minimum amount (23.62 µg/g extract) was determinated in the *in vitro* plantlet. Özçelik *et al.* (2011) proved the

stronger cytotoxicity and anti-viral activity of quinic acid against DNA virus herpes simplex type 1 in Madin-Darby bovine kidney than that of acyclovir used as the references. This potent activity of the compound can be considered of helpful in pharmacy, taking into consideration the need to discover and exploit new natural sources against herpes simplex virus to cause a risk factor for human immunodeficiency virus infection (Kleinstein *et al,.* 2019).

(+)- catechin showed significantly high variation from 1628.20 µg/g extract in the stem to 0.10 µg/g extract in the callus. When compared to previous studies, the stem displayed the higher amount of (+)- catechin than many *Hypericum* species and their plant parts (Camas *et al.,* 2014; Napoli *et al.,* 2018). In contrast, caffeic acid, *p*-coumaric acid, ferulic acid and quercetin were found the highest amount in the flower (5.23, 21.35, 10.80 and 354.61 µg/g extract, respectively). Same results for the amounts of caffeic acid and quercetin were reported for *Hypericum* species by Camas *et al*. (2014).

When the compounds levels of the aerial parts of *H. perforatum* compared with those of the *in vitro* samples, gallic acid and vanillic acid in the *in vitro* plantlet shown the highest levels (10.93 and 55.77 µg/g extract, respectively) among all samples. The amount of vanillic acid in the *in vitro* plantlets was about 1.5 times more than in the flower, 6 times more than in the stem, 8 times abundant than in the leaf. Vanillic acid, a dihydroxybenzoic acid derivative used as a fragrance and flavoring agent has a global market in several areas over 200 million dollars (Ciriminna *et al.,* 2017). This compound has inhibitory and attenuate effects on many neurological diseases (Khoshnam *et al.,* 2018), preventing inflammatory bone disease (Karatas *et al.,* 2019) according to recent researches. Likewise, ferulic acid was abundant amount in the *in vitro* samples, but lower than those in the flower. Moreover, vanillic acid can synthesize from ferulic acid as natural product, and the price of this biotechnological vanillic acid is high (Delisi *et al.,* 2016). Therefore, the fact that these compounds such as vanillic acid and ferulic acid can be increased by *in vitro* culture can be important for the production of natural product.

Free Radical Scavenging Activity: DPPH and ABTS *radical scavenging* activities of all samples of *H. perforatum* were recorded as IC_{50} values (Table 3). *In vitro* samples such as the *in vitro* plantlet and callus of *H. perforatum* shown very low antioxidant activity compared to aerial parts of the plant. Among the all samples, the stem exhibited the strongest DPPH (55.6±0.15 µg/mL) and ABTS (70.6±0.04 µg/mL), as well as the higher than trolox (108.9±0.72 and 100.6±0.13 µg/mL, respectively) as standard. Moreover, there was no statistically significant difference between AA and the stem for DPPH activity. Boga *et al.* (2016) was reported that many *Hypericum* species displayed the more active than BHT (butylated hydroxytoluene) known as a synthetic standard. Contrary to this, some *Hypericum* species displayed the lower antioxidan activity than AA and BHT as standards (Zorzetto *et al.,* 2015). The stem displayed lower than AA for ABTS activity, and there was statistically significant difference.

Table 3. Free radical scavenging activities of the flower, leaf, stem, *in vitro* plantlet, callus of *H. perforatum* (IC_{50} values, µg/mL).

