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Original Article

Assessment of Total Phenolic and Flavonoid Content of Medicinal Plant Extracts from Kosovo

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ABSTRACT

Background and objectives: Phenols and flavonoids are the most common phytoconstituents of medicinal and aromatic plants, and responsible for antioxidant activities. This study aimed to determine the content of phenolics and flavonoids in water and methanolic extracts of six selected medicinal plants (*Betula pendula* Roth, *Betula pubescens* Ehrh., *Trifolium pratense* L., *Verbascum thapsus* L., *Equisetum arvense* L., and *Sambucus nigra* L.) collected from various locations in Kosovo. Methods: The total phenolic content (TPC) was estimated spectrophotometrically using the Folin-Ciocalteu method and expressed as mg of gallic acid equivalents per gram of dry weight sample (mgGAE/gDW). The total flavonoid content (TFC) was measured by the aluminum chloride colorimetric assay and expressed as mg of catechin equivalents per gram of dry weight sample (mgCE/gDW). Results: The TPC of the water extracts ranged from 5.26±0.05 mgGAE/gDW (*Equisetum arvense* L.) to 33.14 ± 0.13 mgGAE/gDW (*Sambucus nigra* L.), while that of the methanolic extracts ranged from 16.85 ± 0.27 mgGAE/gDW (*Equisetum arvense*) to 38.95 ± 0.15 mgGAE/gDW (*Betula pendula*). The TFC of the water extracts ranged from 0.09 ± 0.01 mgCE/gDW (*Verbascum thapsus*) to 1.57 ± 0.01 mg CE/gDW (*Trifolium pratense*), while that of the methanolic extracts ranged from 0.25 ± 0.02 mgCE/gDW (*Verbascum thapsus*) to 1.36±0.02 mgCE/gDW (*Trifolium pratense*). *Equisetum arvense herba* showed the largest difference in TPC and TFC between the water and methanolic extracts, with 8.26 ± 0.05 mgGAE/gDW and 0.25 ± 0.01 mgCE/gDW in water extract and 16.85 ± 0.27 mgGAE/gDW and 1.15 ± 0.08 mgCE/gDW in methanolic extract, respectively. Conclusions: Results shows varying levels of phenolics and flavonoids, with some plants exhibiting higher levels in methanolic extracts than in water extracts. These findings may have important implications for the potential use of these plants in traditional medicine and as sources of natural antioxidants.

INTRODUCTION

Plant and plant-based products are the natural sources of different phytochemicals such as phenols, flavonoids, alkaloids, glycosides, lignins and tannins. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables and medicinal and aromatic plants, which are responsible for antioxidant activities. Due to the potential toxicological effects of synthetic antioxidants, natural antioxidants such as phenols and flavonoid compounds from plant origin are gaining popularity these days [1]. Flavonoids and the other phenolic compounds are commonly known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. More than 8000 phenolic compounds as naturally occurring substances from plants have been reported [2]. Phenolic compounds, including flavonoids, have often been used as chemotaxonomic markers in plants. Phenolics play a fundamental role in the interaction of plants with the environment, including their defense mechanisms against biotic and abiotic stresses and other adaptation processes. It is well known that the presence of phenolics in plants and the subsequent

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antioxidant capacity can also be subjected to significant variation due to environmental conditions and to biotic and abiotic stresses [3]. Phenolic compounds from medicinal plants have several biological effects and can play an important role in the prevention of many diseases. They also play an important role in healthcare, which are well-known in phytotherapy and ethno-veterinary practices too [4].

