Studies on the antioxidant activity, phenol and flavonoid contents of some selected Romanian medicinal plants used for wound healing

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Abstract

The therapeutic benefit of medicinal plants was often attributed to their antioxidant properties. Some plant extracts are believed to have strong antioxidant effects. The aim of this study was to examine the antioxidant activity of ethanol and water extracts from four selected Romanian medicinal plants used traditionally as wound healing agents. Water extracts and ethanol extracts were prepared according to traditional Romanian medicine. Total phenol compounds, flavonoid content and DPPH radical scavenging activity effects of the extracts were spectrophotometrically determined. Using DPPH colorimetric method it was found that the ethanol extracts had higher scavenging activity than the water extracts. The highest radical scavenging effect was observed in Echinaceea purpurea ethanol and water extracts followed by Hyssopus officinalis and Achillea millefolium ethanol extracts. The amounts of phenol compounds present in the ethanolic extracts of Achillea millefolium and Hyssopus officinalis correlate to their scavenging effects, but no correlation was observed between phenol compounds of Echinaceea purpurea and its scavenging activity. These results demonstrated that the antioxidative activities observed can be ascribed both to the different mechanisms exerted by different phenolic compounds and also to the synergistic effects of different phytocompounds.

Keywords: antioxidant activity, flavonoids, phenols, ethanol extract, water extract, Equisetum arvense, Achillea millefolium, Hyssopus officinalis, Echinaceea purpurea

Introduction

In coetaneous tissue repair, oxidants and antioxidants play very important roles. Oxidants are now considered to be involved in a number of aspects of burned injury and tissue repair. Oxygen free radicals contribute to further tissue damage in the events following skin injury and are known to impair healing process. Antioxidants, on the other hand, significantly prevent tissue damage and stimulate wound healing process. The therapeutic benefit of medicinal plants is often attributed to their antioxidant properties [1-4]. Some plants extracts are believed to have strong antioxidant effects. Many plant species have been investigated in the search for strong antioxidants [5-8].

It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [9]. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [10]. Some evidences suggest that the biological actions of these compounds are related to their antioxidant activity [11].

The present study was undertaken to investigate the antioxidant activity and the chemical composition of some selected Romanian medicinal species used traditionally as wound healing agents: *Equisetum arvense* L. (Equisetaceae), *Achillea millefolium* L. (Compositae), *Hyssopus officinalis* L. (Labiatae) and *Echinacea purpurea* (Compositae). We tried to find out the relationship of total flavonoid and phenolic contents with the antioxidant activity. The plant species identified as having high antioxidant activity *in vitro* will be used to design further studies to reveal their vulnerary potential in *in vitro* wound-healing models.

Materials and Methods

Plant material and sample preparation

Aerial parts of the plants *Equisetum arvense* (sterile stems), *Achillea millefolium*, *Hyssopus officinalis* and *Echinaceea purpurea* were collected from Neamt County, Romania. Plant material was dried at room temperature and then ground. Five grams of each plant powder were extracted in 100 ml 70% v/v ethanol or in

100 ml cold distilled water by maceration (8 h), followed by filtration through Whatmann #1 filter paper. The resulting filtrates were used for the following tests.

Determination of flavonoid contents

The aluminium chloride colorimetric method [12] was used to measure the flavonoid content of extract samples. From each plant extract 0.5 ml were added to 1.5 ml methanol, 0.1 ml 1M sodium acetate and 2.8 ml distilled water. The mixture was allowed to stand for 30min at room temperature. The absorbance of reaction mixture was measured at 415 nm with a UV/VIS spectrophotometer (Jasco V-530, Japan). Quercetin was used as standard for the calibration curve. Flavonoid contents were expressed as mg quercetin equivalent /g dry weight (D.W.).

Determination of total phenol compounds

Total phenol content was estimated using Folin-Ciocalteu reagent based assay [13]. To an aliquot (10µl) from each plant extract, 10 ml of water, 1.5 ml of Folin Ciocalteu reagent (Sigma-Aldrich, Switzerland) were added. The mixture was kept for 5 min at room temperature and then 4 ml of 20% Na_2CO_3 were added and the volume brought to 25 ml with double-distilled water. The mixture was allowed to stand at room temperature for 30 min and the absorbance of the developed colour was recorded at 765 nm using an UV-VIS spectrophotometer (Jasco V-530, Japan). Caffeic acid was used as standard for calibration curve. The contents of phenol compounds were expressed as mg caffeic acid /g dry weight (D.W.).

