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Antioxidant Activity of Methanol Extracts Obtained from *Plantago* Species

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Antioxidative activities of methanol extracts from five *Plantago* species (*P. afra*, *P. coronopus*, *P. lagopus*, *P. lanceolata*, and *P. serraria*) were characterized by the DPPH scavenging test and the inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation on bovine brain liposomes. All extracts showed antioxidant activity in both methods. Whereas *P. serraria* exhibited the strongest activity as a DPPH scavenger, *P. lanceolata* and *P. serraria* were found to be the most active in the lipid peroxidation inhibition assay. The extracts were investigated regarding their composition by different colorimetric techniques, such as the content of total phenolic compounds by the Folin−Ciocalteu assay, flavonoids by $AlCl_3$ reagent, phenylpropanoid glycosides (PPGs) by Arnow reagent, and iridoids by Trim−Hill assay. A high correlation was found between the scavenging potency and the total phenolic and phenylpropanoid content of the extracts but not between the lipid peroxidation potency and the extract composition. *P. serraria* is presented as a possible new source of natural antioxidants.

KEYWORDS: ***Plantago*; antioxidant activity; DPPH; lipid peroxidation; phenolic compounds; phenylpropanoid glycosides; flavonoids**

INTRODUCTION

Plantago species are perennial herbs of the Plantaginaceae family widely distributed in Europe and America, and over 256 species have been described. The aerial parts of some species are used, mainly as polar extracts or entire leaves, in folk and phytotherapy medicine, for a wide range of diseases; these include problems related with digestive and respiratory organs, skin and infectious diseases, pain relief, and cancer (*1*). In addition, the leaves and seeds of some *Plantago* species, for example, *P. coronopus*, *P. lanceolata*, and *P. serraria*, are used in certain countries such as France, Italy, and South Africa as part of the diet, particularly as ingredients for salads or for childrens' mush (*2*−*4*). These species are not only useful for human use, but they are also important from the animal feeding point of view. *P. lanceolata* is currently being evaluated in different countries as a potential pasture species because of its medicinal value to animals, improving their physiological conditions and diminishing the need for antibiotic growth promoters (*5*−*9*).

Previous chemical studies on some *Plantago* species have shown the presence of iridoids and various phenolics, such as flavonoids or phenylpropanoid glycosides (PPGs) (*10*−*13*). Many phenolic compounds are antioxidant but, despite the richness of phenolic compounds in *Plantago*, most species have not been screened for antioxidant activity previous to the work reported here (*14*, *15*).

The dual effect exerted by free radicals (ROS) is well-known. On the one hand, they are useful to defend the organism from attack by microorganisms, but, on the other, they can react with biological molecules, such as DNA, proteins, or lipids, generating mutations and damaging membranes, leading to cell and tissue injuries (*16*−*18*). This damage is considered to be, at least in part, responsible for the genesis of different diseases, such as atherosclerosis, arthritis, neurodegenerative disorders, and cancer. ROS can also affect food quality, reducing its nutritional content and promoting the developingment of food rancidity and off-flavors (*19*).

In aerobic organisms, one on the major targets of ROS is the cellular biomembrane, where they induce lipid peroxidation (LPO). Under this process, not only the membrane structure and its functionality seem to be affected, but also some reaction products, for example, malondialdehyde (MDA), can react with biomolecules and exert cytotoxic and genotoxic effects (*16*, *20*). High levels of lipid peroxides have been found in the serum of patients suffering from liver diseases, diabetes, vascular disorders, cataracts, or tumors (*16*).

All of these considerations indicate a possible useful role for antioxidants in disease prevention. Recent publications have mentioned the disadvantages of synthetic antioxidants, for example, butylated hydroxyanisole (BHA) or butylated hy-

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droxytoluene (BHT), and their possible toxic properties for human and animal health (*21*−*23*). The development of alternative antioxidants from natural origin has attracted considerable attention and is thought to be a desirable development by many (*24*−*26*).

