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Research Article

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Antioxidant activity of water extracts of some medicinal plants from Herzegovina region

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ABSTRACT

Natural antioxidants are present in plants in various amounts. This study was undertaken to investigate antioxidant activities of some medicinal plants from Herzegovina region that are daily used as dietary supplement and functional food. The dry leaves and flowers from eleven medicinal plants (Lauris nobilis, Cichorium intybus, Chelidonium majus, Plantago major, Papaver rhoeas, Asplenium ceterach, Taraxacum officinale, Silene vulgaris, Cetraria islandica, Leucanthemum vulgare, and Helichrysum italicum) were extracted with water. Aqueous extracts of 11 plants were investigated for their antioxidant properties using DPPH and ABTS radical scavenging capacity assay and ferric reducing antioxidant potential (FRAP) assay. Totalphenolic content was also determined by the Folin-Ciocalteu method. Results indicated highest phenolic content in Papaver rhoeas aqueous extracts (7551.98±143.25 mg GA/100 g DW). Also Papaver rhoeas showed highest overall antioxidant activity in all of the methods used. Significant relationship between antioxidant capacity and total phenolic content indicates that the phenolic compounds are the major contributors to the antioxidant properties of these medicinal plants.

Key words: *Antioxidant Activities, Medicinal Plants, Total Phenolic Content, Reducing Power*

INTRODUCTION

Recently lots of attention has been devoted to natural oxidants and their health benefits. Plants produce various antioxidant compounds as protection to reactive oxygen species (ROS) and free radicals. ROS are various species of activated oxygen leading to oxidative damage to tissue. Free radicals in the cell may occur due to various external factors such as ultraviolet radiation, chemical reactions and some metabolic processes. Accumulations of them cause considerable diseases, such as cardiovascular diseases, aging, cancer, inflammatory diseases [1,2,3].

Different parts of plants (root, leaf, flower, fruit, stems, bark) have been used successfully to treat many diseases. Their antioxidant and antimicrobial activity affect many physiological processes in the body, thus protecting against free radicals and undesirable microorganisms. Phenols are commonly found in plants and have been reported to have antioxidant activity[4,5].

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Their antioxidant activity is mainly due to the fact that they can act as reducing agents and hydrogen donators. Several studies have been conducted in order to correlate amount of phenolic compounds in plants and antioxidant activity[6,7,8].

Bosnia and Herzegovina is located in the south-eastern part of Europe, more precisely in the central part of the Balkan peninsula. Because of the geographical position on the transition between the eastern Adriatic coast and the central Balkans, Herzegovina region is rich in biological wealth and diversity of habitats. 30% of the total endemic flora of the Balkans is in Bosnia and Herzegovina.

Aim of this study is to investigate antioxidant activity of several medicinal plants from Herzegovina region using different methods and to evaluate relationship between antioxidative activity and total phenolic contents of the plants.

MATERIAL AND METHODS

Chemicals

All chemicals used were purchased from Sigma-Aldrich Ldt (Germany). All absorbance measurements for determination of total phenolic content and antioxidant activity were conducted using a UV Shimadzu spectrophotometer.

Preparation of plant material and extraction method

All plants were collected during May 2014 in Mostar area. Overground parts of fresh samples were washed and shade dried to obtain 5 g dried sample. Ground plant material was used for extraction with 200 ml of hot water under stirring.

Determination of total phenolic content

The total phenolic content was determined with Fiolin-Ciocalteu reagent according to the method of Singelton[9]. Standard curve was generated using gallic acid as a standard. Different concentrations of gallic acid were prepared in distilled water, and their absorbance values were measured at 765 nm. For sample measurement, 5 mL (1/10 dilution) of Folin-Ciocalteu phenol reagent and 900 µL of distilled water were added to 100 µL of plant extract. After 5 min, samples were combined with 4 ml of 15% sodium carbonate (Na_2CO_3), and incubated for 120 minutes. Absorbance at 765 nm was measured. Data are presented as average values of three measurements for each sample.