Scavenging activity against 1,1–dipheyl–2-picryl hidrazyl radical (DPPH) The effect of water and ethanol extracts on DPPH radical was estimated according to the modified method of Huang and Chen [14]. Aliquots of 150 µl of each plant extract were mixed with 0.9 ml 0.1 M Tris-HCl buffer (pH 7.4) and then with 1.5 ml methanolic solution of DPPH (final concentration of 0.25 M). The mixture was vigorously shaken and left to stand at room temperature, for 20 min in the dark. The absorbance (ABS) of the reaction solution was measured at 517 nm with an UV-VIS spectrophotometer. Reduced gluthation (GSH) was used as standard control. The percentage of sample decolorization was calculated according to the equation: % decolorization = [1- (ABS sample/ABS control)] x 100.

Statistical analysis

The results were expressed as mean values (±SD) of 3 determinations. The mean values and standard deviations were calculated with EXCEL program from Microsoft Office package.

Results and Discussions

Total flavonoid, phenolic compounds and protein content of the extracts

It has been long recognized that plant flavonoids possess antioxidant activity, with considerable beneficial effects on human nutrition and health; their mechanisms of action are believed to be through scavenging or chelating process [9, 16]. Phenolic compounds constitute a class of antioxidant agents acting as free radical terminators [17]. We analyzed the flavonoid contents of our extracts in terms of quercetin equivalent/per gram D.W. The highest flavonoid content was confined to the ethanolic extract of *E. arvense* 59.6 ± 3.4 mg quercetin/g D.W., followed by the other ethanolic extracts: *A. millefolium* (36.30 ± 1.6 mg quercetin/g D.W.), and by the almost equivalent levels of *H. officinalis* and *E. purpurea* (see Table 1). The flavonoid content of aqueous extracts is lower as compared to that of corresponding ethanolic extracts. Also shown in Table 1 are the total phenolic contents expressed in terms of caffeic acid equivalent (mg caffeic acid/g D.W.). The total phenolic content varied from 38.20 ± 4.2 mg caffeic acid/g D.W. (*E. purpurea*) to 118.3 ± 1.9 mg caffeic acid/g (*A.millefolium)* among the ethanolic extracts. The total phenolic content of the aqueous extract is rather similar for all plants investigated, with values around 40 mg caffeic acid equivalent/g D.W.. For *A. millefolium* the total phenol content of its ethanol extract is almost 4 times higher as compared to that of its corresponding aqueous extract and for *H. officinalis* this ratio is greater than 2.

Table 1. Flavonoid and phenol content in the studied plant extracts.

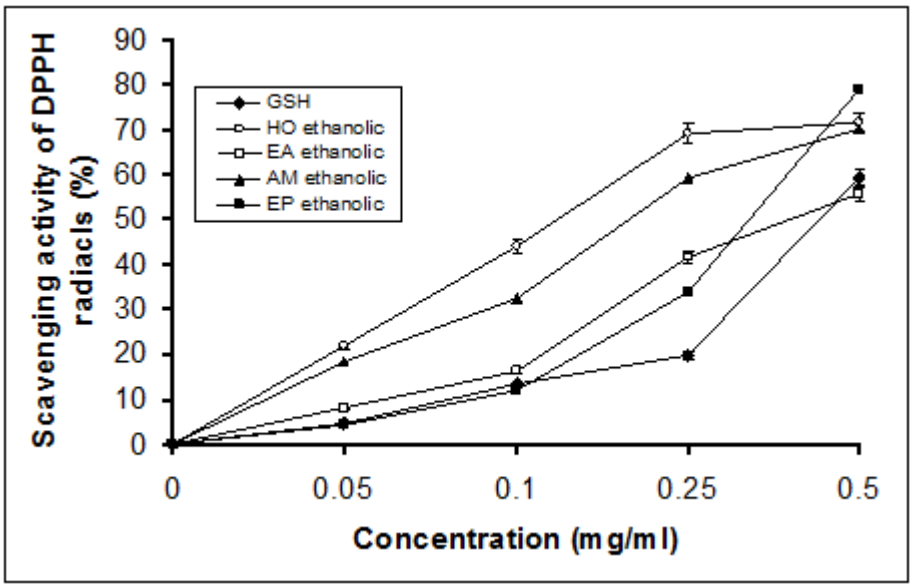
Plant species	Flavonoid content (mg quercetin/g D.W.)	Total phenolic content (mg caffeic acid/g D.W.)
Ethanol extract		
Hyssopus officinalis	31.4 ± 1.3[1]	86.7 ± 2.6
Equisetum arvense	59.6 ±3.4	59.3 ± 3.5
Achillea millefolium	36.3 ±1.6	118.3 ± 1.9
Echinacea purpurea	31.4 ±0.8	60.0 ± 4.2
Water extract		
Hyssopus officinalis	6.5 ± 1.1	40.4 ± 3.4
Equisetum arvense	10.7 ±2.9	40.0 ±3.9
Achillea millefolium	13.5 ±1.8	43.1 ± 2.7
Echinacea purpurea	12.5 ±0.9	41.2 ±4.4

[1]Each value in the table was obtained by calculating average of three experiments ± standard deviation.

