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Influence of Solvents and Solvent Mixtures on the Content of Polyphenols and Antioxidant Activity of *Capsella bursa-pastoris* (L.) Medik. Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Capsella bursa-pastoris (L.) Medik. (known as shepherd's purse) is a plant whose parts are used as medicine in herbal medicine. It is applicable as a medicine in the treatment of all forms of internal bleeding, for the treatment of hemorrhoids, excessive menstruation, but also for the usual stopping of nosebleeds. Through this research, the influence of organic solvents and their aqueous mixtures on the efficiency of polyphenol extraction and antioxidant activity was compared. The inhibition of free radicals was tested by the DPPH method, while the FRAP method was used to test the reduction potential. Analyzes have shown that water is the most effective solvent in the isolation of polyphenols from the aerial parts of shepherd's purse. Mixtures of organic solvents with water also showed high efficiency in the extraction of bioactive components, while the weakest results were obtained for extracts prepared in pure organic solvents.

Keywords: Shepherd's purse; TPC; FRAP; DPPH inhibition.

1. INTRODUCTION

Capsella bursa-pastoris (L.) Medikus, commonly known as 'shepherd's purse', belongs to the family Brassicaceae. It is an annual plant, widely distributed throughout the world. This plant is commonly used in traditional medicine because it exhibits antimicrobial, anti-inflammatory, antioxidant, cardioprotective, antitumour, hepatoprotective, sedative, hemostatic, wound healing and other pharmacological effects [1,2]. In some countries, infusion and decoction of *C. bursa-pastoris* aerial parts are also used in a traditional medicine, considering diuretic, astringent, emmenagogue, and antidiabetic effects of this plant [3]. Traditionally, this plant was also used to treat chronic heavy menstrual bleeding [4]. Previous phytochemical studies of this plant have confirmed the pressence of several flavonoids, terpenoids, phenolic compounds, tannins, choline, acetylcholine, etc. Some of the confirmed phytoconstituents are quercetin-6-C-glucoside, kaempferol-3-O-glucoside, fatty acids, organic acids, aminoacids, and sterols [4,5]. It is suggested that the presence of citric, malic and quinic acids in *C. bursa-pastoris*, is attributed to antihemorrhoidal effects, due to anti-inflammatory effects of these organic acids and ameliorative effects on the antioxidant-oxidant balance [3]. Flavonoids and organosulfur compounds, such as sulforaphane, reportedly exhibit antibacterial and anti-inflammatory activity [6,7].

The aim of this research is to determine the effectiveness of organic solvents and their aqueous mixtures in the isolation of bioactive components responsible for the antioxidative activity of extracts of *Capsella bursa-pastoris* (L.) Medik. For this purpose, the least invasive extraction technique, maceration, was chosen.

2. MATERIAL AND METHODS

Dried aerial parts of *Capsella bursa-pastoris* (L.) Medik. were purchased in a local market in Tuzla. The sample was determined in the pharmacognosy laboratory of the Faculty of Pharmacy, University of Tuzla. The plant was ground into powder using an electric mill. Aqueous solutions needed for the analyzes were prepared using demineralized water. Folin-Ciocalteu reagent for testing polyphenol content, sodium carbonate and anhydrous sodium sulfate were purchased from Semikem, Bosnia and Herzegovina. 2,4,6-Tripyndyl-s-triazine, iron(III) chloride, hydrochloric acid, sodium acetate (for preparation of FRAP reagent) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). All reagents were p.a. purity and were used without further purification. Spectrophotometric measurements were performed on a Perkin Elmer Lambda 25 spectrophotometer, in the wavelength range of 510-765 nm.

2.1 Preparation of Extracts

The extract was prepared by mixing 0.5 grams of chopped plant material with 25 mL of solvent, or solvent mixture. Mixing was carried out at 200 rpm for 20 hours, after which the mixture was filtered and immediately subjected to analyses. All extracts were clear after filtration. For easier discussion of the obtained results, the extracts are labeled as indicated in Table 1.

Table 1. Labels of the samples and color of the extracts after the extraction

Sample	Labels	Color
Methanolic extract	MeOH	dark green
Ethanol extract	EtOH	dark green
Acetone extract	Ace	light green
Aqueous extract	Water	orange
Water-methanolic extract	Water:MeOH	yellow
Water-ethanol extract	Water:EtOH	olive green
Water-acetone extract	Water:Ace	olive green

2.2 Determination of Total Phenolic Content (TPC)

Total phenolic compounds present in the shepherd's purse extracts were quantified spectrophotometrically through the Folin-Ciocalteu test following the protocol [8]. 200 µL of extract was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420 µL of 10% sodium carbonate was added. 910 µL distilled water was added to each sample prior to measuring. The absorbance of the resulting blue-coloured solution was measured at 765 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrammes per gram of shepherd's purse.