The present study was aimed to investigate possible new sources of natural antioxidants, which would be able to help the endogenous antioxidant mechanisms and, therefore, to protect against ROS-involved diseases and also be useful in food conservation and as health-promoting animal fodder. *Plantago* species were selected because of the current interest in them as fodder material, so the antioxidative properties of the methanol extracts (MeOH) of five *Plantago* species, *P. afra*, *P. coronopus*, *P. lagopus*, *P. lanceolata*, and *P. serraria*, were tested. Two assays were employed, the scavenging activity of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and the inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation of bovine brain liposomes. After quantification of the major polar components present in MeOH extracts of each species, possible composition−activity relationships were investigated.

MATERIALS AND METHODS

Plant Material. Aerial parts of *P. afra*, *P. coronopus*, *P. lagopus*, *P. lanceolata*, and *P. serraria* were collected in Don Benito, Badajoz (Spain), in April 2000. They were authenticated by Dr. A. Aparicio from the Department of Botany, Pharmacy Faculty, University of Seville. Voucher specimens (SEV no. 160806, 160808, 160804, 160809, and 160805, respectively) are kept in the Herbarium of University of Seville.

Preparation of Extracts. The extraction procedure has been described previously (*27*). Briefly, 50 g of dried and powdered leaves from each species was extracted by Soxhlet for 48 h with methanol. After filtration, each extract was concentrated to dryness under vacuum and washed with *n*-hexane, using a sonication bath at controlled temperature and times, until all chlorophyll was removed. The washed MeOH extracts were used for the study, with previous filtering to avoid interferences.

Determination of Antioxidant Activity with the DPPH Radical Scavenging Method. For the determination of the antioxidant activity of the samples, the stable DPPH (Sigma-Aldrich Química S.A., Madrid, Spain) radical was used. Qualitative [thin-layer chromatography (TLC)] and quantitative (spectrophotometric) methods were employed.

TLC Autographic Assay (28). A 20 μL aliquot of each of the MeOH extracts was spotted on silica gel plates (20 × 20 cm, silica gel 60 F254, Merck, Darmstadt, Germany) with a solvent system of butanol/acetic acid/water (40:10:50). After developing and drying, TLC plates were sprayed with a 0.2% DPPH solution in methanol and examined 30 min after spraying. Active antioxidant compounds appeared as yellow spots against a purple background.

Spectrophotometric Assay (29). The test was carried out on 96-well microplates. Fifty microliters of a 0.022% DPPH solution in methanol was added to a range of solution of different concentrations (seven serial 3-fold dilutions to give a final range of 1000 to 1.3 μg/mL) of each extract and (seven serial 2-fold dilutions to give a final range of 100 to 5 μM) of compounds to be tested in methanol (230 μL). Absorbance at 515 nm was determined 30 min after the addition of the extracts or compound solutions, and the percentage of activity was calculated. Ascorbic acid was used as positive control (seven serial 2-fold dilutions to give a final range of 100 to 5 μM).

Lipid Peroxidation Assay (*30*). For the determination of the inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation (LPO) on bovine brain liposomes by each of the MeOH extracts, liposomes obtained from bovine brain extract (Sigma-Aldrich Química S.A., Madrid, Spain) were used. Brain extract was suspended in phosphate-buffered saline (PBS) (5 mg/mL) and sonicated in an ice−water bath until the entire lipid was suspended and the suspension appeared to be homogeneous. Glass balls (borosilicate solid-glass beads, Sigma-Aldrich Química) were also included to aid the process. The suspension was not allowed to become hot during the sonication process, as this might have caused lipid degradation.

