Free Radicals and Antioxidants 3 (2013) 43–46

Contents lists available at SciVerse ScienceDirect

Free Radicals and Antioxidants

journal homepage: www.elsevier.com/locate/fra

Short communication

Antioxidant activity profiling by spectrophotometric methods of phenolic extract of *Prasium majus* L

Tarik Md. Chaouche [a,*], Farah Haddouchi [a], Riadh Ksouri [b], Faten Medini [b], Imad A. El-Haci [a], Zahia Boucherit [c], Fatima Zohra Sekkal [d], Fawzia Atik-Bekara [a]

[a] *Laboratory of Natural Products, Department of Biology, Faculty of Sciences, Tlemcen University, Tlemcen, Algeria*
[b] *Laboratory of Extremophile Plants, Borj Cedria Biotechnology Centre (CBBC), Hammam-Lif 2050, Tunisia*
[c] *Antibiotics Antifungal Laboratory: Physical-Chemistry, Synthesis and Biological Activity, Department of Biology, Faculty of Sciences, Tlemcen University, Tlemcen, Algeria*
[d] *Department of Biotechnology, Faculty of Natural Science and Life, Abdelel Hamid Ibn Badiss University, Mostaganem 27000, Algeria*

ARTICLE INFO

Article history:
Received 12 March 2013
Accepted 18 March 2013
Available online 28 March 2013

Keywords:
Prasium majus
Quantification of phenolic classes
Antioxidant activity

ABSTRACT

Introduction: Phytochemicals are extensively found at different levels in many medicinal plants. To investigate the phenolic compound content and *in vitro* antioxidant activity of phenolic extract from *Prasium majus* L (Lamiaceae).
Methods: The present investigation comprises, estimation of total polyphenol, flavonoid, tannin, *in vitro* antioxidant assays such as total antioxidant capacity, DPPH, ABTS, β-carotene and ferric reducing power.
Results: P. majus exhibited 64.25 mg GAE g^{-1} extract of polyphenol phenol content, and better scavenging activity of DPPH (IC_{50} = 7.95 μg mL^{-1}), $ABTS^{\bullet+}$ (IC_{50} = 373.78 μg mL^{-1}), and β-carotene (IC_{50} = 122.56 μg mL^{-1}).
Conclusion: Our results clearly demonstrated that phenolic extract *P. majus* has antioxidant capacity. Therefore is a valuable source of natural antioxidants.

1. Introduction

The family of Lamiaceae consists of about 233 genera and 6900 species worldwide. Many species of the Lamiaceae family are considered of high importance because of their uses in medicine, culinary and cosmetics. *Prasium majus* L. is a wildflower species belonging to this family. An infusion of the leaves and flowers of the plant is used for gastrointestinal diseases.[1] In this paper we screen the phenolic extract for their free radical scavenging and antioxidant activity. That should complement to their therapeutic value and improve the popularization of the species. To the best of our knowledge, we were among the first to provide data on the antioxidant effect of the phenolic extract of *P. majus* by five methods.

2. Material and methods

2.1. Phenolic extraction

P. majus was harvested in April 2012 from northwest of Algeria (Tlemcen). It was identified in the Laboratory of Natural Products, Department of Biology, University of Tlemcen (Algeria). Voucher specimen was deposited at the Herbarium of the Laboratory.

The powder of plant dried (2 g) was first extracted by 25 mL hexane to remove lipids and chlorophylls. After centrifugation, the pellet was resuspended in 25 mL methanol/water (80:20, v:v) for 24 h. The obtained extract was filtered through a Whatman N° 4 filter paper and evaporated at 45 °C under reduced pressure, redissolved in methanol at a concentration of 1 mg mL^{-1}.[2]

2.2. Quantification of phenolic classes

2.2.1. Total polyphenol quantification

Total polyphenol content of the plant extract was determined using Folin–Ciocalteu reagent (FC),[2] using gallic acid as a standard. An aliquot (250 μL) of diluted sample extract was added to 0.5 mL of distilled water and 0.125 mL of the FC. The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 mL of 7% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 3 mL and mixed thoroughly, and held in dark for 90 min at ambient temperature. After incubation, the absorbance at 760 nm was recorded. Total polyphenol content of plant parts was expressed as mg of gallic acid equivalents per gram of dry weight

* Corresponding author. Tel.: +213 772735216.
E-mail address: kiratcqa@yahoo.fr (T.Md. Chaouche).