Plant parts	DPPH	ABTS
Flower	323.1±2.66 [d]	295.0±0.07 [d]
Leaf	231.3±4.41 [c]	275.4±0.55 [c]
Stem	55.6±0.15 [a]	70.6±0.04 [b]
In vitro plantlets	1351.1±7.00 [e]	2208.8±1.11 [e]
Callus	3215.4±5.35 [f]	6225.4±0.84 [f]
Trolox	108.9±0.72 [b]	100.6±0.13 [b]
AA[a]	59.1±0.01 [a]	55.7±1.07 [a]

[a]Ascorbic acid; Statistically, each column was evaluated separately and indicated in small letters (P<0.01)

Principal Component Analysis (PCA): PCA is a commonly used statistical technique to visually present the relationship between variables (Barbu *et al.,* 2015). In this study, the PCA was applied to find the relationship between variables among leaf, flower, stem, *in vitro* plantlet and callus of *H. perforatum*; with respect to TPC, TFC, ABTS, DPPH, quinic acid, gallic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, (+)- catechin, quercetin. The data matrix of phytochemicals and antioxidant activities was rotated using Varimax rotation method. The results shown that two eigen values >3 explained about 79.4% of the total variance. The first principal component (PC1) accounted for 51.9%, the second (PC2) for 27.5%. Their loading and score plots were shown in Fig. 3. Positive part of PC1 was related with TPC, TFC, quinic acid, *p*-coumaric acid, (+)-catechin, quercetin and caffeic acid. The concentrations of TPC, TFC, quinic acid and (+)- catechin that had the predominant phytochemicals in PC1 were highest for the stem. PC2 was positively correlated with the variables of vanillic acid, ferulic acid, *p*-coumaric acid, (+)- catechin, quercetin and caffeic acid. Ferulic acid, p-coumaric acid and quercetin that had higher amounts on PC2 appeared highest for the flower.

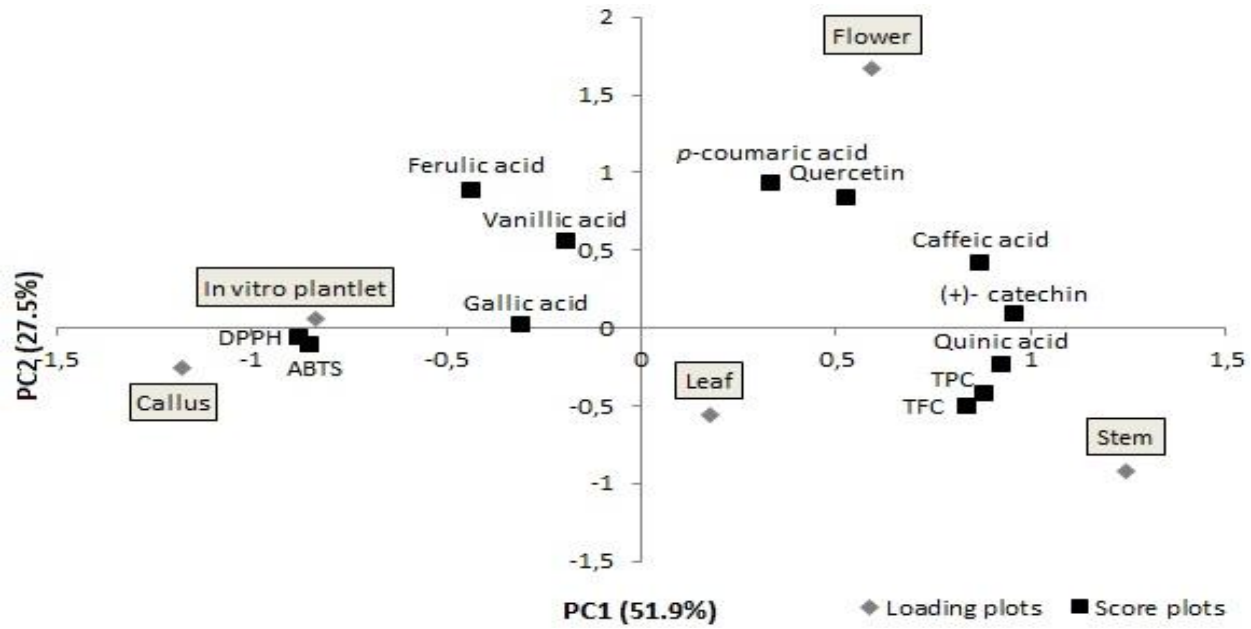

Figure 3. The principle component analysis for phytochemical contents and free radical scavenging activities of *H. perforatum*

Negative part of PC1 and PC2 was related on IC_{50} values of DPPH and ABTS which had higher for the callus and *in vitro* plantlet. High IC_{50} value is an indication that antioxidant activity is low. Ferulic and vanillic acids that had the positive impact on PC2 are higher loadings for *in vitro* plantlet from the callus.

