**Original Article**

# Tyrosinase Inhibitory and Antioxidant Activity of Wild *Prunus spinosa* L. Fruit Extracts as Natural Source of Bioactive Compounds

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## Abstract

In recent years, naturally derived tyrosinase inhibitors and antioxidants have become very important, since the synthetic ones are related with several side effects. As wild fruits are rich source of bioactive compounds, this study focused on the examination of *Prunus spinosa* L. (blackthorn) fruit extracts, widely distributed in our country. However, blackthorn extracts are not enough investigated, and there are only a few published works. Extraction efficiency of *P. spinosa* ultrasonic fruit extracts obtained by using different solvents (methanol, 70% (v/v) ethanol, 45% (w/w) propylene glycol and water) was evaluated, regarding polyphenolic profile, tyrosinase inhibitory and antioxidant activity. Results suggested that extracts with 45% propylene glycol and water were particularly rich in polyphenolic compounds (especially phenols, flavonoids and anthocyanins). The highest extraction yield was for 45% propylene glycol extract. This extract showed higher tyrosinase inhibitory and antioxidant activity compared to others, while the methanolic extracts had the highest chelating capacity. Therefore, *P. spinosa* fruit extracts, especially extracts obtained by ultrasonic extraction with 45% propylene glycol, have a great potential to be incorporated in some pharmaceutical formulations for obtaining tyrosinase inhibitory and antioxidant effects.

*Keywords:* anti-tyrosinase activity; blackthorn; free radicals; polyphenols; ultrasonic extraction

## Introduction

Free radical species (especially reactive oxygen and reactive nitrogen species) generation is caused by harmful exogenous factors of the environment, but they are also produced as the result of normal biochemical reactions in the human body. Free radical overproduction and excessive accumulation increase incidence of damaging macromolecules in cells by oxidative stress mechanism (Temple, 2000; Valko *et al.*, 2007). Since the oxidative stress prevention has become very important, there is considerable interest in natural sources of antioxidants for maintaining cellular homeostasis with the aim to replace the synthetic ones which are related with several side effects (Ratnam *et al.*, 2006). Nothing is more important than the use of naturally derived tyrosinase inhibitors in melanogenesis disorders, skin hyperpigmentation and other melanin-related problems, but also to prevent the enzymatic browning in fruits caused by enzymatic oxidation process (Maisuthisakul and Gordon, 2009). Synthetic tyrosinase inhibitors (hydroquinone, arbutin or kojic acid) are associated with various adverse effects (Ebanks *et al.*, 2009). Plants have a long history of applications in traditional medicine due to their availability and wide diversity of active phytochemical compounds. Usage of plants for medicinal purposes is an integral part of our cultural life and thus is unlikely to change in the years to come (Sun *et al.*, 2002). There are confirmed reports that different fruits are a rich source of antioxidants, with large amounts of phenolic compounds, vitamins, fibers and minerals and therefore are involved in the prevention of various chronic diseases and disorders (Temple, 2000; Szajdek and Borowska, 2008; Nile and Park, 2014).

Consequently, this paper focuses on the examination of *Prunus spinosa* L., commonly known as blackthorn. It is a wild species of the Rosaceae family that is distributed in various regions in Serbia. Ethnobotanical studies have

reported that the fruit of *P. spinosa* has been used as an astringent, diuretic and purgative (Fraternale *et al.*, 2009; Barros *et al.*, 2010). Aqueous fruit extracts showed antibacterial effects (Gegiu *et al.*, 2015). Wild *P. spinosa* fruits are a rich source of sugars, organic acids, carotenoids, tocopherols, chlorophylls, phenolic compounds, fatty acids (Morales *et al.*, 2013; Mikulic-Petkovsek *et al.*, 2016). In our country, this fruit is processed into juice, beverages, frozen products, jams, wine, etc. To our knowledge, this is the first demonstration of tyrosinase inhibitory and metal-chelating potential of examined fruit extracts, while data about the antioxidant properties regarding free radicals scavenging activity of extracts are limited. Hence, an attempt has been made to examine extraction efficiency of *P. spinosa* ultrasonic fruit extracts obtained by using different solvents, regarding polyphenolic profile, tyrosinase inhibitory and antioxidant activity. To evaluate antioxidant activity, several *in vitro* techniques were applied (some of them for the first time in this paper), such as radical scavenging assays against DPPH, nitric oxide and hydroxyl radicals, FRAP assay, assay of inhibition of β-carotene bleaching and metal-chelating activity.