2.3 Determination of Antioxidant Capacity

The antioxidant capacity was tested *in vitro* using the DPPH and FRAP methods. The test of the reducing ability of the extracts was tested using the FRAP (ferric reducing antioxidant power) method, according to the published procedure [9]. 3 mL of prepared FRAP reagent (mixture of acetate buffer, iron(III) chloride hexahydrate and TPTZ reagent in a volume ratio of 10:1:1) was mixed with 100 µL of extracts. Absorbance at 593 nm was recorded after 30 min incubation at 37°C.

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method [10]. Two concentrations of the extract, 0.5 and 1 mg/mL, were used to test the inhibition of the extract. For the volume of the mother extract of 50 and 100 µL, 1950 and 1900 µL of methanol were added. 0.5 mL of 0.5 mM DPPH solution were added and the samples were left to incubate for 30 minutes in a darkened room at a room temperature. The absorbance was measured at 517 nm with methanol as a blank sample. 0.5 mL of 0.5 mM DPPH dilution, diluted with 4 mL of methanol, was used as a control sample. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$[(Ac - As) / Ac] \times 100$$

Where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the DPPH solution.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content

Folin-Ciocalteu reaction is widely used spectrophotometric method to determine total phenolic content. [11]. High concentrations of phenolic contents indicate the medicinal importance of plants as they reduce the chances of cancer obesity, urinary infections, cardiovascular and periodontal diseases [12]. total phenolic content of *Capsella bursa-pastoris (L.)* Medik. is presentend in Fig. 1.

As can be observed from the Fig. 1, the amount of total phenols varied between different extracts with respect of solvents. Organic solvents showed lower extraction yields in comparison to their mixtures with water. Total phenolic content measure decreased in following order: water extract>water:acetone extract>water:ethanol extract>water:methanol extrac>methanol extract>ethanol extract> acetone extract. It is evident that water extract contains the highest amount of phenols compared to the other extracts, with concentration of total phenols being 5.55 mgGAE/g. The lowest extraction yield is observed for acetone extracts, where the concentration of total phenols is 0.97 mgGAE/g, which is more than 5 times lower concentration in comparison to water extracts. This forms the conclusion that water is the best solvent for polyphenol extraction from *Capsella bursa-pastoris (L.)* Medik., followed by water: organic solvent mixtures. In the previous study, it was mentioned that