Correlations Between Antioxidant Activities and Phytochemicals: Pearson correlations was found usually moderate relationship between phytochemical compositions and free radical scavenging activities (Table 4). The correlations were found statistically insignificant. DPPH and ABTS radical scavenging activities was negatively correlated phytochemical compositions except gallic acid and ferulic acid.

Table 4. Correlation coefficient between free radical scavening activities and phytochemical compositions of studied *H. perforatum* samples.

Phytochemicals	DPPH	ABTS
TPC	-0,703	-0,664
TFC	-0,508	-0,524
Quinic acid	-0,679	-0,640
Gallic acid	0,381	0,361
Vanillic acid	-0,120	-0,181
Caffeic acid	-0,661	-0,645
p-coumaric acid	-0,264	-0,285
Ferulic acid	0,405	0,365
(+)- catechin	-0,730	-0,703
Quercetin	-0,508	-0,524

TPC, total phenolic content; TFC, total flavonoid content

Total phenolic and flavonoid contents exhibited high correlation between free radical scavenging activities, but total phenolics shown the higher correlation than total flavonoids. In agreement with these obtained results, Orcic *et al.* (2011) reported that the total phenolics of St John's wort did correlate higher with antioxidant activity than its total flavonoid. The antioxidant activity may be attributed mostly to total phenolics

The strongest correlation for ABTS and DPPH activity (IC_{50} value) was observed in quinic acid, caffeic acid and (+)- catechin phytochemicals ($r > 0.600$) whereas the weakest correlation was found in vanillic acid ($r < 0.200$). This correlation of the compounds suggests that it is related to their amount in the extract. Because, antioxidant activities of these compounds were listed from highest to lowest as follows: gallic acid, quercetin, catechin, caffeic acid, quinic acid, ferulic acid and coumaric acid, respectively (Iwasaki *et al.,* 2011; Cos *et al.,* 2012).

Conclusion: *Hypericum perforatum* is well-known plant for content of pharmacologically important secondary metabolites. The stem of *H. perforatum* was found to have the highest effect for both TPC and TFC and antioxidant activity among other samples. Moreover, the radical scavenging activities of the stem displayed stronger activity than trolox known as standard. The stem can be a potential product as natural antioxidant. Therefore, further analysis is needed to use the stem at the flowering period in food, pharmacology and cosmetic industry, and for active ingredients. The amounts of gallic acid, vanillic acid and ferulic acid in the *in vitro* plantlet

have shown that they can be produced *in vitro* culture if suitable techniques are adapted. The *in vitro* production of these compounds used for many purposes may be very important. Especially, *in vitro* production of ferulic acid and vanillic acid may be considered to be important for the natural production of vanillin.

REFERENCES

Arvouet-Grand, A., B. Vennat, A. Pourrat and P. Legret (1994). Standardization of a propolis extract and identification of the main constituents. J. Pharm. Belgium. 49: 462–468.

Barbu, V., C. Neagu, and M. Dragan (2015). Principal component analysis of some parameters used for lycopene extraction from tomatoes. *Acta Alimentaria*. 44: 473–481.

Blois, M. S. (1958). Antioxidant Determinations by the Use of a Stable Free Radical, *Nature*. 181: 199–1200.

Boga, M., A. Ertas, E. Eroglu-Ozkan, M. Kizil, B. Ceken and G. Topcu (2016). Phytochemical analysis, antioxidant, antimicrobial, anticholinesterase and DNA protective effects of *Hypericum capitatum* var. *capitatum* extracts. S. Afri. J. Botany. 104: 249–257.