## Materials and Methods

### Biological material

Fruits of *Prunus spinosa* L. were collected in October 2015, from plants growing wild on the locality of Vlasina (Natural Park), Serbia. Plant material was identified and the voucher specimen (No. 2221PS) has been deposited at the herbarium of the Institute for medicinal plant research 'Dr Josif Pančić', Serbia. Well maturated fruits were harvested randomly and stored at -20 °C until analyzed.

### Chemicals and reagents

Folin-Ciocalteu reagent, hide powder, sodium nitroprusside, pyrogallol, catechin, ethylenediamine-tetraacetic acid (EDTA), mannitol, L-ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris-(2-pyridyl)-s-triazine (TPTZ), β-carotene, linoleic acid, Tween 20 (polyoxyethylenesorbitan monolaurate), dimethyl sulfoxide (DMSO), L-3,4-dihydroxyphenyl-alanine (L-DOPA), kojic acid, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p′-disulfonic acid monosodium salt hydrate (ferrozine) and methanol were supplied by Sigma-Aldrich (Germany). Tyrosinase from mushroom was the product of Sigma-Aldrich (Germany). The specific activity of the enzyme was 5771 units mg$^{-1}$. Griess reagent, gallic acid, rutin trihydrate and ethylenediaminetetraacetic acid disodium salt dihydrate (Na$_2$EDTA × 2H$_2$O) were from Fluka Analytical (Switzerland). Vanillin and 2-deoxy-2-ribose were purchased from Carl Roth (Germany). Thiobarbituric acid (TBA) was obtained from abcr (Germany). 3,5-Di-tert-4-butylhydroxytoluene (BHT) was purchased from Supelco Analytical (USA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) from Acros Organics (Denmark) and chloroform from VWR International (USA). Sodium carbonate, aluminium chloride, potassium acetate, trichloroacetic acid (TCA) and ferrous sulfate heptahydrate (FeSO$_4$ × 7H$_2$O) were purchased from Centrohem (Serbia). All other chemicals and solvents used were of analytical grade.

### Preparation of extracts and physicochemical characterization

Fresh fruits were mechanically homogenized and subjected to ultrasonic extraction. Fifty grams of the fruits were extracted with four different solvents (D : E = 1 : 5, w/w), including methanol (methanolic extracts - MEs), 70% (v/v) ethanol (ethanolic extracts - EEs), 45% (w/w) propylene glycol (propylene glycolic extracts - PEs) and water (water extracts - WEs), in an ultrasonic bath under maximum operating conditions, at temperature 22 ± 1 °C, for 30 min. After filtration, extracts were evaporated to dryness *in vacuo* at 40 °C to avoid any loss or degradation of phytochemicals. The extracts are labelled as shown in Table 1. Extraction yield (% w/w) was expressed as the amount of dry extract obtained from 100 g of the fresh plant material weight (fw). Measurements of refractive index (RI), relative density (RD) and pH values were based on the official procedures explained in the European Pharmacopoeia 9$^{th}$ edition (European Pharmacopoeia, 2017).

### Determination of polyphenolic content

Total phenolic content (TPC) was measured by the modified Folin-Ciocalteu method (Singleton *et al.*, 1999). Briefly, 50 μL of each extract solution was mixed with 250 μL of Folin-Ciocalteu reagent. The mixture was vortexed, then 750 μL of 20% (w/v) aqueous sodium carbonate solution was added and the volume was made up to 5 mL with distilled water. After 2 h of incubation at room temperature, the absorbance was measured at 760 nm. A calibration curve was constructed using gallic acid standard solutions (0.015 to 0.150 mg mL$^{-1}$). The results were expressed as mg of gallic acid equivalents (GAE) per g of dry plant material (dw).

Total flavonoid content (TFC) was assayed by aluminium chloride colorimetric method (Woisky and Salatino, 1998), with minor modifications. Briefly, a volume of 0.5 mL of extracts were mixed with 1.5 mL of methanol, 100 μL of 10% (w/v) aluminium chloride solution, 100 μL of potassium acetate solution (1.0 M) and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 min and absorbance was measured at 425 nm. Rutin solutions (0.05-0.50 mg mL$^{-1}$) were used for construction of the standard curve. The results were expressed as mg of rutin equivalents (RE) per g of dw.