It is very interesting to note that half of these phenolic compounds are flavonoids presenting as aglycone, glycosides and methylated derivatives. These phytochemical substances are presented in nutrients and herbal medicines, both flavonoids and many other phenolic components have been reported on their effective antioxidants, anti-inflammation [1,2, 4], anticancer, antibacterial, cardioprotective agents, immune system promoting, skin protection from UV radiation, and interesting candidate for pharmaceutical and medical application [2]. Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups [5]. Nowadays traditional medicine, a source of several bioactive molecules for therapeutic purposes, has become a cure for various diseases. In fact, the evaluation of plant exploitation has become progressively significant and the therapeutic effects of many traditional medicines may be due to the immense presence of natural antioxidants [6]. Traditional flavonoid extraction techniques are being replaced by advanced techniques to reduce energy and solvent consumption, increase efficiency and selectivity, to meet increased market demand and environmental regulations [7]. There are different types of extraction techniques for polyphenols such as water bath, microwave-assisted extraction (MAE), solvents, and ultrasound-assisted extraction (UAE) [8]. Ultrasonic-assisted extraction (UAE) is an effective alternative to the conventional extraction method that offers an inexpensive and environmentally friendly extraction process. UAE provides a high production yield, lower energy input, and temperature used, shorter extraction time, low solvent consumption, and improve extraction of thermal-labile compounds at low temperatures [9]. Solvents used during the extraction process are reported to have an influence on nature and the number of secondary metabolites extracted from medicinal plants. Thus, the choice of proper extraction solvent is necessary for the desired pharmacological activity of these extracts [10]. Kosovo has a very good geographical position. It is located almost in the center of the Balkans, having an aerial distance of 90 km from the Adriatic Sea and 220 km from the Aegean Sea. During the summer period, the climate of Kosovo is influenced by the movement of the high-pressure air from the sub-tropic area toward the north that is under influence of the cyclone activity from the Atlantic Ocean and the Mediterranean Sea, and during the winter under the influence of the Siberian anticyclone. In Kosovo, it is possible to differentiate into basic climate regions: mountains and lowlands. Kosovo is rich with medicinal aromatic plants, wild berries, and mushrooms [11].

Taking into account all the considerations mentioned above, the aim of this work was to determine the total phenolics and total flavonoid content in water and methanolic extracts of six medicinal plants from Kosovo, using an ultrasonic-assisted extraction technique.

MATERIALS AND METHODS

Plant Material

Plant material was collected from six different localities in Kosovo (Table 1) in the period May-June 2021. The plant material was air dried, packed in paper bags and kept in a dark and cool place until analysis. Plant identity was verified by professor Shkelzim Ukaj and voucher specimens were deposited at the Herbarium at the Department of Pharmacognosy, Faculty of Pharmacy, UBT, Pristina, Kosovo (PhGNEF/UBT-03).

Extract Preparation

Dried plant material from each sample was used for the preparation of extract. Plant extracts were obtained using ultrasonic-assisted extraction process at room temperature. 70% methanol (for methanolic extracts) and distillated water (for water extract) was used for the extraction in 2 portions in a ratio to plant material of 1:100 (0.5 g plant material was extracted twice with 25 ml solvent). The duration of the extraction was 2 x 5 min. After each phase, filtration was made into a 50 mL volumetric flask; the volume was made up with 70 % methanol or water. The

obtained extract was used for determination of total phenols and total flavonoids content.

Determination of Total Phenolic Content (TPC)

TPC was determinated with the Folin-Ciocalteu reagent [12], with some modifications. To 1 mL of methanol extract or water extract, 0.5 mL Folin-Ciocalteau 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 2h. After incubation, absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Genesys 10S UV-Vis spectrophotometer).

Determination of Total Flavonoid Content (TFC)

TFC was determinated using the aluminum chloride assay [13] with some modifications.

1 mL methanol extract or water extract, 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 for 15 min, the absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Genesys 10S UV-Vis spectrophotometer).

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

This study focuses on six different plants that are of potential interest to consumers. They were characterized in terms of total phenolic content and total flavonoid content potential. The TPC was determinated as mg of gallic acid equivalents per g of dried plant material (mg GAE/g DW) using an equation obtained from a standard gallic acid calibration graph (R2 = 0.996). Data obtained from determination of TPC are expressed as mean values ±SD (n = 3).

The highest content of phenolics in water extract was found in Sambucus nigra flower (33.14±0.13 mg GAE/g DW) and Betula pendula leaf (32.16±0.10 mg GAE/g DW), followed by Betula pubescens leaf and Trifolium pretense flower.

Table 1 Plant material

No	Plant	Family	Plant organ	Localities	Latitude (La) Longitude (Lo) Altitude (Al)
1.	*Betula pendula*	Betulaceae	leaf	Suharekë	La: 42˚20′09″ N Lo: 20˚50′50″ E Al: 443 m
2.	*Betula pubescens*	Betulaceae	leaf	Krushë e madhe (Gjakovë)	La: 42˚19′13″ N Lo: 20˚37′58″ E Al: 314 m
3.	*Trifolium pratense*	Fabaceae	flower	Klinë	La: 42˚37′01″ N Lo: 20˚34′01″ E Al: 383 m
4.	*Verbascum thapsus*	Scrophulariaceae	flower	Nerodime e epërme (Ferizaj)	La: 42˚22′13″ N Lo: 21˚04′35″ E Al: 638 m
5.	*Equisetum arvense*	Equisetaceae	aerial part	Suharekë	La: 42˚20′09″ N Lo: 20˚50′50″ E Al: 443 m
6.	*Sambucus nigra*	Caprifoliaceae	flower	Klinë	La: 42˚37′01″ N Lo: 20˚34′01″ E Al: 383 m

The lower content of TPC (< 20 mg GAE/g) was found in Verbascum thapsus flower and Equisetum arvense aerial part.