Camas, N., J. Radusiene, L. Ivanauskas, V. Jakstas, S. Kayikci and C. Cirak (2014). Chemical composition of *Hypericum* species from the Taeniocarpium and Drosanthe sections. Plant Systematics and Evolution. 300: 953–960.

Ciriminna, R., R. Delisi, F. Parrino, L. Palmisano and M. Pagliaro (2017). Tuning the photocatalytic activity of bismuth wolframate: towards selective oxidations for the biorefinery driven by solar-light. Chemical Communications. 53: 7521-7524.

Cos, P., Rajan, P., Vedernikova, I., Calomme, M., Pieters, L., Vlietinck, A.J., Augustyns, K., Haemers, A., and D.V. Berghe (2012). In Vitro Antioxidant Profile of Phenolic Acid Derivatives. *Free Radical Research*. 36: 711-716.

Delisi, R., R. Ciriminna, F. Parrino, L. Palmisano, Y. J. Xu and M. Pagliaro (2016). One-Pot, Clean Synthesis of Vanillic Acid from Ferulic Acid. ChemistrySelect. 1: 626–629.

Fobofou, S. A., T. Franke, K. Sanna, G. Porzel, A. Bullita, E. La, P. Colla and L. A. Wessjohann (2015). Isolation and anticancer, anthelminthic, and antiviral (HIV) activity of acylphloroglucinols, and regioselective synthesis of empetrifranzinans from *Hypericum roeperianum*. Bioorganic & Medicinal Chemistry. 23: 6327-6334.

Iwasaki, Y., T. Hirasawa, Y. Maruyama, Y. Ishii, R. Ito, K. Saito, T. Umemura, A. Nishikawa and H. Nakazawa (2011). Effect of interaction between phenolic compounds and copper ion on antioxidant and pro-oxidant activities. Toxicology in Vitro. 25: 1320.

Karatas, O., H. Balci Yuce, M. M. Taskan, F. Gevrek, F. Ucan Yarkac, A. Keskin, S. F. Ocak Karatas and H. Toker (2019). The effect of vanillic acid on ligature-induced periodontal disease in Wistar rats. *Archives of Oral Biology*. 103: 1–7.

Kasper, S., F. Caraci, B. Forti, F. Drago and E. Aguglia (2010). Efficacy and tolerability of *Hypericum* extract for the treatment of mild to moderate depression. European Neuropsychopharmacology. 20: 747-765.

Khan, S. A., P. Verma, A. Arbat, S. Gaikwad. and V.A. Parasharami (2018). Development of enhanced hypericin yielding transgenic plants and somaclones: High throughput direct organogenesis from leaf and callus explants of *Hypericum perforatum*. Industrial Crops and Products. 111: 544-554.

Khoshnam, S. E., Y. Farbood, H. Fathi Moghaddam, A. Sarkaki, M. Badavi and L. Khorsandi (2018). Vanillic acid attenuates cerebral hyperemia, blood-brain barrier disruption and anxiety-like behaviors in rats following transient bilateral common carotid occlusion and reperfusion. Metabolic Brain Disease. 33: 785–793.

Kleinstein, S. E., P. R. Shea, A. S. Allen, D. M. Koelle, A. Wald and D.B. Goldstein (2019). Genome-wide association study (GWAS) of human host factors influencing viral severity of herpes simplex virus type 2 (HSV-2). Genes and Immunity. 20: 112–120.

Kumar, V., M. Moyo, J. Gruz, M. Šubrtová and J. Van Staden (2015). Phenolic acid profiles and antioxidant potential of Pelargonium sidoides callus cultures. Industrial Crops and Products. 77: 402–408.