2231-2536/$ – see front matter
http://dx.doi.org/10.1016/j.fra.2013.03.004

(mg GAE g^{-1}DW) through the calibration curve (0–400 μg mL^{-1} range).

2.2.2. Flavonoid quantification

Total flavonoid content was measured using a colorimetric assay.[2] An aliquot (250 μL) of diluted sample or standard solution of catechin was added to 75 μL of NaN2 solution (7%), and mixed for 6 min, before adding 150 μL $AlCl_3$ (10%). After 5 min, 0.5 mL of NaOH solution (1 M) was added. The final volume was adjusted to 2.5 mL, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoid content was expressed as mg catechin equivalent per gram of dry weight (mg CEf g^{-1}DW), through the calibration curve of catechin (0–400 μg mL^{-1} range).

2.2.3. Tannin quantification

The tannin content was measured using a colorimetric assay.[2] Briefly, 50 μL of diluted extract solution was mixed with 3 mL of 4% vanillin–methanol solution and 1.5 mL of concentrated hydrochloric acid, and the mixture was allowed to stand for 15 min. Absorbance was read at 510 nm against the blank (water). Tannin content was expressed as mg catechin equivalents (CE) per gram of dry weight (mg CEt g^{-1}DW), through the calibration curve of catechin. The calibration curve range was 0–400 μg mL^{-1}.

2.3. Determination of antioxidant activities

2.3.1. Total antioxidant capacity

This assay is based on the reduction of Molybdene (VI) to Molybdene (V) by the sample extract, which produces a green phosphomolybdenum (V) complex under acidic pH conditions.[3] An aliquot (0.1 mL) of phenolic extract was combined to 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated in a thermal block at 95 °C for 90 min. After, the mixture had cooled to room temperature. The absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE g^{-1}DW). The calibration curve of gallic acid range was 0–400 μg mL^{-1}.

2.3.2. DPPH assay

The ability of the corresponding extracts to donate hydrogen atoms or electrons was measured from the bleaching of purple coloured methanol solution of DPPH.[4] Fifty microlitre of various concentrations of phenolic extract was added to 1950 μL of 6.34×10^{-5} M DPPH radical solution in methanol. The mixture was shaken vigorously and allowed to stand for 30 min in the dark. The absorbance of the resulting solution was measured at 517 nm and butylated hydroxytoluene (BHT) was used as a positive control (standard). Inhibition of DPPH radical was calculated as follows:

$$\text{DPPH scavenging effect}(\%) = [A_0 - A_1/A_0] \times 100 \quad (1)$$

where A_0 and A_1 are the absorbance at 30 min of the control and the sample, respectively.

The antiradical activity was expressed as IC_{50} (μg mL^{-1}), the extract dose required to cause a 50% decrease of the absorbance at 517 nm. A lower IC_{50} value corresponds to a higher antioxidant activity.

2.3.3. ABTS assay

ABTS radical scavenging activity of extracts was determined according to Re et al.[5] The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 5 mL of 14 mM $ABTS^{\bullet+}$ solution and 5 mL of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with methanol to get an absorbance of 0.700 ± 0.020 at 734 nm. In a final volume of 1 mL, the reaction mixture comprised 950 μL of $ABTS^{\bullet+}$ solution and 50 μL of the phenolic extract, BHT, Trolox and Methanol blanks at various concentration. The absorbance ware recorded at 734 nm. The inhibition percentage of $ABTS^{\bullet+}$ radical was calculated using the following formula (2):

$$ABTS^{\bullet+}\text{scavenging effect}(\%) = [A_0 - A_1/A_0] \times 100 \quad (2)$$

where A_0 and A_1 have the same meaning as in formula (1).

2.3.4. β-Carotene bleaching test

A modification of the method described by Koleva et al[6] was employed. β-carotene (2 mg) was dissolved in 20 mL chloroform and to 4 mL of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under vacuum at 40 °C and 100 mL of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Sample extract and standards group (BHA and BHT) were prepared in methanol. An aliquot (150 μL) of the β-carotene/linoleic acid emulsion was distributed in each of the wells of 96-well microtitre plates and 10 μL of various concentrations of phenolic extract was added. The microtitre plates were incubated at 50 °C for 120 min, and the absorbance was measured using a model EAR 400 microtitre reader at 470 nm. Readings of all samples were performed immediately ($t = 0$ min) and after 120 min of incubation. The inhibition percentage of β-carotene was calculated using the following formula (3):

$$\beta - \text{carotene bleaching inhibition}(\%) = [S - C_{120}/C_0 - C_{120}] \times 100 \quad (3)$$

where C_0 and C_{120} are the absorbance values of the control at 0 and 120 min, respectively, and S is the sample absorbance at 120 min. The results were expressed as IC_{50} values (μg mL^{-1}).