Scavenging activity against DPPH radical

The DPPH radical was widely used as a model system to investigate the scavenging activities of several natural compounds such as phenolics and antocyanins or crude mixture such as ethanol extract of plants [17,18]. DPPH is scavenged by antioxidants through the donation of a proton forming the reduced DPPH. Radical scavenging activity increased with increasing percentage of the free radical inhibition. Figure 1 shows the dose response curves for the radical-scavenging activity of the different water and ethanol extracts, and GSH as results from the DPPH colorimetric method. Ethanol extracts had higher radical-scavenging activity than water extracts. It was found that in 0.5 mg dry matter/ml, ethanol extract of *H. officinalis* (71.59 ± 0.72%) and *A. millefolium* (70.36 ± 0.49%) had the highest radical scavenging activities followed by water (72.02 ± 0.43 %) and ethanol extract (78.9 ± 0.51%) of *E. purpurea*. The other extracts also showed DPPH radical scavenging activities, but the activities were lower than that of GSH. Figure 2 shows the scavenging activities of the plant extracts at 0.5 mg dry matter/ml as compared to that of GSH, considered to be 1 (100%).

A



B

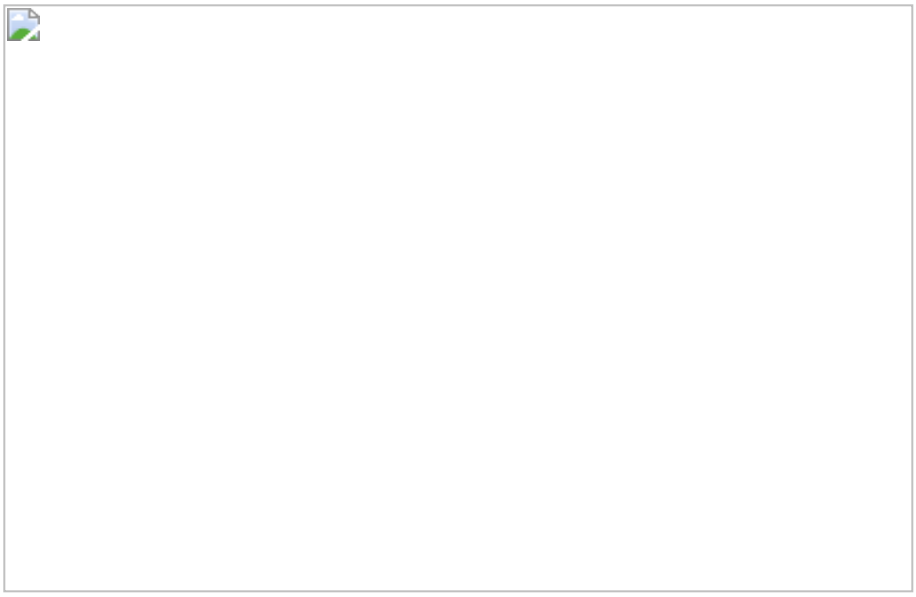


Figure 1. DPPH radical scavenging activity of ethanol (A) and water (B) plant extracts compared to that of GSH. Absorbance values represent means of triplicates of different samples analyzed. *Hyssopus officinalis* = HO, *Equisetum arvense* = EA, *Achillea millefolium* = AM, *Echinacea purpurea* = EP and control, GSH = reduced gluthatione.

Figure 2. Comparison of the DPPH radical scavenging activity of water and ethanol plant extracts to that of GSH (100%) at 0.5 mg/ml dry matter. *Hyssopus officinalis* = HO, *Equisetum arvense* = EA, *Achillea millefolium* = AM, *Echinacea purpurea* = EP and control, GSH = reduced gluthatione.

Conclusions

The results of the current study showed that the water and ethanol extract of *E. purpurea* had the highest scavenging activity followed by *H.officinalis* and *Achillea millefolium* ethanol extracts. The amounts of phenol compounds of *A. millefolium* and *H.officinalis* correlates to their scavenging effect, but no correlation was observed between phenolic compounds of *E. purpurea* and its scavenging activity. These results demonstrate that the antioxidant activities observed can be ascribed both to mechanisms exerted by phenolic compounds and also to synergistic effects of different phytocompounds.

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