The highest TPC (> 30 mg GAE/g DW) of methanolic extract was found in Betula pendula, Sambucus nigra and Trifolium pretense (38.95 ± 0.15; 32.55 ± 0.89 and 30.68 ± 0.42 mg GAE/g DW), respectively. In samples of Betula pendula, Trifolium pratense and Equisetum arvense, the content of TPC in methanolic extracts is higher than in aqueous extracts, while in sample Betula pubescens, the highest content of TPC in aqueous extract is observed.

Generally, in the Equisetum arvense sample a very large difference of the TPC content is observed between the aqueous extract and the methanolic extract (5.26 ± 0.05 and 16.85 ± 0.27 mg GAE / g DW), respectively. In the Sambucus nigra sample a small difference in TPC content is observed between the aqueous extract and the methanolic extract (33.14 ± 0.13 and 32.55 ± 0.89 mg GAE / g DW), respectively.

Total Flavonoid Content (TFC)

The TFC was expressed in mg of catechin equivalents per g of dried plant material (mg CE/g DW) using the linear equation based on the standard catechin calibration curve (R2 = 0.9997). Data obtained from determination of TFC are expressed as mean values ±SD (n = 3).

On Table 2 were represented the total phenolic content in water extract and methanolic extract of these plants.

The TFC was measured in water extract and methanolic extract of six medicinal plants from Kosovo. The results are shown in Table 3.

The highest content of flavonoids in water extract was found in Trifolium pratense (1.57 ± 0.01 mg CE/g), followed by Betula pubescens and Sambucus nigra. In the methanolic extract the highest content of TFC was found in Trifolium pratense and Equisetum arvense, followed by Sambucus nigra. In samples Equisetum arvense and Verbascum Thapsus, the content of TFC in methanolic extracts is higher than in aqueous extracts, while in sample Betula pubescens, the highest content of TPC in aqueous extract was observed.

Generally, in the Equisetum arvense sample a very large difference of the TFC content is observed between the aqueous extract and the methanolic extract (0.25 ± 0.01 and 1.15± 0.08 mg CE /g DW), respectively. In the Betula pendula and Sambucus nigra samples a small difference in TFC content is observed between the aqueous extract and the methanolic extract.

According to literature data TPC in ethanolic-aqueos extract of leaves of Betula pendula with different ratio of ethanol: water, were in the ranges from 10.8 ± 0.05 mg GAE/g (etOH: H2O, 90:10 v/v), 9.11 ± 0.03 mg GAE/g (etOH: H2O, 75:25 v/v) and 11.23 ± 0.02 mg GAE/g (etOH:H2O, 50:50 v/v) [14]. Compared with these data, the TPC values of our tested sample of water extract and methanolic extract from leaves of Betula pendula were much higher (32.63 ± 0.10 and 38.95 ± 0.15 mg GAE/g DW), respectively. Bljajic et al reported total flavonoid content in ethanolic and water extract of Betula pendula leaf expressed in quercetin (45.0 ± 2.8 and 20.1 ± 1.6 mg QE/g), respectively [15]. The various polyphenol constituents present in the Betula pendula extract contribute to the antioxidant activity and total polyphenol content [14].

