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Effect of different extraction solvents on total phenolic and flavonoid contents and antioxidant activity of the *Bolboschoenus laticarpus* rhizome extracts

Original Article

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Abstract:

The aim of this study was to determine and compare antioxidant activity and total phenol and flavonoid content in various rhizome extracts of *Bolboschoenus laticarpus.* Methanolic, ethanolic, chloroform, and ethyl acetate extracts were tested at 250, 500, 625, 750, 825, and 1,000 µg/ml of dry extract concentrations. The antioxidant activity of the extracts was evaluated using the DPPH method, the total flavonoid content by the aluminium chloride ($AlCl_3$) method and the total phenolic content by the Folin-Ciocalteau method. The results revealed that ethanolic extract had the highest antioxidant capacity (IC_{50}, 0.981 mg/ml) while the chloroform extract showed the lowest activity (IC_{50}, 11.78 mg/ml). Analysis of total phenolic content in different extract types showed that ethyl acetate extract had the highest concentration of phenols while chloroform extracts had the lowest values. The highest concentration of flavonoids was noticed in ethyl acetate extract while the lowest concentration was determined in methanolic extract. This study confirmed that rhizome of *B. laticarpus* has significant antioxidant capacity and is a good source of phenols and flavonoids.

Key words:
Bolboschoenus laticarpus, different solvents, antioxidant activity, phenols, flavonoids

Apstrakt:

Efekat različitih rastvarača za ekstrakciju na ukupnu količinu fenola i flavonoida kao i na antioksidativnu aktivnost ekstrakata rizoma vrste *Bolboschoenus laticarpus*

Cilj ovog rada je bio da se utvrdi prisustvo ukupnuh fenola i flavonoida kao i antioksidativni potencijal različitih tipova ekstrakata iz rizoma vrste *Bolboschoenus laticarpus*. Testirani su metanolni, etanolni, hloroformski i etil acetatni ekstrakti pri koncentracijama od 250, 500, 625, 750, 825 i 1,000 µg/ml. Antioksidativna aktivnost ekstrakta je utvrđena korišćenjem DPPH metode, ukupna količina flavonoida je određena aluminijum-hlorid ($AlCl_3$) metodom dok je ukupna količina fenola određena metodom po Folin-Ciocalteou. Rezultati su pokazali da je etanolni ekstrakt imao najvišu antioksidativnu aktivnost (IC_{50}, 0.981 mg/ml), dok je hloroformski ekstrakt pokazao najnižu aktivnost (IC_{50}, 11.78 mg/ml). Analiza ukupne količine fenola je pokazala da etil acetatni ekstrakt ima najveću količinu ukupnih fenola, dok je hloroformski ekstrakt imao najmanju. Najveća koncentracija flavonoida je zabeležena u etil acetatnom ekstraktu, dok je najmanja koncentracija flavonoida određena u metanolnom ekstraktu. Ovi rezultati pokazuju da rizom *B. laticarpus* vrste pokazuje značajan antioksidativni kapacitet i dobar je izvor fenola i flavonoida.

Ključne reči:
Bolboschoenus laticarpus, različiti rastvarači, antioksidativna aktivnost, fenoli, flavonoidi

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Introduction

For a long time *Bolboschoenus laticarpus* Marhold, Hroudová, Duchácek & Zákravsky, was considered a hybrid between the species *B. yagara* (Ohwi) Y. C. Yang & M. Zhan and *B. maritimus* (L.) Palla (Browning et al., 1996). However, Hroudová et al. (1999) proposed a different parentage for *B.*

laticarpus - *B. planiculmis* × *B. yagara* which is more likely according to ecological requirements and chromosome numbers (Jarolimova & Hroudová, 1998). In 2004 Marhold et al. compared morphological characteristics of populations belonging to hybrid *B. maritimus* × *B. yagara* with other related taxa native to Central Europe and described *B. laticarpus* as a new species, undoubtedly of hybrid origin but represents a well-established taxon occurring independently of the area of putative parents.

Bolboschoenus laticarpus is a perennial herbaceous plant with a branched underground rhizome, which bears a 2-3 cm elliptical to spherical tuber. Stems are erect, trigonous, about 0.7-1.1 m tall, with leaves spirally arranged in three lines (Hroudová et al., 2007). According to the identification key for European species of the genus *Bolboschoenus* (Hroudová et al., 2007), the main morphological characteristics for distinguishing *B. laticarpus* from other European *Bolboschoenus* species are: branched inflorescence, consisting of a central group of 2-7 clustered, sessile spikelets and of 2-5 rays bearing fascicles of 2-4 spikelets, the rays more than two times longer than sessile spikelets, total number of spikelets on rays higher than or same as number of sessile spikelets, broad achenes (2.0-2.4 mm wide), compressed obtusely-trigonous in cross-section, thin exocarp but visibly developed, formed of slightly elongated cells.