2.3.5. Iron reducing power

The capacity of plant extracts to reduce Fe^{3+} was assessed by the method of Trabelsi.[2] One millilitre of various concentrations of phenolic extract was mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After that, 2.5 mL of 10% trichloroacetic acid were added, and the mixture was centrifuged at 650× g for 10 min. The upper layer fraction (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride and thoroughly mixed. The absorbance was measured at 700 nm and ascorbic acid was used as a positive control. A higher absorbance indicates a higher reducing power. IC_{50} value (μg mL^{-1}) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from linear regression analysis.

2.4. Statistical protocol

Values shown in tables were means ± standard deviations of three parallel measurements. The IC_{50} values were calculated from linear regression analysis.

3. Results and discussion

3.1. Total polyphenol, flavonoid and condensed tannin contents

The gallic acid linear curve was obtained using the formula $y = 0.00253x + 0.01344$ ($R^2 = 0.9954$). Using this gallic acid linear curve, total polyphenol content of *P. majus* value was 64.25 ± 0.30 mg GAE g^{-1}DW. The total flavonoid content was

obtained using the regression calibration curve $y = 0.00355x+0.09903$ ($R^2 = 0.997$) with catechin equivalent, the value was 31.56 ± 0.20 mg CEf g^{-1}DW. The total tannin content was obtained using the regression calibration curve $y = 0.00076x-0.00760$ ($R^2 = 0.9948$) with catechin equivalent, the value was 7.37 ± 0.20 mg CEt g^{-1}DW.

The findings of the phytochemical screening indicated that extract phenolic is rich in polyphenol, flavonoids and tannins which may be responsible for the antioxidative efficacy.[7] Due to redox properties, these compounds play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.[8] It has been also recognized that flavonoids show antioxidant activity through scavenging or chelating process and their effects on human nutrition and health are considerable.[9]

Lamiaceae species are known to produce a diverse array of phenolic compounds, these phytochemicals can occur either as aglycones or glycosides. Concerning our specie no references could be found despite the thorough literature survey. Comparing the obtained results with the previously data published by Stagos et al,[10] on polyphenol contents of 24 species of Lamiaceae, whose contents obtained are between 91 and 575 mg GAE g^{-1}DW, we have found that total phenolic contents in our extract is lower.

3.2. Antioxidant activity of phenolic extract

3.2.1. Total antioxidant capacity

The gallic acid linear curve was obtained using the $y = 0.3072 \ln x - 0.7541$ ($R^2 = 0.992$) and the results are expressed as mg gallic acid equivalent. The result for the total antioxidant capacity of phenolic extract was 85.12 ± 0.80 mg GAE g^{-1}DW.

3.2.2. DPPH assay

The scavenging activity of the investigated extract varied widely from 12% to 98.5% (IC_{50} = 7.95 ± 0.60 μg mL^{-1}) (Table 1) and in standard from 10.78% to 79.88% (IC_{50} = 15.6 ± 0.45 μg mL^{-1}). The DPPH radical contains an odd electron which is responsible for the absorbance at 517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance.[11] It was observed that the radical scavenging activity is increasing with the increase of phenolic compound content. The extract was also reported a high concentration between DPPH radical scavenging potential and total polyphenol content.[2,12]

Table 1
DPPH, ABTS, β-carotene, reducing power activities of phenolic extract of *P. majus*.

	Concentration (μg mL^{-1})	Percentage activity (%)	IC_{50} (μg mL^{-1})
DPPH	1.25	12	7.95 ± 0.60
	3.75	45.21	
	15	91.37	
	22.5	98.52	
ABTS	80	17.82	373.78 ± 5.1
	160	28.11	
	320	47.32	
	620	73.07	
β-carotene	125	42.07	122.56 ± 4.3
	250	56.81	
	500	61.48	
	1000	70.43	
Reducing power	150	0.244	354 ± 4.6
	300	0.408	
	600	0.903	

Compared with positive control, the phenolic extract was presented a high antioxidant activity, higher than BHT. That result suggests that *P. majus* possess phenolic compounds that have a high potential to neutralize free radicals.

