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PREFACE

The Proceedings contains 25 papers presented at X International Symposium on Agricultural Sciences "AgroReS 2021" in Trebinje, Bosnia and Herzegovina, from 27 to 29 May, 2021. In the Proceedings are published only papers for which their authors choose that way of publishing

All papers were subject to anonymous double reviews and the category of papers were determined by the editors based on the recommendation of the reviewers.

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Original scientific paper

The influence of extraction solvents on the antioxidant potential of St. John's wort (*Hypericum perforatum* L.)

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Abstract

Hypericum perforatum L. (St. John's wort) is medicinal plant with high antioxidant, anti-inflammatory, antiviral, antimicrobial and antitumoral activities, used in treatments of many diseases. In this paper content of polyphenols compounds (total phenols, tannins and flavonoids) and antioxidant potential of methanol, ethanol, acetone and aqueous extracts of Hyperici herba were evaluated. The highest concentration of total phenols and total tannins were found in acetone extracts. The highest total flavonoids amount was detected in alcohol extracts. Acetone extracts showed the strongest antioxidant capacity. The results suggested that polyphenols are one of the main compounds responsible for antioxidant activity of *Hipericum perforatum* L. extracts. Due to its chemical composition *Hipericum perforatum* L. is valuable raw material for pharmaceutical and cosmetical industry.

Key words: St. John's wort, antioxidant capacity, polyphenols

Introduction

In recent years, antioxidants from plant sources have gained increasing interest because of its capability to neutralize or scavenge free radicals and protect cells from oxidative damages. Free radical is defined as an atom, molecule, or ion with one or more unpaired electron in its outer shell and normally generated in organisms when cells use oxygen to produce energy. The imbalance between production of free radicals and antioxidant defence system lead to oxidative

stress, which has implications for the progression of many degenerative diseases (cancer, cardiovascular illnesses, Alzheimer's disease, Parkinson's disease and others) (Pham-Huy et al., 2008; Sindhi et al., 2013). Plants are rich in bioactive compounds with high antioxidant activity such as tocopherols, tocotrienols, ascorbic acid, flavonoids, carotenoids and phenolic acids (Zehiroglu and OzturkSarikaya, 2019; Tecucianu and Oanacea, 2020).

Hypericum perforatum L. (St. John's wort) is a rich source of various groups of biologically active compounds e.g. naphthodianthrones (hypericin, pseudohypericin), phloroglucinol (hyperforin, adhyperforin), flavonoids (hyperoside, quercitrin, quercetin, rutin phenolic acid (chlorogenic acid) (Koyu and Haznedaroglu, 2015) and tannins (Maleš et al., 2006). Antioxidant, anti-inflammatory, antiviral, antimicrobial, antioxidant, antitumoral activities of this plant is well documented and related to its complex chemical composition and high concentration of mentioned bioactive compounds, located in buds, blossoms, and tips of twigs. However, the content of these compounds depends on species, growing regions, time of harvesting, extraction process and storage condition (Oliveira el al., 2016; Makarova et al., 2021).

St. John's wort is a member of Hypericaceae family with long traditional use in folk medicine and high distribution all over the world. It is also accepted by conventional medicine due to its positive pharmacological activities and benefits for human health. Dried flowering tops or aerial parts of plants represent the crude drug (Hyperici herba) commercially used in a form of tea, oil extracts, tinctures or sophisticated phytopharmaceutical products (i.e. capsule) (Šavikin et al., 2017) in treatment of internal and external diseases such as gastrointestinal diseases, bronchitis, sore throat infections (Güzel et al., 2019), nerve pain, wounds, skin inflammation, sleep disorders, depression, and haemorrhoids (Müller, 2003; Shrivastava et al., 2015).

The aim of this paper was to investigate the influence of different extraction solvents (70% methanol, 70% ethanol, 70% acetone and distillate water) on polyphenols content (total phenols, total tannins and total flavonoids) and antioxidant capacity of St. John's wort's dry areal parts.

Material and Methods

The wild-growing populations of St. John's wort harvested at full flowering stage were used as plant material in this study. Plants were collected at mountain Kopaonik, Treska, Serbia, during the summer 2020. After harvest, plant material (herba) was air-dried at dark place at room temperature till constant weight and grounded to a fine powder using a blender mill. One

gram of samples was extracted with 10 ml 70% methanol, 70% ethanol, 70% acetone or distillate water during 24 hours. The extracts were centrifuged, filtered and kept in fridge.

Folin-Ciocalteu colorimetric method as described by Nagavani and Raghava Rao (2010) with slight modification was used for determination of total phenolic compounds (TP) and total tannins (TT). Quercetin was used as standard and the amount of total phenolic compounds and total tannins were expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW). Total flavonoids content (TF) was estimated spectrophotometrically using aluminium chloride ($AlCl_3$) method, previously described by Saha el al. (2013). The concentration of total flavonoids was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW).