According to Hroudová et al. (2007), *B. laticarpus* is a frequent species mostly distributed in Central Europe while it is rare in Southern and Southeastern Europe and inhabits river floodplains, next to canals and occasionally flooded areas (Hroudová et al., 1999). It can form large littoral zones in aquatic habitats, but it also thrives in terrestrial habitats that were temporarily flooded (Mikulka et al., 1999). In Serbia *B. laticarpus* occupies different habitat types: river floodplains, places near water channels, and the shores of lakes and ponds. It is recorded along the Danube and Tisa rivers in Vojvodina and in a few localities in the south-eastern and southern parts of Serbia (Nikolić et al., 2019).

It is known that some representatives of the genus *Bolboschoenus* are used for practical purposes and in medicine (Ewing, 1983; Powell et al., 1987; Simpson & Inglis, 2001). The stems of *B. maritimus* are woven into baskets and made into blankets in Ethiopia, while in India the seeds are used in food or made into flour (Simpson & Inglis, 2001). *Bolboschoenus maritimus* is also used in medicine as an agent against tumors and leukaemia (Powell, 1987). Powell (1987) examined the alcoholic extract from the seeds of *B. maritimus* and analysed the bioactive components as well as their effect on leukaemia. In China, the root of *B. maritimus* is used as an astringent and diuretic (Ewing, 1983). Grigore & Oprica (2015) analysed the possibility of antioxidant potential in some halophytes, including *B. maritimus*. Numerous methods exist for extracting antioxidants from plants, including Soxhlet extraction, maceration, supercritical fluid extraction, subcritical water extraction, and ultrasound-assisted extraction (Do et al., 2014). The extraction yield and antioxidant activity are influenced not only by the chosen extraction technique but also by the solvent utilized (Dapkevicius et al., 1998). The solubility of different antioxidant compounds, which vary in their chemical characteristics and polarities, may vary depending on the solvent employed (Turkmen et al., 2006). Polar solvents are commonly employed to extract polyphenols from plant matrices (Anokwuru et al., 2011). Among them, aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate are the most suitable (Sultana et al., 2009). Ethanol is recognized as an effective solvent for polyphenol extraction and is considered safe for human consumption. Methanol is often preferred for extracting lower molecular weight polyphenols, while aqueous acetone is known for its efficacy in extracting higher molecular weight flavanols (Dai & Mumper, 2010).

The species *B. laticarpus* has not been investigated in terms of antioxidant activity and total phenolic and flavonoid content so far.

Accordingly, the following goals were established:

1) Determination of the antioxidant activity of different extracts of the rhizome of *B. laticarpus.*
2) Determination of the total amount of phenol and flavonoids in the analysed different extracts of the rhizome of *B. laticarpus.*

Materials and Methods

Plant material

For the purposes of this research, rhizomes of the species *B. laticarpus* were collected from May to September 2018 at the Oblačina location (SE Serbia). The material was identified based on the key for European and Serbian species (Hroudová et al., 2007; Nikolić et al., 2019) and deposited in the Herbarium Moesiacum Niš (HMN) of the Faculty of Sciences and Mathematics (13511 HMN). The plant material was air-dried and stored in a dry room at room temperature until further processing.

Obtaining extracts

Dried and crushed plant material (10 g) was extracted using different organic solvents: ethanol, methanol, ethyl acetate, and chloroform (100 ml).

The extraction was done within 24 h in a dark place at room temperature. Extraction in the first and the last hour of the scheduled time took place in an ultrasonic bath. After filtering, the extracts are evaporated to dryness using a vacuum evaporator. All extracts were prepared to the concentration of 1 mg/ml. The extracts were packed in bottles and stored in a dark and cool place.

Chemicals and reagents

Chemicals and reagents used for antioxidant analysis were butylated hydroxytoluene (BHT) („Zorka pharma" Šabac) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Chemicals Co. (St. Louis, MO, USA)). The Folin-Ciocalteau phenolic reagent (Merck (Darmstadt, Germany) and gallic acid (Numex chemical products (India)) were used to determine the total phenolic content. Na_2CO_3, $AlCl_3$ and NaOH („Centrochem Doo" Stara Pazova, Serbia) and quercetin (Med Chem Expres USA) were used. All solvents and substances were p.a. purity.