Lipid peroxidation was assayed using the formation of malondialdehyde (MDA) as indicator. For each extract, a range of concentrations was tested. The extract test reaction mixture (ET) consisted of 0.2 mL of liposomes, 0.1 mL of aqueous $FeCl_3$ (1 mM), 0.1 mL of aqueous ascorbic acid (1 mM), 0.5 mL of PBS, and 0.1 mL of the MeOH extract solution to be assessed. Different controls were used, being, first, the full reaction mixture (FRM), where the extract was omitted and the solvent (methanol) was added instead. Second, a suspension of liposome alone (B) was employed, to test if liposomes underwent a self-peroxidation process during the incubation period, and, third, each extract alone with PBS (EA), to take into consideration its own absorbance reading. Propylgallate (10^{-4} M) in FRM was used as a positive control. Four replicates were carried out for each test mixture (extract test, FRM, and all controls).

Each mixture was incubated at 37 °C for 20 min. After this time, the TBA test was performed by adding 0.1 mL of 2% butylated hydroxytoluene (BHT) in EtOH followed by 0.5 mL of 1% w/v thiobarbituric acid (TBA) in 50 mM NaOH and 0.5 mL of 25% HCl. The system was heated to 90 °C for 30 min. After cooling, 2.5 mL of butanol was added to each tube. The mixture was vortexed and centrifuged at 3500 rpm for 20 min at room temperature. The absorbance of the MDA−TBA complex in the upper layer was determined at 532 nm by a spectrophotometer (Perkin-Elmer Lambda 3).

The percentage of lipid peroxidation inhibition was assessed by comparing the absorbance of the full reaction mixture with that of the extract test reaction mixtures where the substance to be assessed was included:

$$\% \text{ inhibition} = 100 \times [(\text{FRM-B}) - (\text{ET-B-EA})]/(\text{FRM-B})$$

Determination of Total Phenolic Compounds. The concentration of total phenolic compounds in the MeOH extracts was determined spectrophotometrically using the Folin−Ciocalteu reagent, following the method described by Julkunen-Tiito with few modifications (*31*). Briefly, 100 μL of crude extracts and the standard, previously dissolved in methanol, was diluted with water to 8 mL, 0.5 mL of Folin−Ciocalteu phenol reagent was added, and the flasks were shaken vigorously. After 8 min, 1.5 mL of 20% sodium carbonate solution was added, and the mixtures were mixed thoroughly again. The mixtures were allowed to stand for 1 h protected from light. The absorbance of the blue color produced was measured with a spectrophotometer (Perkin-Elmer Lamda 3) at 750 nm. The concentration of total phenolic compounds for each extract was calculated on the basis of a standard curve obtained using protocatechuic acid (six serial 2-fold dilutions to give a range of 0.02−0.002 mg/mL in triplicate).

Determination of Total Flavonoids. The determination of flavonoids was performed according to the colorimetric assay established by Lamaison and Carnat adapted to a microplate assay (*32*): 100 μL of each sample was added to a 96-microwell plate and then 100 μL of a 2% $AlCl_3$ solution in methanol. After 10 min, the absorbance was measured with an ELISA reader at 415 nm, a yellow color indicating the presence of flavonoids. A standard curve was developed using luteolin-7-*O*-β-glucoside isolated from *P. lagopus* (*27*) (six serial 2-fold dilutions to give a range 100−3.12 μg/mL; $n = 4$).

Determination of Total Phenylpropanoid Glycosides (PPGs). The determination of phenylpropanoids was determined according to a colorimetric method based on *o*-dihydroxycinnamic derivatives estimation, by the Arnow reaction, validated by the European Pharmacopeia (*33*). One milliliter of each sample was added to 2.0 mL of aqueous 0.5 M HCl, 2.0 mL of a 10% (w/v) aqueous solution of sodium nitrite and a 10% (w/v) aqueous solution of sodium molybdate (Arnow reagent), and 2.0 mL of aqueous 2 M NaOH. The solution was adjusted to 10.0 mL with water. After 10 min, the absorption was measured at 525 nm, a purple color indicating the presence of phenylpropanoids. The concentration was calculated on the basis of the standard curve of verbascoside isolated from *P. serraria* (six serial 2-fold dilutions to give a range of 20−0.31 μg/mL in triplicate).