Ferric-reducing antioxidant power of extracts (FRAP) was assayed following method reported by Valentão et al. (2002), based on reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to blue colored ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by antioxidants present in samples. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was carried out according to method based on reaction between stable DPPH radical and a substance that can donate a hydrogen atom (Lai and Lim, 2011). ABTS radical cation assay, based on reduction dark blue colored 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$) to colourless ABTS by antioxidants, was estimated by the protocol of Zheleva-Dimitrova et al. with minor modification. The total antioxidant activity (TAA) was carried out by phosphomolybdenum method (Kamath et al., 2015). According to Saha et al. (2013) was estimated the total reduction capacity of extracts (TRC). The results of antioxidant activity estimated by DPPH, FRAP, ABTS, TAA and TRC were expressed as milligrams of trolox equivalents per gram of dry weight (mg Trolox/g DW). The superoxide free radical scavenging activity was estimated using a riboflavin/nitro blue tetrazolium (NBT) method based on ability of superoxide dismutase (SOD) to inhibit photochemical reduction of NBT. The results were expressed as number of International Units (IU) of SOD equivalents per gram of dry weight (IU SOD/g DW) (Kalaskar and Surana, 2014).

The data in triplicate were reported as mean ± standard deviation (Table 1). Statistic evaluation of data was analysed using software STATISTICA ver. 13.2 (StatSoft, Inc., USA). A one-way analysis of variance with the *post hoc* Fisher LSD was used to compare significant difference between the groups at the 5% significance level ($p< 0.05$). The correlation coefficients were done by Pearson.

Results and Discussion

The amounts of polyphenolic compounds (total phenols, tannins and flavonoids) as well as antioxidant activity measured by DPPH, FRAP, ABTS, TRC, TAA and NBT assays are summarized in *Table 1*. The range of total phenols varied between 42.70 and 85.82 mg QE/g DW in different solvent extraction systems. The concentration of total tannins was from 55.75 to 40.47 mg QE/g DW and the content of total flavonoids was from 9.14 to 13.24 mg QE/g DW. In this study, acetone extracts of St. John's wort were showed the highest concentration of TP (85.82 mg QE/g DW) and TT (55.75 mg QE/g DW). The amount of TP in ethanol extracts (63.41mg QE/g DW) was higher than in methanol (57.27 mg QE/g DW) and aqueous extracts (42.70 mg QE/g DW). According to analysis of variance followed by Fisher LSD *post hoc* test, the difference among TP and TT contest in acetone extracts and other tested extracts was statistically significant (p <0.05). Bonoli et al. (2004) were found that extraction of TP from barley flour by mixture of ethanol and acetone was most effective. Our results show that content of TF is not significantly higher in ethanol extracts (13.24 mg QE/g DW) than in methanol (12.30 mg QE/g DW) as well as the significant difference between its content in aqueous (9.14 mg QE/g DW) and acetone extracts (9.69 mg QE/g DW) no exist. In one previously research, Wang and Helliwell (2001) were reported that aqueous ethanol solvent had better performance for extraction of TF from tea, than aqueous methanol and aqueous acetone. Do et al. (2014) were found that 75% acetone extracts of *Limnophila aromatica* had the highest concentration of TP and TF. The differences between TP and TF extraction efficiency in this study and those of other studies may be associated with different chemical properties and polarity of flavonoids and others polyphenol compounds.

Our results demonstrate that *H. perforatum* extracts is a good source of polyphenols compounds and this is in agreement with previous research (Fathi and Ebrahimzadeh 2013; Šavikin et al., 2017). Phenolic compounds (flavonoids, phenolic acid, and polyphenol compounds) are plant secondary metabolites responsible for colour and sensory characteristics of vegetables and fruits as well as they have a significant role in plants` growth, reproduction, protection against pathogen and predators. In human diet phenolic acids, flavonoids, and tannins represent the most abundant antioxidants (Vuolo et al., 2019).

The extraction yields are strongly depending on the solvent polarity and chemical nature of polyphenols, under the same temperature, pH and extraction time (Do et al., 2014). One of widely used solvent for antioxidants and phenols extraction is methanol (Hertog et al., 1993) especially in extraction of lower molecular weight polyphenols while acetone is better

extraction solvent for the higher molecular weight flavanols. However, there is no universal extraction procedure suitable for extraction of all phenolic compounds (Dai and Mumper, 2010).