Determination of total phenolic content

The total phenolic content of the extracts was determined spectrophotometrically according to Folin-Ciocalteau (Singleton et al., 1999) with some modifications. The 300 µl extract solution and 1,500 µl 1:10 of Folin-Ciocalteau reagent were mixed and after 6 min in the dark 1,200 µl (7.5%) of Na_2CO_3 was added. After standing in the dark for 2 h at room temperature, the absorbance was measured at 740 nm wavelength (UV-1650PC, Shimadzu 1650, Europe). The total phenolic content was calculated based on the calibration curve of gallic acid (GA) in concentrations of 0.02-0.1 mg/ml. Data are expressed as mg gallic acid equivalents per gram of extract (GAE/g).

Determination of total flavonoid content

The $AlCl_3$ (aluminium chloride) method (Chang et al., 2002) was used to determine the total flavonoid content. Aliquots of extracts and standards were dissolved in methanol. The 1 ml was added to a standard 10 ml dish containing 4 ml of distilled water (dH_2O). Then 0.3 ml of 5% $NaNO_2$ was added. After 5 min, 0.3 ml of $AlCl_3$ (10%) was added. After 6 min, 2 ml of NaOH (1M) was added and the total volume of the reaction mixture was adjusted with 2.4 ml of dH_2O. The mixture was shaken well and allowed to incubate at room temperature for 30 min. Absorbance was measured at 510 nm. The total flavonoid content is expressed as mg equivalent of quercetin per gram of extract (mg QuE/g extract). A standard curve was created for quercetin in the concentration range of 0.02, 0.04, 0.06, 0.08, and 0.1 mg/ml. Measurements were made in relation to a blank sample (all substances used in the same ratio without extracts or standards).

Determination of antioxidant activity - DPPH method

The antioxidant activity of the extracts was determined using the DPPH method (Blois, 1958). Immediately before the experiment, a fresh DPPH working solution (concentration 0.004%) was prepared. The initial stock of the extract had a concentration of 1 mg/ml. Dilutions were made in concentrations of 1,000 µg/ml, 825 µg/ml, 750 µg/ml, 625 µg/ml, 500 µg/ml, and 250 µg/ml. The following procedure was used for each tested concentration and for each type of extract: 300 µl of extract of a certain concentration was mixed with 2,700 µl of a methanol solution of DPPH, and the mixture was left in the dark for 30 min at a room temperature and after the reaction time, the absorbance was measured at 517 nm at spectrophotometer (A_1). Each sample as well as BHT (positive control) was measured in 3 replicates. Results are presented as mean ± standard deviation.

The percentage of DPPH radical inhibition (it can also be indicated as the percentage of antioxidant activity, %AA) is calculated using the formula:

$$\% \text{ DPPH radical inhibition (at 517 nm)} = [(A_0 - A_1) / A_0] \times 100$$

$\mathbf{A_0}$ – absorbance of the blank sample (DPPH dissolved in methanol);
$\mathbf{A_1}$ – sample absorbance.

Concentrations that inhibit 50% of DPPH radicals (IC_{50}) were obtained from the curve (Microsoft Excel 2007) where the dependence between DPPH radical inhibition and concentration is presented for each type of extract as well as for the control. To obtain the IC_{50} value, an XY-scatter graph is used, on the basis of which the equation of the curve is determined. Solving the curve equation for X gives the IC_{50} value. The R^2 - coefficient of correlation represents the probability of the accuracy of the constructed curve.

A Shimadzu UV-Vis 1650PC spectrophotometer was used for the determination of antioxidant activity (DPPH), as well as for the determination of total phenols and flavonoids.

All measurements were repeated 3 times and presented as the mean value obtained from 3 repetitions ± standard deviation. Calculations and construction of curves were performed using the MS Office Excel 2007 software package.