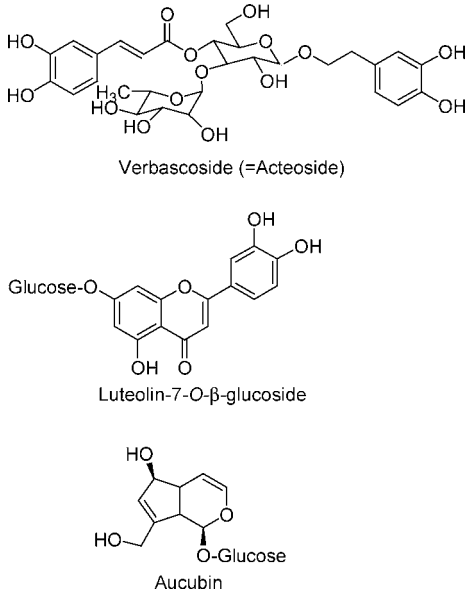

Figure 1. Structural formulas of the isolated compounds from *Plantago* species: the phenylpropanoid glycoside, verbascoside; the flavonoid, luteolin-7-*O*-β-glucoside; and the iridoid, aucubin.

Determination of Total Iridoids. The content of iridoids was determined according to a colorimetric method based on the Trim and Hill reaction (*34*). Briefly, 400 μL of each extract was mixed with 4.0 mL of Trim−Hill reagent (acetic acid/0.2% $CuSO_4$/HCl concentration 10:1:0.5). After the samples had been heated at 100 °C for 5 min, the absorption was read at 609 nm, a blue color indicating the presence of iridoids. The concentration was calculated on the basis of the standard curve of aucubin (Extrasynthese, Genay, France) (six serial 2-fold dilutions to give a range of 48.80−4.90 μg/mL in triplicate).

Isolation of Pure Compounds. Luteolin-7-*O*-β-glucoside was isolated from *P. lagopus* as described in ref *27*, and verbascoside was isolated from *P. serraria* (*35*) (**Figure 1**). The identity of the compounds was confirmed by comparing their spectroscopic data with literature values.

Statistical Analysis. The antioxidant activity of each sample for both assays was expressed as IC_{50} value, which is the concentration in micrograms per millilter or micromolar (extracts and compounds, respectively) that inhibited DPPH radical or MDA formation by 50%. Values were calculated from the corresponding log−dose inhibition curve using GraphPad Prism software.

The concentrations of phenolics, flavonoids, PPGs, and iridoids for each species were expressed as percentage in the extract (w/w). For antioxidant assays and extract composition determinations, all of the results were expressed as mean ± SD of three or four different trials. Analysis of variance (ANOVA) and the Student−Newman−Keuls' test were used to assess significant differences ($p < 0.05$) between extracts.

RESULTS AND DISCUSSION

Antioxidant Activity of Extracts. In the TLC−DPPH assay with MeOH extracts of *P. afra*, *P. coronopus*, *P. lagopus*, *P. lanceolata*, and *P. serraria*, some active components were detected as DPPH scavengers as zones with R_f values at 0.66 for *P. afra*, *P. coronopus*, and *P. serraria*; at 0.33 and 0.66 for *P. bellardii*; and at 0.55 and 0.66 for *P. lagopus* and *P. lanceolata*. These results directed the investigations in two ways: one to the study of the antioxidant activity of the extracts in two assays, DPPH scavenging and Fe^{2+}/ascorbate-induced LPO inhibition expressed through calculation of their IC_{50} value, and the other being the analysis of their chemical composition. A comparison of these two sets of results would enable some composition−activity relationship to be deduced.