Determination of proanthocyanidins was based on the vanillin assay (Price *et al.*, 1978). The absorbance was measured at 500 nm. Standard curve was constructed using the catechin solutions (0.03-0.30 mg mL$^{-1}$). Total content of proanthocyanidin (TPAC) was expressed as mg of catechin equivalent (CE) per g of dw. Official methods from the European Pharmacopoeia 9$^{th}$ edition (European Pharmacopoeia, 2017) was used for determination of total tannins and anthocyanins content. The total tannin content (TTC) was expressed as pyrogallol (% w/w). Total anthocyanins content (TAC) was expressed as mg cyanidin-3-glucoside chloride (CG) per 100 g of dw.

*Tyrosinase inhibitory activity assay*

The assay of the tyrosinase inhibitory activity (TIA) was performed as previously described (Fu *et al*., 2005), with some adjustments. Mushroom tyrosinase was used, with L-DOPA as the substrate. The diphenolase activity of mushroom tyrosinase was monitored at 475 nm. Briefly, 40 μL of 10 mM L-DOPA and 100 μL of 50 mM sodium phosphate buffer (pH 6.8) were inserted in a 96-well plate and incubated at 30 °C for 5 min. Then, 100 μL of various concentrations of extracts dissolved in DMSO followed by 30 μL of the aqueous solution of mushroom tyrosinase (217 units mL$^{-1}$) were added to the mixture and incubated at 30 °C for 25 min. Enzymatic activity was quantified by measuring the absorbance at 475 nm. Negative controls, without inhibitor, but containing 3.3% DMSO were also determined. The percentage of tyrosinase inhibition was obtained by equation 1:

$$Percentage\,(\%) = \frac{Acontrol - Asample}{Acontrol} \times 100$$

*Antioxidant activity assays*

The DPPH scavenging assay was done according to the method reported by Brand-Williams *et al*. (1995), with some changes. Briefly, an aliquot of extract (100 μL) was mixed with 2.9 mL of methanol and 1.0 mL of freshly prepared DPPH working solution (90 μM). The colorimetric decrease in absorbance was measured at 517 nm.

Nitric oxide radical scavenging (NOS) activity was measured according to the modified method described by Rao (1997). About 1 mL of sodium nitroprusside (10 mM) in phosphate buffer (0.1 M, pH 7.4) was mixed with different concentrations of extract or standard solutions in phosphate buffer. After incubation at 25 °C for 120 min 0.5 mL of mixture was pipetted and 0.5 mL of Griess reagent was added. The absorbance of the chromophore formed was measured spectrophotometrically at 546 nm.

The ability of extracts to inhibit non-site specific hydroxyl radical scavenging (HRS_NS) activity was estimated according to the method of Halliwell *et al*. (1987), slightly modified. The reaction mixture (in a final volume of 1 mL) contained 100 μL of extract or standard solutions, 500 μL of 2-deoxy-D-ribose (5.6 mM) in phosphate buffer (50 mM, pH 7.4), 200 μL of pre-mixed solution of 100 μM FeCl$_3$ and 104 μM EDTA (1:1 v/v), 100 μL of H$_2$O$_2$ (1.0 mM) and 100 μL of L-ascorbic acid (1.0 mM). After incubation at 50 °C for 30 min, 1 mL of 2.8% TCA and 1 mL of 1.0% TBA were added and the mixture was incubated again under the same conditions. The absorbance was measured at 532 nm. The ability of extracts to inhibit site-specific hydroxyl radical scavenging (HRS_SS) activity was done according to the procedure described for the HRS_NS assay, except that EDTA was replaced by phosphate buffer (Aruoma *et al*., 1987).

The ferrous ion-chelating (FIC) activity of extracts was estimated using the method of Dinis *et al*. (1994), with some modifications. Briefly, an aliquot (150 μL) of extract was mixed with 50 μL of FeSO$_4$ × 7H$_2$O (2.0 mM) in a 96-well plate and incubated for 5 min at room temperature. The reaction was initiated by the addition of 50 μL of ferrozine (5.0 mM). Reaction mixture was shaken vigorously and left to stand at room temperature for 10 min and then the absorbance was measured at 562 nm.

The scavenging activities obtained by described assays (DPPH, NOS, HRS_NS and HRS_SS) and FIC activity of the extracts were calculated as percentage of inhibition (%) using the equation 1.