Results and discussion

Total phenolic and flavonoid content of different extracts of Bolboschoenus laticarpus rhizome

The ethanolic extract had the highest yield, while the lowest yield was noticed in chloroform extract (**Tab. 1**). The total phenolic content determined within the investigated extracts ranges from 7 to 80 mg GAE/g of extracts (**Tab. 1**). These values are higher than value 4.28 mg GAE/g obtained for species *B. maritimus* (Grigore & Oprica, 2015). Although the ethanolic extracts had the highest yield, the highest phenolic content was present in the ethyl acetate extract. The ethanolic extract had a higher amount of phenolic content compared to the methanolic and chloroform extract, and a lower amount compared to the ethyl acetate extract. The advantage of the ethanolic extracts compared to the methanolic extracts is also confirmed by Yu et al. (2005) who investigated the effects of extraction solvents on concentration and antioxidant activity of peanut skin. The methanolic extract shows a lower phenolic content compared to the ethanolic and ethyl acetate extracts, and a higher content compared to the chloroform extract. The smallest amount of phenolic content is present in the chloroform extract (**Tab. 1**).

Antioxidant activity of different extracts of Bolboschoenus laticarpus rhizome

Analysis of the antioxidant activity showed that the ethanolic extract showed the highest antioxidant activity, while the lowest activity was recorded in the chloroform extract (**Tab. 2**). Similar results were reported by Popescu et al. (2016) for *Scirpus holoschoenus* L. rhizome extracts, namely between different solvents such as water, acetone, ethyl acetate and ethanol, where the highest antioxidant activity was noticed with ethanolic extract. The positive control had a low IC_{50} value, which is explained by its high antioxidant activity. Ethyl-acetate extract showed higher antioxidant activity compared to methanol extract (**Tab. 2, Fig. 1**). Depending on the different concentrations, the antioxidant activity of the examined extracts was calculated. The highest value of antioxidant potential was noticed in the ethanolic extract (50.13±0.78) at a concentration of 1,000 µg/ml, while the chloroform extract had the lowest value (5.30±2.33) at the same concentrations. It was observed that the ethyl acetate extract at lower concentrations (750, 625 and 500 µg/ml) had a higher antioxidant activity compared to the ethanol extract (**Tab. 2**, **Fig. 1**).

Table 1. Yield, total phenolic content (TPC) and total flavonoid content (TFC) of tested extracts

Extract	Extraction yield (g)	TPC (mg of GA/g of extracts)	TFC (mg of QuE/g of extracts)
Methanolic	1.47	29	103
Ethanolic	2.87	69	209
Ethyl acetate	0.24	80	539
Chloroform	0.22	7	417

Flavonoids have the ability to scavenge reactive oxygen species due to their phenolic hydroxyl groups, which gives them good antioxidant potential (Cao et al., 1997). The presence of a high content of flavonoids (yellow complex) in the extracts directly contributes to a strong antioxidant activity whose role is to remove free radicals.

The amount of total flavonoids in different extracts of the analyzed plant species ranges from 103 to 539 mg QuE/g extract (**Tab. 1**). The highest concentration of flavonoids was found in the ethyl acetate extract. The chloroform extract showed a lower concentration of flavonoids compared to the ethyl acetate extract, and a higher concentration compared to the methanolic and ethanolic extracts. In the case of the ethanolic extract, a lower content of flavonoids was determined compared to the ethyl acetate and chloroform extracts. The lowest concentration was found in the methanolic extract (**Tab. 1**).

It is considered that the antioxidant capacity of the analysed plant species depends on the type of solvent and concentration which is also confirmed in many studies (Zhou & Yu, 2004; Yu et al., 2005; Turkmen et al., 2006).

The positive correlation between the concentration of phenolic content and antioxidant activity is already reported in many studies (Yu et al., 2005; Ghasemzadeh et al., 2010; Anokwuru et al., 2011) which is partially in agreement with our results. In our study ethyl acetate extract which had the highest phenolic and flavonoid content showed the highest antioxidant activities at concentrations of 750, 625, and 500 µg/ml, while ethanol extracts at higher concentrations (1,000 and 825 µg/ml) showed the highest antioxidant activities. It was expected that ethyl acetate extract would have higher antioxidant activity at all concentrations but surprisingly the ethanol extract has the higher antioxidant activity which is similar to study by Mahboubi et al. (2013) where the aqueous extract of *Scrophularia striata*

Table 2. DPPH radical scavenging activity and IC_{50} values by different extracts of *B. laticarpus* rhizomes