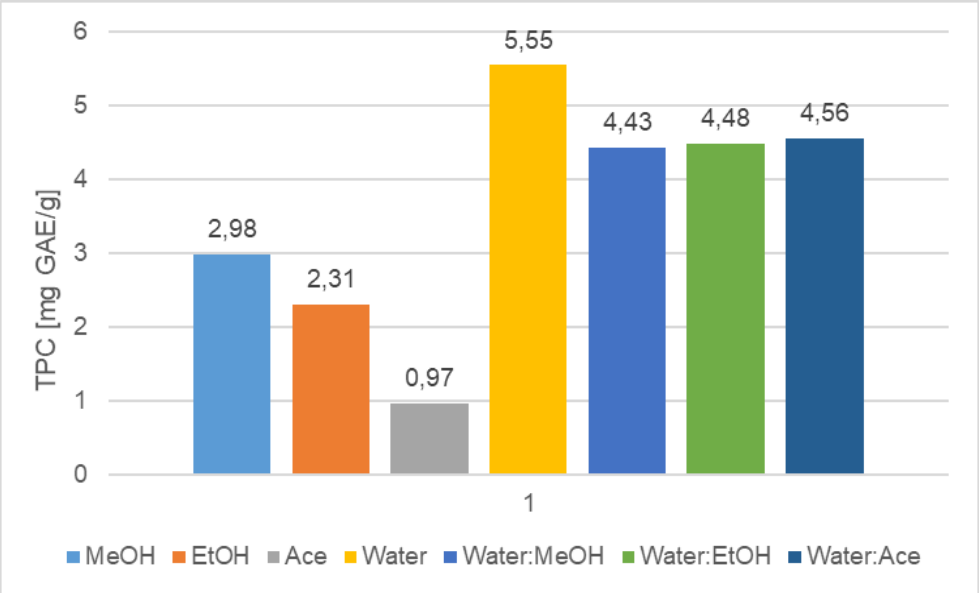

Fig. 1. Content of total polyphenols in extracts of shepherd's purse

phenolic substances are extracted in greater quantities with highly polar solvents [13]. This thesis is confirmed in also our study. The observed differences among the solvents are due to the polarity of solvent, where acetone is the least polar solvent, hence performing lowest extraction efficiency. Mixing organic solvents with water decreases dielectric constant of solvent, resulting with increased extraction efficiency. In adition, water causes the plant material to swell and facilitates the penetration of organic solvent into the plant material. In this study the effect of decreasing dielectric constant is highly evident when comparing acetone extract (0.97 mgGAE/g) and water:acetone extract (4.56 mgGAE/g). Mixing acetone with water results with better extraction yield, obtaining almost 5 times higher total phenolic content in comparison to acetone extract. In the study of Mărghitaş et al., methanolic extract of honey bee-collected pollen of *Capsella bursa pastoris* L. (Medik.) showed concentration of total polyphenols 15.2 mgGAE/g [14]. This higher content of polyphenols can be due to different plant parts, and different extraction time used in mentioned study. A great influence on total phenol content in *Capsella bursa pastoris* L. (Medik.) has the method of extract preparation. Much higher concetration of total polyphenols was reported in study of Yousuf S. et al.It was reported that water extract contained 24.25 mgGAE/g and ethanolic extracts 35.52 mgGAE/g [15], possibly due to use of Soxhlet extraction method. Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent [13]. Therefore, this comparison indicates that solubility of phenolic components of *Capsella bursa pastoris L*. (Medik.) is low and requires adequate extraction conditions for appropriate extraction yield.

3.2 Antioxidant Capacity

It was reported that the antioxidant activity of a plant sample is associated with the amount of phenolic compounds in the plant, due to the phenolic compounds have hydroxyl groups that will act as hydrogen atoms donors [16]. DPPH radical scavenging activity is greatly associated with total phenolic content. Higher phenolic content gives higher DPPH scavenging activity [17]. According to the studies, polyphenols present in plant extracts are mainly responsible for their antioxidant properties [18]. Results of DPPH and FRAP assay in this study are in agreement with the previous stations. The differences and changes in antioxidant capacity for *Capsela bursa pastoris* L. (Medik.) extracts are presented in Fig. 2 and Fig. 3.

The results of DPPH and FRAP assay among the *Capsella bursa pastoris* L. (Medik.) extracts are showing the similar trend demonstrated in total phenolic content assay. The antioxidant capacity of tested extracts is in following order: Water extract>water:acetone extract>water:ethanol extract>water:methanol extract>methanol extract>ethanol extract> acetone extract. Water extracts of *Capsella*

bursa pastoris L. (Medik.) showed the highest reducing properties and the highest % of DPPH radical inhbitibon, whereas the lowest results are obtained for acetone extracts. In respect to different concentrations of extract samples, 1 mg/ml samples presented almost 3 times higher DPPH inhibition % in comparison to 0.1 mg/ml samples. The conducted studies on extracts of *Capsella bursa pastoris* L. (Medik) presented high antioxidant capacity, whereas for the study of Salayová A. et al. DPPH inhibition % was 89.68 [1]. Statistically significant difference in ferric ions reducing capacity of Shepherd's purse extract was found in study of its honey bee collected pollen [14]. FRAP assay for methanol extract of honey bee collected pollen demonstrated about 8 times lower values (2.412 µmol/g) in comparison to our study result (16.01 µmol/g). In the study of Neagu E. et al., it is confirmed that sonication method of extraction results in much higher antioxidant values for this plant [19]. The DPPH inhibition % for ethanol extracts collected in autumn are approximately 3 times higher comparing to our study results. This significant difference can be attributed to the influence of demographic factors and environmental factors as well.