Table 1. IC_{50} Values[a,b] of Scavenging Activity and Inhibition of Fe^{2+}/Ascorbate-Induced Lipid Peroxidation on Bovine Brain Liposomes by the Studied *Plantago* Methanol Extracts

	IC_{50} (μg/mL)	
methanol extract	DPPH scavenging activity	lipid peroxidation inhibition
P. afra	85.22 ± 7.56 a	152.21 ± 6.14 a
P. coronopus	47.43 ± 3.42 c	92.91 ± 9.03 c
P. lagopus	31.31 ± 5.55 b	103.09 ± 19.76 c
P. lanceolata	30.86 ± 4.66 b	24.69 ± 3.05 b
P. serraria	7.60 ± 0.46 d	29.84 ± 4.17 b

[a] The IC_{50} (μg/mL) values were calculated from the dose–response curves. [b] Values are means ± SD of three or four different determinations. Means within each column with different letters (a–d) differ significantly ($p < 0.05$). Positive controls used: for DPPH, ascorbic acid, with an $IC_{50} = 4.26$ μg/mL. In the case of LPO inhibition, propylgallate was tested at 10^{-4} M, inducing 85% inhibition.

The MeOH extracts of all investigated species demonstrated considerable scavenging of DPPH and inhibition of Fe^{2+}/ascorbate-induced LPO on bovine brain liposomes (**Table 1**).

In the case of the DPPH assay, when the IC_{50} values (expressed as micrograms per milliliter) were compared for the five extracts tested, *P. serraria* showed the highest scavenging activity ($p < 0.001$), with an IC_{50} value of 7.60 ± 0.46 μg/mL. Two other extracts, that is, *P. lagopus* and *P. lanceolata*, showed the next highest activity with no significant difference between them. The extracts with the weakest scavenging potency were *P. coronopus* and *P. afra*, which had significantly lower activities than the other three tested. Therefore, the scavenging activity of the extracts in decreasing order was *P. serraria* > *P. lagopus* ≥ *P. lanceolata* > *P. coronopus* > *P. afra*.

In the case of values of IC_{50} corresponding to the inhibition of Fe^{2+}/ascorbate-induced LPO, two extracts, *P. lanceolata* and *P. serraria*, possessed the highest activity, with no significant difference between them. As in the previous assay, a decreasing LPO inhibition activity order of the extracts could be established: *P. lanceolata* ≥ *P. serraria* > *P. coronopus* ≥ *P. lagopus* > *P. afra*, with all values significantly different at $p < 0.001$ except for *P. bellardii*, *P. lanceolata*, and *P. serraria*.

Determination of Total Phenolic Compounds. Because the extracts studied were obtained with methanol, the first approach to the characterization of each extract was to determine their amount of total phenolic compounds by the Folin−Ciocalteu assay. Each set of calibration standards, absorbance (AU) versus concentration, was fitted to a least-squares linear plot. All plots were found to be linear across the range assayed (12.00−2.00 μg/mL, $R^2 = 0.9981$). The calibration curve was used to calculate the percentage of phenolic content for each extract. **Table 2** presents the yield obtained for each extract as percentage w/w and the percentage of total phenolic content calculated for each extract. When the phenolic content of each extract was compared with the others, it could be observed that *P. serraria* MeOH extract was significantly higher ($p < 0.001$). The order obtained in relation with the total phenolic content for the MeOH extracts was (indicating the p value of significance with respect to the following sample) *P. serraria* ($p < 0.001$) > *P. lanceolata* ($p < 0.05$) > *P. coronopus* ($p < 0.01$) > *P. lagopus* ≥ *P. afra*.