Kwiecień, I., J. Smolin, L. Beerhues and H. Ekiert (2018). The impact of media composition on production of flavonoids in agitated shoot cultures of the three *Hypericum perforatum* L. cultivars 'Elixir,' 'Helos,' and 'Topas'. In Vitro Cellular & Developmental Biology – Plant. 54: 332–340.

Napoli, E., L. Siracusa, G. Ruberto, A. Carrubba, S. Lazzara, A. Speciale, F. Cimino, A. Saija and M. Cristani (2018). Phytochemical profiles, phototoxic and antioxidant properties of eleven *Hypericum* species - A comparative study. Phytochemistry. 152: 162–173.

Nigutová, K., S. Kusari, S. Sezgin, L. Petijová, J. Henzelyová, M. Bálintová, M. Spiteller and E.

Čellárová (2019). Chemometric evaluation of hypericin and related phytochemicals in 17 *in vitro* cultured *Hypericum* species, hairy root cultures and hairy root-derived transgenic plants. J. Pharmacy and Pharmacology. 71: 46–57.

Orcic, D. Z., N. M. Mimica-Dukic, M. M. Franciskovic, S. S. Petrovic and E. D. Jovin (2011). Antioxidant activity relationship of phenolic compounds in *Hypericum perforatum* L. Chemistry Central J. 5: 34.

Özçelik, B., M. Kartal and I. Orhan (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. Pharmaceutical Biology. 49: 396–402.

Ozturk, N., M. Tuncel and I. Potoglu-Erkara (2009). Phenolic compounds and antioxidant activities of some *Hypericum* species: a comparative study with *H. perforatum.* Pharmaceutical Biology. 47: 120-127.

Porto, J. M. P., R. Paiva, A. C. de Souza, A. Santos and F.T. Braga (2014). Induction and determination of total phenols of callus of barbatimão. Australian J. Basic and Applied Sciences. 9.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 26: 1231–1237.

Saddiqe, Z., I. Naeem and A. Maimoona (2010). A review of the antibacterial activity of *Hypericum perforatum* L. J. Ethnopharmacology. 131: 511-521.

Shakya, P., G. Marslin, K. Siram, L. Beerhues and G. Franklin (2019). Elicitation as a tool to improve the profiles of high-value secondary metabolites and pharmacological properties of *Hypericum perforatum*. *J. Pharm. Pharmacol.* 71 (1): 70-82.

Shrivastava, A. and V.B. Gupta (2011). Methods for the determination of limit of detection and limit of quantitation of the analytical methods, Chronicles of Young Scientists. 2: 21.

Singleton, V. L., R. Orthofer and R. M. Lamuela-Raventós (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: Methods in Enzymology, Oxidants and Antioxidants Part A. Academic Press. 152–178 p.

Swain, D., S. Lenka, T. Hota and G.R Rout (2016). Micro-propagation of *Hypericum gaitii* Haines, an endangered medicinal plants: assessment of genetic fidelity. The Nucleus. 59: 7–13.

Yaman, C., O. Tugay and D. Ulukuş (2020). Endemik Haplophyllum A. Juss. Türlerinin Antioksidan Aktivitesi Üzerine Lokasyon ve Tür Farkının Etkisi. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 10(1): 648-657.

Yamaner, O and B. Erdag (2020). Callus Induction and Adventitious Shoot Regeneration of *Hypericum adenotrichum* SPACH. Eskişehir Technical University J. Science and Technology C- Life Sciences and Biotechnology, 9 (1): 98-108.

Zorzetto, C., C. C. Sánchez-Mateo, R. M. Rabanal, G. Lupidi, D. Petrelli, L. A. Vitali, M. Bramucci, L. Quassinti, G. Caprioli, F. Papa, M. Ricciutelli, G. Sagratini, S. Vittori and F. Maggi (2015). Phytochemical analysis and *in vitro* biological activity of three *Hypericum* species from the Canary Islands (*Hypericum reflexum*, *Hypericum canariense* and *Hypericum grandifolium*). Fitoterapia. 100: 95–109.