In addition, the antioxidant activity was evaluated by the modified β-carotene bleaching (BCB) assay (Koleva *et al*., 2002). Shortly, 1 mL of β-carotene solution in chloroform (0.2 mg mL$^{-1}$), 25 μL of linoleic acid and 200 mg of Tween 20 emulsifier were transferred to a round-bottomed flask. The chloroform was then evaporated under a nitrogen stream. Oxygenated ultrapure water (50 mL) was added into the flask and the obtained mixture was shaken vigorously. Further, 200 μL of the β-carotene–linoleic acid emulsion was added to the 25 μL of extracts in each well. The plates were shaken at 300 rpm for 5 min and incubated at 55 °C in the laboratory incubator. The absorbance was read at 450 nm. BCB inhibition was calculated as a percentage of inhibition (%) according to equations 2 and 3:

$$BCB\,(\%) = \frac{Rcontrol - Rsample}{Rcontrol} \times 100$$

$$R = \frac{\ln\left[At_0 / At_{120}\right]}{120}$$

where R= bleaching rate of β-carotene; ln = natural log; $At_0$ = the initial absorbance measured at t = 0 min and $At_{120}$ = the absorbance measured after t = 120 min. The concentration of extract providing 50% of β-carotene bleaching inhibition, IC$_{50}$ (μg mL$^{-1}$), was calculated.

The ferric reducing antioxidant power (FRAP) assay was used to estimate total antioxidant activity of extracts (Benzie and Strain, 1996). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ solution in 40 mM HCl and 20 mM FeCl$_3$ × 6H$_2$O solution (10:1:1, v/v/v). An aliquot of 100 μL of extract solution was mixed with 3.0 mL of FRAP reagent. The absorbance readings were taken at 593 nm, after 10 min of incubation at room temperature. Aqueous solutions of FeSO$_4$ × 7H$_2$O (100-1000 μM) were used for calibration. The FRAP value was expressed as μmol Fe$^{2+}$ equivalents per g of dw.

*Statistical analysis*

Data of all measurements obtained in triplicate are presented as mean values ± standard deviation (SD). All statistical analyses were performed using the statistical program SPSS, version 25.0. Analysis of variance (ANOVA) was followed by Tukey's test for the determination of significant differences. The TIA activity, the quality of the radical scavenging property and the FIC activity of the extracts were determined by calculating the IC$_{50}$ (μg mL$^{-1}$) values (concentration of each extract required to inhibit 50% of enzymatic activity/to scavenge 50% of free radicals/to chelate 50% of the ferrous ions).

**Results and Discussion**

*Ultrasonic extraction efficiency and polyphenolic content of extracts*

The extraction method is a very important step for

further qualitative and quantitative analysis of the obtained extracts. One of the main factors influencing the extraction process is certainly the choice of extraction solvents. Yields reported for ultrasonic extraction and physicochemical properties of *P. spinosa* fruit extracts are given in Table 1. The ultrasonic extraction with 45% propylene glycol resulted in the highest extraction yield. Extraction yields were lower when methanol and 70% ethanol were employed, while the water extraction capacity was the lowest. To the best of our knowledge, there are no previous reports on physicochemical properties of examined extracts. WE and PEs showed lower pH value compared to extracts with other solvents. In current scientific literature, pH values of whole fresh *P. spinosa* fruits varied between 3.43 and 3.92 (Ganhão *et al.*, 2010; Morales *et al.*, 2013). All extracts showed statistically significant differences in pH values. RI and RD values varied due to the usage of different extraction solvents. PEs demonstrated the highest RI and RD values, as it was expected. Physicochemical characterization is important for the quality evaluation of extracts, for their standardized production and application in the pharmaceutical industry. It can be observed that the solvent used in the extraction affected the examined bioactivity of the extracts, by its effect on the content of the phenolic compounds.

Polyphenolic content of extracts is presented in Table 2. TPC was higher for PSPE and PSWE extracts, more than three times higher than the content in PSEE sample, which was found to have the smallest amount of phenolics. The current scientific literature gave us a wide range of data when it comes to the TPC of examined fruits, from 1.34 to 4.73 mg GAE g$^{-1}$ (Jabłońska-Ryś *et al.*, 2009; Ganhão *et al.*, 2010), due to the different extraction procedures, plant genotype and differences in the fruits' maturity stage. The other researchers reported higher TPC in WEs compared to the EE and MEs, which is in agreement with this paper

findings (Ganhão *et al.*, 2010). More polar solvents, like 45% propylene glycol and water, extracted higher amounts of phenolics from *P. spinosa* extracts. Similar results were found for TFC, where samples PSWE and PSPE had several times higher content of flavonoids (up to 85% higher) in comparison to extract PSME. According to Velickovic *et al.* (2016), TFC for *P. spinosa* WEs was 0.42 mg g$^{-1}$, while the EEs had TFC of 0.70 mg g$^{-1}$. Barros *et al.* (2010) showed higher TFC for MEs (8.68 mg g$^{-1}$) in comparison to results from this study.