Determination of Flavonoids. For a further specification of the phenolic compounds, the contents of flavonoids as well as PPGs were determined. Each set of calibration standards, absorbance (AU) versus concentration, was fitted to a least-squares linear plot. All plots were found to be linear across the range assayed (50.00−3.12 μg/mL, $R^2 = 0.9941$). Each calibra-

Table 2. Extract Yield, Total Phenolic Compounds (Measured by Folin–Ciocalteu Assay), Flavonoids (Measured by $AlCl_3$ Reagent), Phenylpropanoid Glycosides (PPGs) (Measured by Arnow Reagent), and Iridoids (Measured by Trim–Hill Reagent) of Methanol Extracts of the Studied *Plantago* Species Expressed as Percentage in Extracts[a]

methanol extract	extract yield (%)	total phenolic compounds (%)	flavonoids (%)	PPGs (%)	iridoids (%)
P. afra	18.04	4.84 ± 0.39 a	1.12± 0.05 a	2.15 ± 0.03 a	0.95 ± 0.06 a
P. coronopus	21.32	7.11 ± 0.37 c	1.05 ± 0.04 a	2.45 ± 0.20 b	nd
P. lagopus	19.46	5.44 ± 0.32 a	3.09 ± 0.08 b	1.38 ± 0.03 c	0.83 ± 0.01 b
P. lanceolata	29.13	8.16 ± 0.45 b	1.24 ± 0.09 c	2.18 ± 0.06 a	traces
P. serraria	24.17	16.83 ± 0.88 d	0.69 ± 0.09 d	7.17 ± 0.85 d	traces

[a] Values are means ± SD of three or four different determinations. Means within each column with different letters (a–e) differ significantly ($p < 0.05$). nd, not detected.

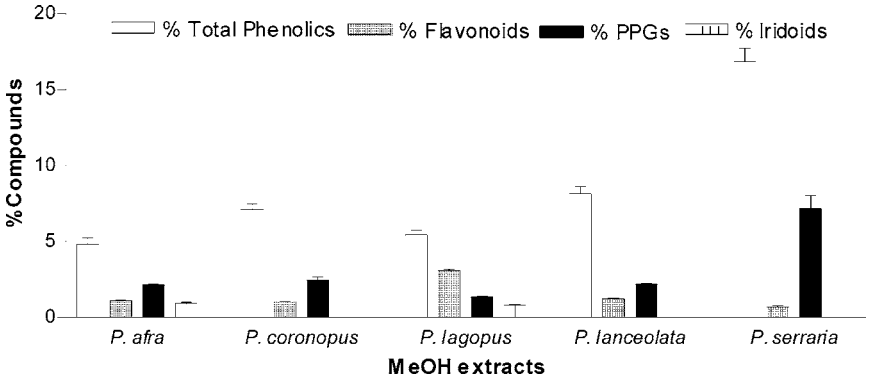

Figure 2. Percentage of total phenolic compounds, flavonoids, phenylpropanoid glycosides (PPGs), and iridoids calculated for the MeOH extracts by colorimetric techniques: Folin–Ciocalteu, $AlCl_3$, Arnow, and Trim–Hill reagents, respectively. Data are represented as mean ± SD ($n = 3$).

tion curve was used to calculate the percentage total flavonoid content for each extract (**Table 2**).

The MeOH extract richest in flavonoids was that of *P. lagopus*, highly significant compared to the others ($p < 0.001$), whereas the MeOH extract of *P. serraria* had the lowest flavonoid content. The decreasing order of flavonoid content of the MeOH extracts was *P lagopus* ($p < 0.001$) > *P. lanceolata* ($p < 0.05$), *P. afra* ≥ *P. coronopus* ($p < 0.001$) ≥ *P. serraria*.

Determination of Phenylpropanoid Glycosides. The quantification of PPGs was developed using Arnow reagent and verbascoside as standard. Each set of calibration standards, absorbance (AU) versus concentration, was fitted to a least-squares linear plot. All plots were found to be linear across the range assayed (10.00−0.31 μg/mL, $R^2 = 0.9963$). The calibration curve was used to calculate the percentage of PPGs content for each extract (**Table 2**). A decreasing order of PPG content was established for the samples, being *P. serraria* ($p < 0.001$) > *P. coronopus* ($p < 0.05$) > *P. lanceolata* ≥ *P. afra* ($p < 0.05$) ≥ *P. lagopus*.

