ORIGINAL ARTICLE

POLYPHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *CHRYSANTHEMUM PARTHENIUM* EXTRACT

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Abstract

The main objective of this study was the phytochemical and biological analysis of the polyphenolic compounds of *Chrysanthemum parthenium*. The phenolic compounds were analysed using the reference high performance liquid chromatography (HPLC) method. The evaluation of the polyphenolic content was performed by colorimetric analysis. The ethanolic extract was assessed for the antioxidant activity using three *in vitro* assay models, the 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) radical scavenging assay, silver nanoparticles-based assay (SNPs), and an electron paramagnetic resonance (EPR) radicals' detection. SNPs method was described here for the first time in the *C. parthenium* extract. Using high performance liquid chromatography - mass spectrometry (HPLC-MS) analysis, phenolic acid derivatives and free aglycones of flavonoids were detected. The extract of *C. parthenium* with medium total polyphenol content (3.48%) showed a moderate antioxidant activity, as demonstrated by the three methods, and these results could serve as a scientific basis for its therapeutic uses.

Rezumat

Obiectivul principal al acestui studiu a fost analiza fitochimică şi biologică a compuşilor polifenolici din *C. parthenium*. Compuşii fenolici au fost analizaţi utilizând o metodă de cromatografie lichidă de înaltă performanţă (HPLC). Evaluarea conţinutului polifenolic s-a realizat prin analiză colorimetrică. Extractul etanolic a fost testat pentru activitatea antioxidantă prin trei modele *in vitro:* 2,2-difenil-1-picril-hidrazilhidrat (DPPH), metoda bazată pe nanoparticule de argint (SNPs) şi rezonanţă paramagnetică electronică (EPR), metoda SNPs fiind descrisă pentru prima dată în această lucrare. Folosind analiza cromatografică lichidă de înaltă performanţă cuplată cu spevtroscopia de masă (HPLC-MS), s-au detectat derivaţii de acid fenolic şi agliconii flavonoidici. Extractul de *C. parthenium* cu conţinut mediu total de polifenoli (3,48%) a prezentat o activitate antioxidantă moderată, după cum o arată cele trei metode, aceste rezultate putând servi drept bază ştiinţifică pentru utilizările sale terapeutice.

Keywords: *Chrysanthemum parthenium*, polyphenols, EPR method, SNPs assay

Introduction

Chrysanthemum parthenium (L.) Bernh. *syn*. *Tanacetum parthenium* L. Sch.-Bip., feverfew is a medicinal herb spread in the Romanian cultivated flora [1]. The active principles of this species are sesquiterpene lactones, essential oils, polyphenolic compounds. Feverfew is a medicinal plant traditionally used for the treatment of fever, migraine headaches, rheumatoid arthritis, stomach aches, toothaches, insect bites, and infertility. It has multiple pharmacologic properties, such as anticancer, anti-inflammatory, cardiotonic, antispasmodic, and antioxidant. The extract of feverfew provided from Charleston has an antioxidant activity [2-5]. In literature there are not available data of the chemical composition of the polyphenols of *C. parthenium* from Romania. The purpose of the present study was the screening of the major polyphenolic compounds of the ethanolic extract from the aerial parts of *C. parthenium,* and the evaluation of its *in vitro* antioxidant activity.

Materials and Methods

The aerial parts of *C. parthenium* (L.) Bernh. (*Tanacetum parthenium* L. Sch.-Bip.) (Voucher No. 28) were collected at flowering stage in July 2013 (Cluj-Napoca). Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Cluj-

Napoca, Romania. The powder obtained from the aerial parts was extracted with 70% ethanol, at 60°C [6].

HPLC-MS determination. HPLC was used to analyse the phenolic derivatives. HPLC-MS analysis was performed on an Agilent 1100 HPLC Series system using the chromatographic conditions previously described [6-8]. Quantitative determinations were performed using an external standard method. The HPLC peaks were identified by comparing their retention times with those of the standard samples, which were determined under the same chromatographic conditions. Calibration curves in the 0.5 - 50 mg/mL range with good linearity ($R^2 >$ 0.999) for a five points plot, were used to determine the concentration of polyphenols in plant samples.