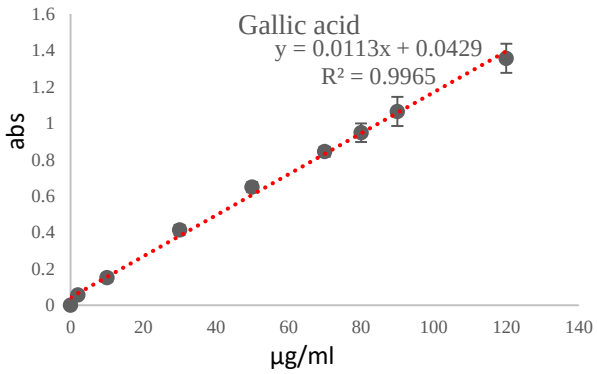

Fig. 1 Calibration curve of gallic acid

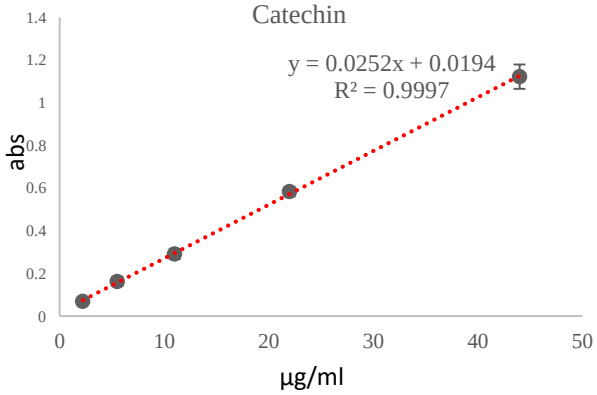

Fig. 2 Calibration curve of catechin

Akbaribazm *et al*. determinated phenolics and flavonoids (expressed in rutin) content in ethanolic extract of Trifolium pretense leaf (58.12 ± 6.21 mgGAE/g and 39.21 ± 4.26 mg RUE/g) [16]. Horvat

et al. reported TPC in methanolic extract of lyophilized plant material of Trifolium pretense flower (38.67-59.96 mg GAE/g DW) [17]. We analyzed flower of Trifolium pretense (water and methanolic extract) and found lower content of phenolics and flavonoids. Besides being rich in proteins, Trifolium species have been reported to contain a wealth of biologically active secondary metabolites of which phenolic compounds are one of the main classes [3].

Abidet et al revealed that for Verbascum thapsus, the n-butanol extract and chloroform extract show the highest total phenolic content (126.35 mg GAE/g) and flavonoids content (83.88 mg QE/g) respectively [18]. Compared with these data, the TPC and TFC values of our tested sample of water and methanolic extract from Verbascum Thapsus flower were much lower. A literature searches also revealed that the efficiency of phenolic extraction depends on the solvent type used for the plant material [19]. We used water and 70% methanol as solvent. Approximate values with our results, Petkova et al determined in microwave-assisted extracts of Sambucus nigra flower (30.28 + 0.35 mg GAE/g) [20].

The values of phenolic content in this current study varied slightly compared to those in the literature. This may be due to the presence of different amounts of sugars, carotenoids or ascorbic acid, or the duration, geographical variation or methods of extraction, which may alter the amount of phenolics [5]. The total phenolic content varied in the different varieties of plants and each plant extract contained a lower total flavonoid content than the total phenolic content, due to the presence of non-flavonoid phenolic substances in plants [20].

CONCLUSION

In the current study, extracts of water and 70% methanol were used to evaluate and validate their impact on the extraction of phenolic compounds and flavonoids from the leaves, flowers, and aerial parts of some selected medicinal plants from Kosovo. The nature of the solvent and its polarity significantly impacted the phenolic extraction. The content of phenolics and flavonoids in these extracts varied among the different studied plants. The content of phenolics and flavonoids in these extracts varied among the different studies plants. Phenolic compounds contribute to quality and nutritional value and also providing health beneficial effects. This study revealed the phenolic and flavonoid spectrum of some medicinal plants from Kosovo.

Table 2 The content of total phenols in water and methanolic extract (mg GAE/g DW) of six medicinal plants from Kosovo

No	Plant	Plant organ	C mgGAE/g DW	
			Water extract	Methanolic extract
1.	*B. pendula*	folium	32.63 ± 0.10	38.95 ± 0.15
2.	*B. pubescens*	folium	28.01 ± 0.08	20.60 ± 0.29
3.	*Tr. pratense*	flos	24.73 ± 0.27	30.69 ± 0.42
4.	*V. thapsus*	flos	19.71 ± 0.05	17.06 ± 0.21
5.	*E. arvense*	herba	5.26 ± 0.05	16.85 ± 0.27
6.	*S. nigra*	flos	33.14 ± 0.13	32.55 ± 0.89

n=3; GAE-gallic acid equivalents; DW- dry weight.

Table 3 The content of total flavonoids in water extract and methanolic extract (mg CE/g DW) of six medicinal plants from Kosovo

No	Plant	Plant organ	C mgCE/g DW	
			Water extract	Methanolic extract
1.	*B. pendula*	folium	0.41 ± 0.19	0.42 ± 0.01
2.	*B. pubescens*	folium	0.90 ± 0.23	0.47 ± 0.00
3.	*T. pratense*	flos	1.57 ± 0.01	1.36 ± 0.02
4.	*V. thapsus*	flos	0.09 ± 0.01	0.25 ± 0.02
5.	*E. arvense*	herba	0.25 ± 0.01	1.15 ± 0.08
6.	*S. nigra*	flos	0.82 ± 0.01	0.79 ± 0.02

n=3; CE- catechin equivalent; DW- dry weight.

Declaration of Interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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