Determination of Iridoids. The quantification of iridoids was developed using Trim−Hill reagent and aucubin as standard. Each set of calibration standards, absorbance (AU) versus concentration, was fitted to a least-squares linear plot. All plots were found to be linear across the range assayed (39.00−4.90 μg/mL, $R^2 = 0.996$). The calibration curve was used to calculate the percentage of iridoids for each extract (**Table 2**). A decreasing order of iridoid content was established for the samples as *P. afra* ≥ *P. lagopus* Only traces of iridoids were detected in *P. lanceolata* and *P. serraria*, whereas iridoids were not detected at all in *P. coronopus*.

Figure 2 summarizes the total phenolic, flavonoid, PPG, and iridoid contents for each MeOH extract for an easier appreciation of the obtained values. As can be observed in this figure, the phenolic compounds mainly comprise flavonoids and PPGs. It can be also observed that *Plantago* species have a much higher concentration of phenolic compounds than iridoids.

Table 3. Correlation Factors between Paramaters Describing the Amount of Phenolic Compounds, Flavonoids, Phenylpropanoid Glycosides (PPGs), and Iridoids and the Two Methods Describing the Antioxidant Activity of the Methanol Extracts

	DPPH scavenging	LPO inhibition
total phenolics	0.9469	0.6368
flavonoids	0.4892	0.2731
PPGs	0.9139	0.2544
iridoids	0.2634	0.5037

It is important to note the result calculated for *P. serraria* extract, because it is richest in PPGs, and, moreover, it possess a higher PPG concentration than those described previously for other species, such *P. lanceolata* (*36*). Due to this high concentration, *P. serraria* might become a promising source of PPGs. It has been shown that the content of verbascoside in *P. serraria* is at its highest when the plant reaches maturity (*9*).

After these analyses of the antioxidative activities and the major components of the MeOH extracts, the relationship between the antioxidant potency (expressed as the reciprocal of the calculated IC_{50} for both assays) and each kind of compound calculated above was compared (**Table 3**). A tendency was detected between the scavenging potency and the total phenolic content ($R^2 = 0.9465$) (**Figure 3**) and the content of PPGs ($R^2 = 0.895$) in the extracts (**Figure 4**). This correlation suggests that PPG content may be responsible for a significant part of the antioxidant effect of the MeOH extracts of *Plantago* species. However, no similar relationship was observed for the total flavonoid or iridoid content of the extracts. This lack of relationship between the antioxidant potency and the flavonoid content is in agreement with other literature (*37*, *38*). In the case of inhibition of LPO potency, no relationship was detected

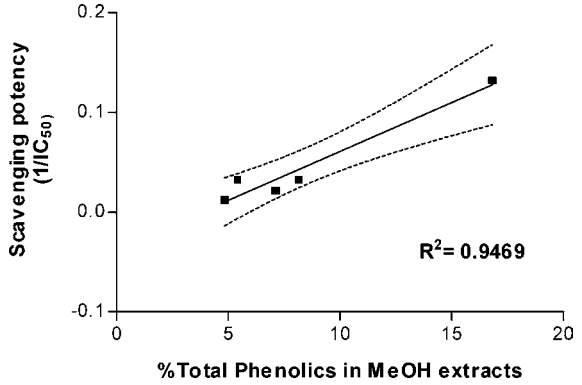

Figure 3. Relationship between the scavenging potency of the studied *Plantago* MeOH extracts and their total phenolic compounds content.