Spectrometric determination of polyphenolic content. Quantitative determination of caffeic acid derivatives**,** flavonoids and polyphenols (TPC) was carried out using spectrophotometric methods. Caffeic acid, gallic acid and rutin reagents were used as standards for calibration for these phenols [6, 8-10].

Determination of antioxidant activity. The extracts were screened for antioxidant activities using three *in vitro* assay models, the 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) assay, silver nanoparticles-based assay (SNPs), and an electron paramagnetic resonance (EPR) radicals detection. The antioxidant activity is described as having activity against the stable form of the synthetic substance DPPH [6, 8, 11]. A sensitive colorimetric method for the detection of polyphenols was used in this research based on the reduction of Ag (+) ions by polyphenols in the presence of citrate-stabilized silver seeds [12-13]. EPR measurements were performed on a Bruker Elexsys E500 spectrometer operating in X band (~ 9.4 GHz) with 100 kHz modulation frequency, at room temperature [14].

Results and Discussion

Phenolic compounds were analysed using the reference HPLC method (Table I). The method allowed a simultaneous analysis of different classes of polyphenols by a single pass column [6-8].

Table I

Polyphenolic compounds content in *C. parthenium* (µg/g plant material)

Polyphenolic compounds	Rt ± SD (min)	*m/z*	*C. parthenium*
Gentisic acid	2.15 ± 0.07	179	< 0.2
Caffeic acid	5.60 ± 0.04	179	< 0.2
Chlorogenic acid	5.62 ± 0.05	353	< 0.2
Quercetin	26.80 ± 0.15	301	27.61 ± 0.39
Luteolin	29.10 ± 0.19	285	< 0.2
Apigenin	33.10 ± 0.15	279	9.71 ± 0.18

Note: Values are the mean ± SD (n = 3)

Six polyphenols were identified and six of them were quantified in the extract of *C. parthenium* (Table I). The HPLC-MS analysis revealed the presence of few phenolic acids e.g. gentisic acid, caffeic acid and chlorogenic acid. Their concentrations were lower than 0.2 µg/g. Also, in the same sample, were quantified three flavonoid aglycones (quercetin, luteolin and apigenin). Quercetin was the flavonoid found in the largest amount (27.61 ± 0.39 µg/g), followed by apigenin (9.71 ± 0.18 µg/g). Luteolin was identified, but it was in too low concentrations to be quantified. Considering the 19 standard compounds used in this study, some other peaks were not identified.

Estimation of polyphenol content. Plant phenols have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases. In the last few years, the results provided by some pre-clinical studies have shown interesting activities of polyphenols against the most common oral diseases (caries, periodontitis and candidiasis) [15, 16]. Estimation of the polyphenol contents of this extract is shown in Table II. The extract of *C. parthenium* contains small amounts of polyphenolic compounds: total polyphenols, flavonoids and caffeic acid derivatives (3.48 ± 0.17 g GAE (gallic acid equivalents)/100 g, 1.27 ± 0.07 g RE (rutin equivalents)/100 g, and 1.30 ± 0.11, respectively). Other authors reported that the total phenolic content of *C. parthenium* from Charleston was 21.21 ± 2.11 µg/mg [5].

Determination of antioxidant activity by DPPH assay. The antioxidant capacity of the extract of *C. parthenium* by using DPPH bleaching assay is shown in Table II. Quercetin was used as the positive control. IC_{50} value represents the concentration of test extract or compound where the inhibition of test activity reached 50%. The extract of *C. parthenium* aerial parts exhibited a moderate antioxidant capacity, related with the polyphenolic total content. Comparing the antioxidant activity of *C. parthenium* flowers from Bulgaria and *C. parthenium* aerial parts from Romania, the ethanolic extract of the Bulgarian species showed higher values (39.23 µg/mL) than the extracts of the Romanian species [17].