The results of antioxidant tests suggested that *H. perforatum* extracts have strong antioxidant capacity and this is in agreement with study of Güzel et al. (2019). The acetone extracts showed the highest value of TAA (478.46 mg Trolox/g DW), TRC (310.54 mg Trolox/g DW) as well as NBT (14.03 IU SOD/mg DW), ABTS (311.31 mg Trolox/g DW), DPPH (239.40 mg Trolox/g DW) free radical scavenging activity. The lowest antioxidant activity obtained by FRAP, DPPH, TRC tests was observed in aqueous extracts (119.00, 98.74 and 143.98 mg Trolox/g DW, respectively) while ABTS, TAA, NBT assays showed the lowest antioxidant activity in methanol extracts (206.70, 306.94 mg Trolox/g DW and 10.45 IU SOD/mg DW, respectively). These assays are one of the most frequently used for evaluating antioxidant activity. But different antioxidant tests are based on different chemical reactions and in order to comparing antioxidant properties of selected compounds must be used more than one method (Shalaby and Shanab, 2013; Shahidi and Zhong, 2015).

Table 1. Content of polyphenol compounds and antioxidant activity in St. John`s wort extracted by four different solvents

	Extraction solvent			
	Water	70% Methanol	70% Ethanol	70% Acetone
TP[1] (mg QE/ g DW)	42.70 ± 0.88^{d}	57.27 ± 3.35^{c}	63.41 ± 0.93^{b}	85.82 ± 0.29^{a}
TT[2] (mg QE/ g DW)	40.47 ± 0.94^{c}	52.75 ± 0.32^{b}	53.68 ± 0.69^{b}	55.75 ± 0.98^{a}
TF[3](mg QE/ g DW)	9.14 ± 0.16^{b}	12.30 ± 0.73^{a}	13.24 ± 0.32^{a}	9.69 ± 1.78^{b}
FRAP[4](mg Trolox/g DW)	119.00 ± 2.05^{c}	140.27 ± 20.00^{b}	181.49 ± 4.45^{a}	155.56 ± 0.34^{b}
DPPH[5](mg Trolox/g DW)	98.74 ± 13.96^{c}	131.77 ±27.02^{c}	181.80 ± 9.62^{b}	239.40 ± 27.07^{a}
TRC[6](mg Trolox/g DW)	143.98 ± 2.55^{d}	192.20 ± 7.96^{c}	271.54 ± 0.94^{b}	310.54 ± 18.62^{a}
NBT[7] (IU SOD/mg DW)	12.14 ± 0.25^{c}	10.45 ± 0.57^{d}	12.92 ± 0.40^{b}	14.03 ± 0.37^{a}
ABTS[8] (mg Trolox/g DW)	235.33 ± 1.36^{c}	206.70 ± 4.78^{d}	249.51 ± 1.76^{b}	311.31 ± 6.86^{a}
TAA[9] (mg Trolox/g DW)	372.38 ± 7.02^{b}	306.94 ± 20.87^{c}	379.42 ± 6.26^{b}	478.46 ± 21.81^{a}

Value is a mean of three replicates ± standard deviation (SD)

Value without the same superscript within each row differ significantly at $p<0.05$ (Fisher LSD *post hoc* test)

[1] TP total polyphenol content [2] TT total tannin content [3] TF total flavonoid content [4]FRAP Ferric-reducing antioxidant power [5] DPPH 2,2-diphenyl-1-picrylhydrazyl [6] TRC total reduction capacity [7] NBT Nitrobluetetrazolium[8] ABTS 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) [9] TAA Total antioxidant activity

Pearson coefficient (*Table 2*) showed positive correlation among TP and FRAP, DPPH, TRC, NBT, ABTS and TAA assays. FRAP, DPPH and TRC were in strong positive correlation with

TT while TF was in positive correlation with FRAP assay. The correlation analysis in Tusevski et al. (2019) study also indicated that antioxidant activity of *H. perforatum* is related to phenolic compound content.

Table 2. Pearson correlation coefficient between biochemical assays.

	FRAP[2]	DPPH[2]	TRC[2]	NBT[3]	ABTS[2]	TAA[2]
TP[1]	0.5815	0.980*	0.9417	0.6660	0.7443	0.8200
TT[1]	0.7685	0.8138	0.8532	0.2712	0.2892	0.4037
TF[1]	0.694	0.059	0.221	-0.336	-0.503	-0.419

*. Correlation is significant at the 0.05 level (2-tailed).

[1]Expressed as mg Quercetin/ g DW[2]Expressed as mg Trolox/g DW[3] IU SOD/ mg DW

Conclusions

The results of this study showed that the extraction solvent has significant influence on all measured phenolic compounds as well as antioxidant capacity estimated by six different assays. Our results suggested that extraction by using 70% acetone can provide significantly higher yield of total phenolic compounds from St. John's wort's raw material than other tested extraction systems. The strongest antioxidant potential measured by NBT, ABTS, DPPH, TAA, TRC was detected in 70% acetone extracts. Antioxidant activity of *H. perforatum* is directly connected with phenol compounds content in investigated extracts.

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