DPPH % scavenging activity					
Concentration (µg/ml)	**Methanolic extract**	**Ethanolic extract**	**Ethyl acetate extract**	**Chloroform extract**	**BHT**
1,000	19.91±0.93*	50.13±0.78	49.53±4.98	5.30±2.33	68.39±1.30
825	16.20±0.41	44.35±1.45	42.16±3.71	5.72±0.50	58.62±1.37
750	14.98±0.63	39.65±0.58	40.96±0.10	5.05±0.59	55.41±1.96
625	12.28±0.63	32.99±0.36	35.28±0.82	4.18±0.47	48.84±0.77
500	13.47±1.43	26.47±4.58	28.72±0.61	3.66±0.82	33.01±4.35
250	6.78±0.40	16.91±2.11	16.62±0.29	2.63±0.67	21.25±1.11
IC_{50}	2,897.97	981.02	988.13	11,782.22	695.05

* values are expressed in mean ± SD of triplicate measurement

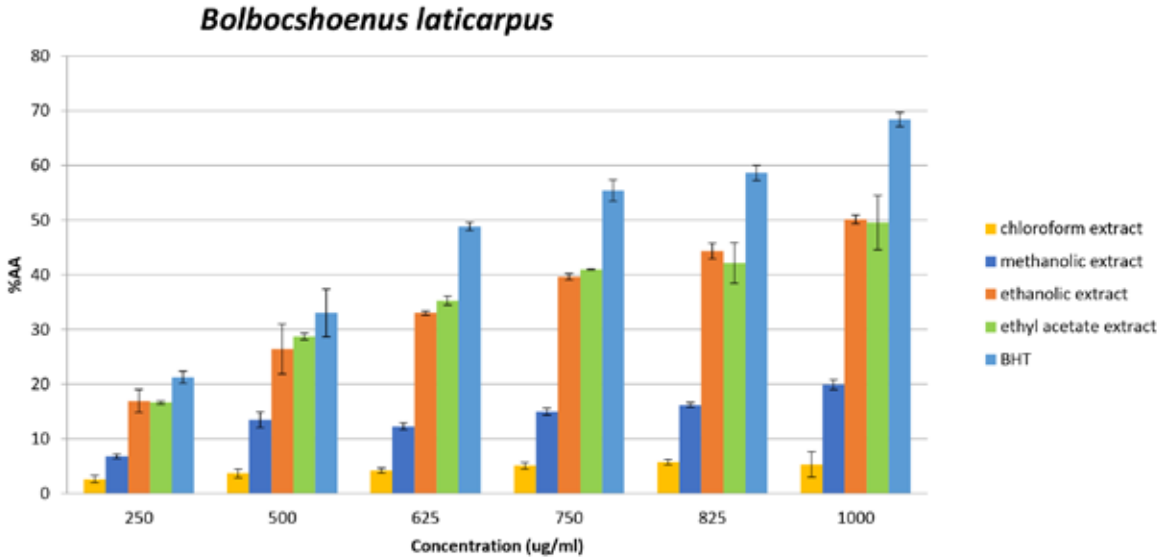

Fig. 1. Comparative overview of DPPH radical inhibition percentage (%AA) in different *B. laticarpus* rhizome extracts and BHT

Boiss. had higher antioxidant activity than ethyl acetate extracts. The observed IC_{50} values indicated that the ethanol extract (IC_{50}=0.981 mg/ml) exhibited the highest antioxidant activity, followed by the ethyl acetate extract (IC_{50}=0.988 mg/ml) and the methanolic extract (IC_{50}=2.897 mg/ml). The chloroform extract (IC_{50}=11.78 mg/ml) showed the lowest antioxidant activity (**Tab. 2**).

Our study confirmed that extract yields, total phenolic and flavonoid contents, and resulting antioxidant activities of the plant materials are strongly dependent on the nature of the extraction solvent, due to the presence of different antioxidant compounds with various chemical characteristics and polarities that may or may not be soluble in a particular solvent which is also confirmed by Jakopic et al. (2009) and Sultana et al. (2009).

Conclusion

Bolboschoenus laticarpus rhizome represents a good source of phenolics and flavonoids and showed significant antioxidant activity. Ethyl acetate and ethanol were found to be the most effective solvents for extracting these compounds from *B. laticarpus*, yielding the highest antioxidant activity and the greatest amounts of total phenolics and flavonoids. Further research is necessary to identify its chemical composition and bioactive compounds responsible for the antioxidant properties.

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References

Anokwuru, C.P., Anyasor, G.N., Ajibaye, O., Fakoya, O., & Okebugwu, P. (2011). Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three nigerian medicinal plants. *Nature and Science*, *9*(7), 53-61.