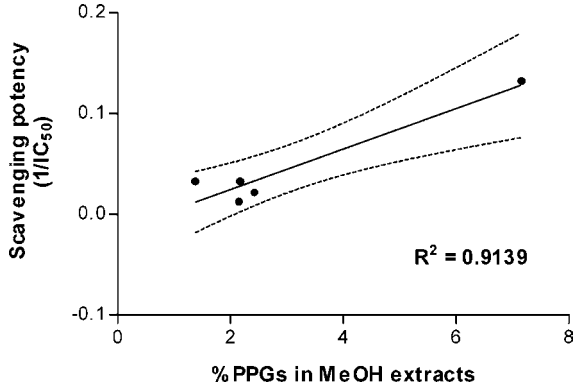

Figure 4. Relationship between the scavenging potency of the studied *Plantago* MeOH extracts and their total phenylpropanoid glycosides (PPGs) content.

Table 4. IC_{50} Values[a,b] of Scavenging Activity Induced by the Isolated Compounds from the Studied *Plantago* Methanol Extracts

compound	IC_{50} (μM) for DPPH scavenging activity
luteolin-7-*O*-β-glucoside	8.98 ± 1.40 a
verbascoside	11.52 ± 1.60 a
aucubin	na
ascorbic acid	24.19 ± 4.21 b

[a] The IC_{50} (μg/mL) values were calculated from the dose–response curves. [b] Values are means ± SD of three or four different determinations. Means within each column with different letters differ significantly ($p < 0.001$). na, not active at the assayed doses (>100 μM).

with any kind of compound (**Table 3**). Further analysis to find out if other substances not measured or detected might be responsible will be performed.

In addition, the scavenging activity of these compounds (verbascoside, luteolin-7-*O*-β-glucoside, and aucubin) against DPPH was calculated as their IC_{50} (micromolar). The results showed that luteolin-7-*O*-β-glucoside and verbascoside are at least as active as the positive control, ascorbic acid ($p < 0.001$), but there was no significant difference between them, whereas the iridoid, aucubin, did not shown any activity at the doses used (<100 μM) (**Table 4**). These results are in agreement with previous reports, where PPGs are described as potent antioxidant agents (*39*−*41*), as well as flavonoidal compounds (*42*, *43*), due to their catechol groups.

With regard to LPO inhibition, luteolin-7-*O*-β-glucoside and verbascoside are known to have powerful activity (*41*, *44*−*46*).

The results of this study on the MeOH extracts indicate that they might prevent ROS attack on biomolecules such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins, and sugars in biological and food systems. Hence, these extracts, especially that from *P. serraria*, could possess therapeutic effects, arising from their antioxidant activity, in areas such as tumors, inflammatory diseases, and cardiovascular protection. Some epidemiological studies have associated the high intake of fruits and vegetables, which are rich sources of polyphenols, with a lower incidence of some forms of cancer or cardiovascular diseases due to the antioxidant activity of the polyphenols present (*47*−*49*).

Because of this high antioxidant activity, animals fed with these species, not only with *P. lanceolata*, may have an enhancement of their physiological condition. *Plantago* species have also immunostimulating properties (*50*, *51*), helping the animal's defense system, and therefore fewer antibiotic growth promoters might be needed.

The high scavenging activity of these extracts could be due to the mixture in their composition of flavonoids and verbascoside, compounds that may exert a synergistic effect. Further studies to establish this fact will be performed, but, if this hypothesis were true, it would provide even more importance to *Plantago* species as a fodder material, because only one other food source that contains verbascoside and luteolin-7-*O*-β-glucoside has been reported, the olive fruit (*52*).

This work demonstrates that *Plantago* species should be considered as a useful source of material for human health, as an antioxidant food preservative, and as an alternative to *P. lanceolata* in animal feed.

ABBREVIATIONS USED

BHA, butylated hydroxyanisole; BHT, butylated hydrxytoluene; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ESCOP, European Scientific Cooperative on Phytotherapy; LPO, lipid peroxidation; MDA, malondialdehyde, MeOH, methanol; PBS, phosphate-buffered saline; PPGs, phenylpropanoid glycosides or phenylpropanoid esters; ROS, reactive oxygen species, also named free radicals; TBA, thiobarbituric acid; TLC, thin-layer chromatography.

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