Table II

Total phenolic content and the values of their antioxidant capacity established by several methods

Samples	Flavonoids (g RE/100 g)	TPC (g GAE/100 g)	Caffeic acid derivatives (g CAE/ 100 g)	IC_{50} (µg/mL)	SNPs (µmol gallic acid/gram)	EPR (µmol gallic acid/gram)
C. parthenium	1.27 ± 0.07	3.48 ± 0.17	1.30 ± 0.11	149.76 ± 6.23	29.85	106.717
Quercetin	-	-	-	5.47 ± 0.03	-	-
DPPH	-	-	-	-	-	797.011

Each value is the mean ± SD of three independent measurements. RE = rutin equivalents; GAE = gallic acid equivalents; CAE = caffeic acid equivalents; TPC = total phenolic content; IC_{50} = concentration with 50% inhibition

Evaluation of antioxidant activity by silver nanoparticles-based assay (SNPs). Silver nanoparticles have attracted many applications in diverse areas [12, 13]. To the best of our knowledge, there has been no reference on the determination of antioxidant capacity of hydrophilic compounds in different herb extracts by the silver nanoparticles-based method. The absorption spectrum of the silver nanoparticles reveals that SNPs prepared by citrate reduction shows a surface plasmon absorption band with a maximum of 423 nm indicating the presence of roughly spherical silver nanoparticles. The spherical shape was further confirmed by transmission electron microscopy (TEM) (Figure 1). The results of antioxidant capacities determined by SNPs method are presented in Table II (expressed as µmol gallic acid/gram). The extract of *C. parthenium* has a moderate antioxidant capacity. One possible explanation is the presence in the ethanolic extracts of other antioxidants, which are not active reducing agents for the catalytic growth of SNPs [18].

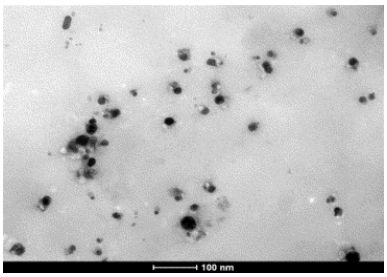

Figure 1.
Transmission electron microscopy (TEM) image of SNPs synthesized with trisodium citrate

Electron paramagnetic resonance (EPR) spectroscopy method. A very used method to study the qualitative antioxidant properties is EPR spectroscopy, using stable free radicals. In this study we made a mixture of free radical (DPPH) and antioxidant extract. The rate of reaction between antioxidant compounds and DPPH radical was monitored by using normalized double integrated residual EPR signal which is correlated with the number of paramagnetic species (Figure 2). The EPR spectra presented in the Figure 2 show that we have a smaller intensity of the signal function of the antioxidant extract. It represents the oxidation-reduction rate of the DPPH radical. The values of the integral intensity of this sample were compared with the DPPH. One can observe that the extract of *C. parthenium* has a higher antioxidant capacity (Table II). Surprisingly, the EPR results are not in good correlated with the DPPH radical-scavenging assays results.

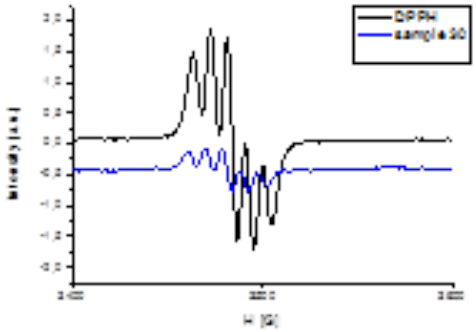

Figure 2.
The rate of reaction between antioxidant compounds and DPPH radical

Conclusions

In this work, during the phytochemical screening, we have determined the phenolic profile and the antioxidant activity for *C. parthenium* and we have completed the literature data with new information concerning the polyphenolic compounds and their bioactivity. The simultaneous determination of a wide range of phenolic compounds was performed using a rapid, highly accurate and sensitive HPLC method assisted by mass spectrometry detection. The antioxidant activity evaluated using the DPPH bleaching method, the silver nanoparticles-based assay, and an EPR spectroscopy method; indicate that the extract of *C. parthenium* exhibited a moderate antioxidant capacity, related with the polyphenolic total content. Additional investigations of this species should be directed to carry out *in vivo* studies of its active compounds in order to develop scientifically based natural pharmaceuticals.

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