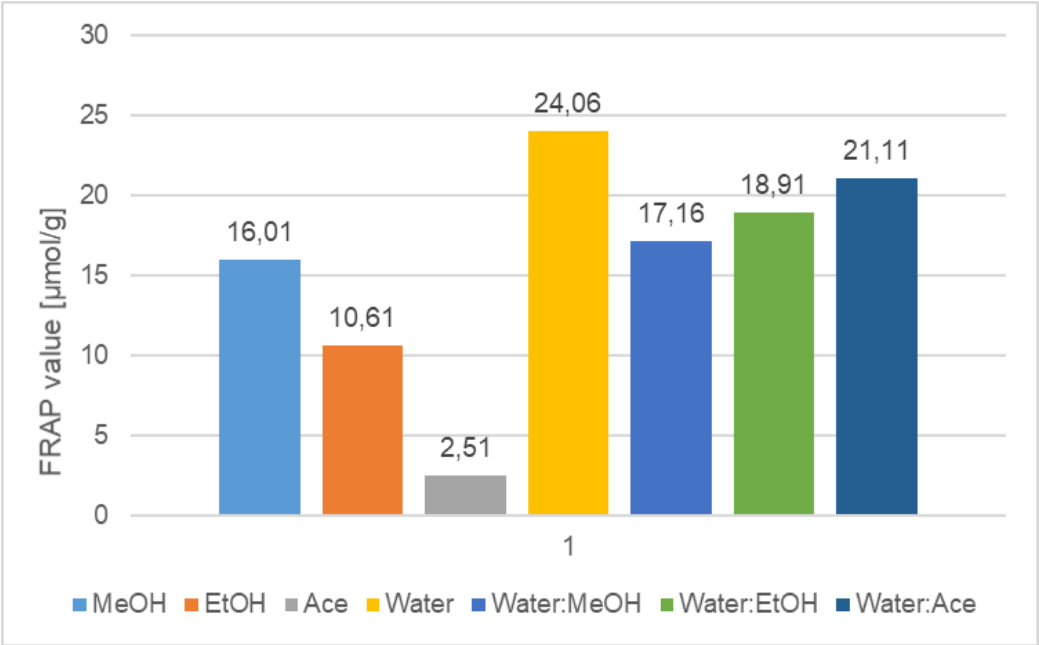

Fig. 2. Reducing ability of the extract

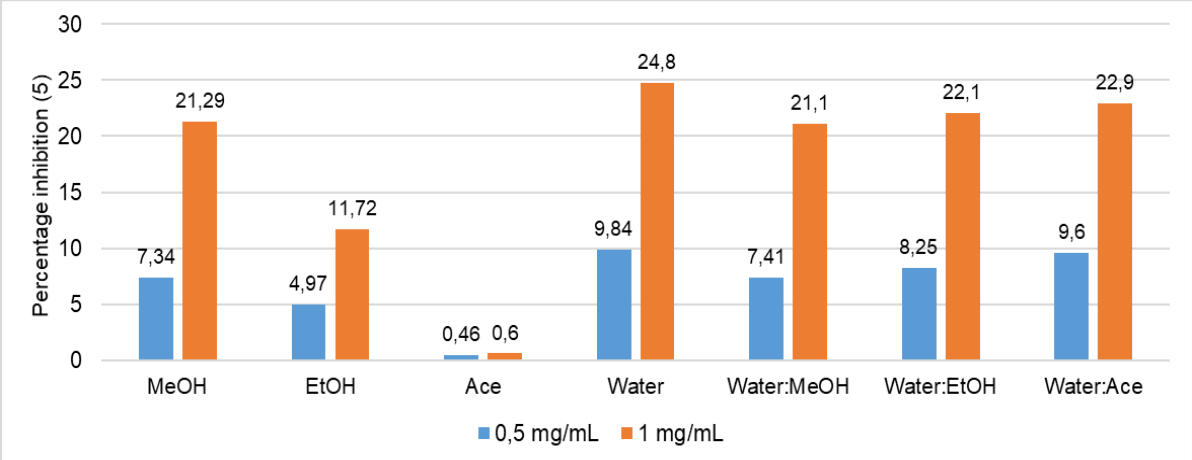

Fig. 3. DPPH radical inhibition percentage for shepherd's purse extract

4. CONCLUSION

In this research, different solvents and their aqueous mixtures were used to test the efficiency of extraction of bioactive components with antioxidant activity. Water proved to be the most efficient solvent for the extraction of polyphenolic compounds. Mixing water with organic solvents also improved the extraction efficiency of bioactive components compared to pure organic solvents, which in the case of this plant proved to be poorly efficient in the extraction of polyphenolic compounds. This research is limited only to maceration, but other extraction techniques should certainly be examined with the aim of better insight into the effectiveness of extraction techniques on the antioxidant activity of *Capsella bursa-pastoris* (L.) Medik.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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