Determination of antioxidant activity

DPPH radical scavenging activity assay:

The free radical scavenging activity was measured *in vitro* using 1,1-diphenyl-2-picrylhydrazyl assay (DPPH) according to the method described by Brand-Williams *et al.*[10]. DPPH radicals have an absorption maximum at 515 nm, which disappears with reduction by an antioxidant compound. 0.1 M solution of DPPH in methanol was prepared immediately before the experiment. 100 µl of the aqueous extract of the sample is mixed with 3.9 ml of 0.1 mM DPPH solution. Immediately after the addition of DPPH, the absorbance is measured at 515 nm at intervals of 1 minute, until a constant value of the absorption is recorded (steady state). Scavenging activity is expressed as the inhibition percentage calculated using the **following equation:**

Anti-radical activity (%) = {(control absorbance – sample absorbance) / control absorbance} × 100

Each determination was carried out in triplicate. The EC 50 values for the concentration required for 50% scavenging activity were calculated from the above equation.

Ferric reducing antioxidant potential (FRAP) assay:

The ferric reducing power of plant extract was determined using method of Benzie and Strain[11], which is based on reduction of a colorless ferric complex (Fe^{3+}tripyridyltriazine) to a blue-colored ferrous complex (Fe^{2+}-tripyridyltriazine) by the action of electron-donating antioxidants. Working FRAP reagent was prepared by mixing 20 ml of 300 mM acetate buffer (pH 3.6) with 20 ml of 10 mM TPTZ (2,4,6-tri (2-pyridyl) -S-triazine) in 40 mM HCl acid with 20 ml of 20 mM ferric chloride and 24 ml of distilled water. The standard curve is made using different concentrations of $FeSO_4 \times 7H_2O$ (0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mM). 50 µl of the sample solution is added to 950 µL of freshly prepared FRAP reagent. The reaction mixture was incubated for 4 min at room temperature. The reduction is monitored by measuring the change in absorbance at 593 nm and the results were

expressed as mmol of Fe (II) / 100 g of dry matter. All measurements were performed in triplicate.

Free Radical Scavenging by the Use of the ABTS Radical:

The free radical scavenging capacity of plant extracts was investigated using the ABTS radical cationdecolorization assay[12]. This method is based on the reduction of ABTS+• radicals by antioxidants of the plant extracts tested. ABTS cation radical (ABTS•) was prepared by dissolving 19.5 mg of ABTS and 3.3 mg of potassium persulfate in 7 ml of distilled water and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. In this study, a solution of ABTS • + was diluted in ethanol to obtain 0.70 ± 0.02 absorbance at 734 nm. An aliquot of 20 µL sample was mixed with 2 ml of the ABTS radicals in the cuvette and the absorbance was measured at 734 nm after 6 minutes. All solutions were prepared on the day of the experiment, and all measurements were performed in triplicate.

RESULTS

The concentrations of total phenols (TPC) (mg / 100 g dry matter) are shown in Table 1.

Table 1. Total phenol content in aqueous extracts of different medicinal plants

Water extract of medicinal plants	Total phenol content (mg GAE/100g dry weight)
Lauris nobilis	884.36 ± 13.29
Cichorium intybus	802.27± 37.51
Chelidonium majus	2267.25± 44.29
Plantago major	1895.91± 98.69
Papaver rhoeas	7551.98± 143.25
Asplenium ceterach	2754.00± 34.64
Taraxacum officinale	5307.33±221.21
Silene vulgaris	662.33±118.46
Cetraria islandica	95.67±7.64
Leucanthemum vulgare	372.33±118.99
Helichrysum italicum	1569.00±17.32

As seen in the Table 1, significant amounts of TPC of the water extracts were determined in all plant species. TPC amounts of eleven species ranged from 372.33±118.99 to 7551.98±143,25 mg GAE/ 100 g dry weight (DW). *Papaver rhoeas* shows the highest total phenolic content of the samples, while *Cetraria islandica* has the lowest content of total phenols.

Antioxidant activity cannot be measured directly. The antioxidant activity of a compound can be measured by the ability of the compound to intercept free radicals by scavenging or trapping methods. Great number of research articles using different *in vitro* and *in vivo* methods to evaluate antioxidant activity has been published. By examining the literature, we can conclude that 19 different in vitro methods are used [13].

In this study we used three different methods to evaluate antioxidant activity of plant material: (DPPH, ABTS and FRAP method) because the use of more than one method is recommended for the sake of a broader prediction of the antioxidant activity.