Blois, M. (1958). Antioxidant Determinations by the Use of a Stable Free Radical. *Nature 181*, 1199–1200. https://doi.org/10.1038/1811199a0

Browning, J., Gordon-Gray, K.D., Smith, S.G., & Van Staden, J. (1996). *Bolboschoenus yagara (Cyperaceae)* newly reported for Europe. *Annales Botanici Fennici*, *33*, 129-136.

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free radical biology and medicine*, *22*(5), 749-760.

Chang, C.C., Yang, M.H., Wen, H.M., & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3), 178-182.

Dai, J. & Mumper, R.J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.

Dapkevicius, A., Venskutonis, R., van Beek, T.A., & Linssen, J.P. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Journal of the Science of Food and Agriculture*, *77*(1), 140-146.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., & Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, *22*(3), 296-302.

Ewing, K. (1983). Environmental controls in Pacific Northwest intertidal marsh plant communities. *Canadian Journal of Botany*, *61*, 1105–1116.

Ghasemzadeh, A., Jaafar, H.Z., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, *15*(6), 4324–33.

Grigore, M.N. & Oprica, L. (2015). Halophytes as possible source of antioxidant compounds, in a scenario based on threatened agriculture and food crisis. *Iranian Journal of Public Health, 44*(8), 1153-5.

Hroudová, Z., Zákravský, P., Duchaček, M., & Marhold, K. (2007). Taxonomy distribution and ecology of *Bolboschoenus* in Europe. *Annales Botanici Fennici, 44*, 81-102.

Hroudová, Z., Zákravský, P., & Frantik, T. (1999). Ecological differentiation of Central European *Bolboschoenus* taxa and their relationship to plant communities. *Folia Geobotanica 34*, 77–96.

Jakopic, J., Veberic, R., & Stampar, F. (2009). Extraction of phenolic compounds from green walnut fruits in different solvents. *Acta Agriculturae Slovenica*, *93*(1), 11-15.

Jarolimova, V. & Hroudová, Z. (1998). Chromosome numbers within the genus *Bolboschoenus* in Central Europe. *Folia Geobotanica*, *33*, 415-428.

Mahboubi, M., Kazempour, N., & Boland Nazar, A.R. (2013). Total phenolic, total flavonoids, antioxidant and antimicrobial activities of *Scrophularia striata* Boiss. extracts. *Jundishapur Journal of Natural Pharmaceutical Products 8*(1), 15-9.

Marhold, K., Hroudová, Z., Duchaček, M., & Zákravský P. (2004). The *Bolboschoenus maritimus* group (Cyperaceae) in central Europe, including *B. laticarpus*, spec. nova. *Phyton*, *44*, 1-21.

Mikulka, J., Chodova, D., & Abrahamova, I. (1999). Expandujici Kamyšnik primorsky (*Bolboschoenus maritimus* (L.) Palla) na orne pude. *Farmar*, *11*, 27-28.

Nikolić, D., Gocić, D. J., Jušković, M., & Ranđelović, V. (2019). Morphological differentiation of populations of *Bolboschoenus* taxa in Serbia. *Plant Biosystems - An International Journal Dealing with All Aspects of Plant Biology*, *154*(4), 488–502. https://doi.org/10.1080/11263504.2019.1651772

Popescu, A., Pavalache, G., Pirjol, T. N., & Istudor, V. (2016). Antioxidant comparative activity and total phenolic content of *Scirpus holoschoenus* L. (*Holoschoenus vulgaris* Link) depending on extraction condition and the solvent used. *Revista de Chimie*, *67*(2), 255-259.

Powell, R.G., Bajaj, R., & McLaughlin, J.L. (1987). Bioactive stilbenes of *Scirpus maritimus*. *Journal of Natural Products*, *50*, 293.

Simpson, D.A. & Inglis, C.A. (2001). Cyperaceae of economic, ethnobotanical and horticultural importance: a checklist. *Kew Bulletin*, 257-360.

Singleton, V.L., Orthofer, R., & Lamuela-Raventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants

by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, *299*, 152-178.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*, *14*(6), 2167-2180.

Turkmen, N., Sari, F., & Velioglu, Y.S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. *Food chemistry*, *99*(4), 835-841.

Yu, J., Ahmedna, M., & Goktepe, I. (2005). Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. *Food Chemistry*, *90*, 199–206.

Zhou, K. & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. *Lebennsmittel Wissenschaft und Technologie*, *37*, 717–721.