Peterson and Simmonds.[13] who reported the existence of rosmarinic acid–caffeic acid conjugates in Lamiaceae plants, this may explain the increased the scavenging ability of DPPH radical.

3.2.3. ABTS assay

The scavenging ABTS effect increases with the concentration of standard and samples. At 620 μg mL^{-1} concentration, *P. majus* possessed 73.07% (Table 1), IC_{50} = 373.78 ± 5.1 μg mL^{-1} showed lower activity than the standard BHT (73.1 ± 0.85 μg mL^{-1}).

3.2.4. β-Carotene bleaching test

The percentage of scavenging effect on the β-carotene was increased with the increase in the concentrations of the extract from 125 to 1000 μg mL^{-1}(Table 1). The percentages of inhibition were varying from 42.07% (in 125 μg mL^{-1} of the extract) to 70.43% (in 1 μg mL^{-1} of extract). The IC_{50} value of the phenolic extract was estimated at 122.56 ± 4.3 μg mL^{-1}. The BHT in all concentrations showed higher percentage of inhibition of free radicals than the extract, CI_{50} = 11.5 ± 1.5 μg mL^{-1}, ten times more effective than the extract.

3.2.5. Iron reducing power

In the presence of antioxidants in the sample, would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the absorbance at 700 nm. In the present study, this activity also showed with an IC_{50} value of 354 ± 4.6 μg higher than the standard BHT (130 ± 3.5 μg mL^{-1}). Their absorption values increased with increase in concentration, showing that as the concentration of the extract was increased, their ability to reduce Fe^{3+} to Fe^{2+} was also increased (Table 1).

4. Conclusion

The results of the study clearly indicate that the phenolic extract of *P. majus* possess average antioxidant activity. Hence, it is worthwhile to isolate and elucidate the bioactive principle(s) responsible for the antioxidant activity against DPPH radical.

Conflicts of interest

All authors have none to declare.

References

1. Chia-Jung L, Lih-Geeng C, Ting-Lin C, Wei-Ming K, Ying-Fei L, Ching-Chiung W. The correlation between skin-care effects and phytochemical contents in *Lamiaceae* plants. *Food Chem*. 2011;124:833–841.
2. Trabelsi N, Megdiche W, Ksouri R, et al. Solvent effects on phenolic contents and biological activities of the halophyte *Limoniastrum monopetalum* leaves. *Food Sci Technol*. 2010;43:632–639.
3. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999;269: 337–341.
4. Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants. *J Agric Food Chem*. 1999;47:1749–1754.
5. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-evans C. Antioxidant activity applying an improved ABTS radical decolorization assay. *Free Radic Biol Med*. 1999;26:1231–1237.
6. Koleva II, Teris AB, Jozef PH, Linssen AG, Lyuba NE. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem Anal*. 2002;13:8–17.
7. Gautam A, Jhade D, Ahirwar D, Sujane M, Sharma GN. Phamacognostic evaluation of *Toona ciliate* bark. *J Adv Pharm Technol Res*. 2010;1:216–220.

8. Paliwal P, Pancholi SS, Patel RK. Pharmacognostic parameters for evaluation of the rhizomes of *Curcuma caesia*. *J Adv Pharm Technol Res*. 2011;2:56–61.
9. Kessler M, Ubeaud G, Jung L. Anti and pro-oxidant activity of rutin and quercetin derivatives. *J Pharm Pharmacol*. 2003;55:131–142.
10. Stagos D, Portesis N, Spanou C, et al. Correlation of total polyphenolic content with antioxidant and antibacterial activity of 24 extracts from Greek domestic Lamiaceae species. *Food Chem Toxicol*. 2012;50:4115–4124.
11. Pal D, Mitra SA. Preliminary study on the *in vitro* antioxidant activity of the stem of *Opuntia vulgaris*. *J Adv Pharm Technol Res*. 2010;1:268–272.
12. Ksouri R, Megdiche W, Falleh H, et al. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. *C R Biol*. 2008;331:865–873.
13. Petersen M, Simmonds MSJ. Rosmarinic acid. *Phytochemistry*. 2003;62: 121–125.