Table 2. Antioxidative activity of selected medicinal plants using DPPH, FRAP i ABTS methods

Water extract	**DPPH method** EC50 (mg / 100gDW)	**FRAP method (**mM Fe^{2+}/100 g DW**)**	**ABTS method** (mM TE/100 g DW)
Lauris nobilis	4.88 ± 0.21	15.36± 0.50	0.63± 0.05
Cichorium intybus	8.21 ± 0.10	18.75± 0.80	5.11± 0.22
Chelidonium majus	9.17 ± 0.30	47.94± 2.06	3.13 ± 0.23
Plantago major	3.77 ± 0.17	43.04± 1.26	3.50± 0.23
Papaver rhoeas	28.72 ± 0.44	185.29± 10.34	12.07± 0.75
Asplenium ceterach	5.49 ± 0.72	12.26± 0.15	5.79± 0.74
Taraxacum officinale	8.78 ± 1.08	13.90± 0.07	6.02± 1.35
Silene vulgaris	1.10 ± 0.08	4.71± 0.45	0.40± 0.21
Cetraria islandica	0.11 ± 0.01	1.48± 0.08	0.17± 0.03
Leucanthemum vulgare	0.34 ± 0.10	3.47± 0.09	0.39± 0.06
Helichrysum italicum	3.19 ± 0.11	11.01± 0.20	1.91± 0.23

The results presented in Table 2. demonstrated that all the investigated plant extracts contain phytochemicals who have ability to donate hydrogen to prevent potential damage from free radicals. Out of eleven examined plant species, using the DPPH method for the *in vitro* determination of the antioxidant activity, the aqueous extracts of all species have demonstrated antioxidant activity (Table 2). The highest antioxidant activity was detected in aqueous extracts of *Papaver rhoeas.*

Reductive capacity of aqueous plant extracts determined by the FRAP method is in the range of 1.48±0.08, which is detected for *Cetraria islandica* to 185.29±10.34 mM TE / 100 g DW for *Papaver rhoeas*.

The ability of plant extracts to bind (capture) ABTS radicals ranged from 0.17±0.03 to 12.07±0.75 mM TE / 100 g FW. Again, the greatest antioxidant activity was detected in the sample *Papaver rhoeas* while *Cetraria islandica* had the lowest antioxidant activity.

DISCUSSION

At the moment, there are very few published data on the content of phenolic compounds of plant species in Herzegovina. There are several studies of the total phenol content of medicinal plant species published for samples taken from different areas in Europe [14,15,16,17,18]. Different total phenol content presented in these studies can be attributed to a plant species, climate, testing methods and standards used by the individual researchers. The method used for determination of total phenol content by Folin-Ciocalteu reagent is based on measurements of changes in colour, which is non-specific for phenols. Perhaps the results are influenced by other components present in the plant extracts which may react with the Folin-Ciocalteu reagent such as ascorbic acid [19]. Also, various phenolic compounds react differently in this method. However, measuring the change in colour after two hours of incubation may be used to determine the presence of phenols in the sample.

DPPH method is the most frequently used one for *in vitro* antioxidant activity evaluation. It is demonstrated that phenolic compounds generally exhibit significant scavenging effects against the DPPH free radical[20,21]. On the other hand ABTS assay is applicable for both hydrophilic and lipophilic antioxidants. Both these methods are substrate-free. Popularity of these methods can be attributed to simplicity and speed of analysis. Due to great structural diversity, the antioxidant profiles differ greatly from one plant to another. Activity of natural extracts depends on the plant compounds as well as type and polarity of the extraction solvent and the isolation procedure.

CONCLUSION

This study reported the total phenolic content and the antioxidant activity of eleven medicinal plants (*Lauris nobilis, Cichorium intybus, Chelidonium majus, Plantago major, Papaver rhoeas, Asplenium ceterach, Taraxacum officinale, Silene vulgaris, Cetraria islandica, Leucanthemum vulgare* and *Helichrysum italicum*). We found a positive correlation between the total phenol content and antioxidant activity of investigated medicinal plants. Medicinal plant extracts that showed the highest antioxidant activity had the highest total phenol content and vice versa. Use of medicinal plants have been encouraged and promoted in recent years, but in order to realize their health benefits it is important to measure antioxidant activity. In the future, extensive work in isolation and characterization of the active biomolecules in these plants are required.

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