It can be observed that samples PSEE and PSPE showed similar percentage of tannins, while sample PSEE had significantly higher concentration of proanthocyanidins (1.7 to 4.2 times higher) than extracts with other solvents. Literature data related to TTC and TPAC in investigated extracts are very scarce. According to Ganhão *et al.* (2010) *P. spinosa* extracts of fruits (without seeds) contained 5.88 mg g$^{-1}$ of proanthocaynidins, although different extraction procedure and method of analysis were used. The specific color of the *P. spinosa* fruits indicated high content of TAC in them (Espín *et al.*, 2000), as confirmed in the present study. Samples PSWE and PSPE were distinguished by the high content of anthocyanins (413.06-517.23 mg/100 g). The obtained results for TAC were much higher from those of other researchers from Serbia, which were 11 mg/100 g for EEs and 12 mg/100 g for WEs (Velickovic *et al.*, 2016). On the other hand, Sikora *et al.* (2013) recorded TAC of *P. spinosa* fruit MEs originated from Poland of 415.04 mg/100 g, which is similar with our findings.

Based on the results of polyphenolic content analysis and extraction yield, 45% propylene glycol was the most efficient solvent for ultrasonic extraction of bioactive compounds from *P. spinosa* wild fruits. Results indicated that proanthocyanidins, anthocyanins and flavonoids are the main groups of phenolic compounds.

Table 1. Extraction efficiency and physicochemical characterization of investigated *P. spinosa* extracts[*]

| Extracts | Solvent | Extraction yield (% fw[#]) | pH value | Refractive index | Relative density (g cm$^{-3}$) |
|---|---|---|---|---|---|
| PSME | methanol | 23.32 ± 0.03[a] | 6.79 ± 0.03[a] | 1.336 ± 0.004[a] | 0.830 ± 0.036[a] |
| PSEE | 70% (v/v) ethanol | 27.69 ± 0.05[b] | 5.24 ± 0.04[b] | 1.362 ± 0.003[b] | 0.943 ± 0.042[a,b] |
| PSPE | 45% (w/w) propylene glycol | 31.82 ± 0.06[c] | 4.86 ± 0.01[c] | 1.383 ± 0.002[c] | 1.037 ± 0.022[b] |
| PSWE | water | 19.51 ± 0.03[d] | 4.08 ± 0.03[d] | 1.340 ± 0.001[a] | 1.013 ± 0.027[b] |

Note: [*] The presented values are mean ± standard deviation of three measurements (n = 3). In each column different letters mean significant differences (p < 0.01); [#] calculated on the basis of fresh plant material weight (fw).

Table 2. Polyphenolic profile of *P. spinosa* fruit extracts. TPC - total phenolic content; TFC - total flavonoid content; TTC - total tannin content; TPAC - total proanthocyanidin content; TAC - total anthocyanin content[*]

| Extracts | TPC (mg GAE g$^{-1}$) | TFC (mg RE g$^{-1}$) | TTC (%) | TPAC (mg CE g$^{-1}$) | TAC (mg CG/100 g) |
|---|---|---|---|---|---|
| PSME | 0.55 ± 0.18[a] | 0.50 ± 0.03[a] | 0.16 ± 0.02[a] | 12.86 ± 0.87[a] | 347.87 ± 35.85[a] |
| PSEE | 0.46 ± 0.16[a] | 2.03 ± 0.05[b] | 0.27 ± 0.05[a,b] | 22.10 ± 0.41[b] | 197.26 ± 43.80[a,b] |
| PSPE | 1.82 ± 0.14[b] | 2.62 ± 0.06[c] | 0.23 ± 0.02[a,b] | 6.81 ± 0.16[c] | 413.06 ± 53.32[a,c] |
| PSWE | 1.51 ± 0.19[b] | 3.29 ± 0.08[d] | 0.11 ± 0.01[a,c] | 5.29 ± 0.14[c] | 517.23 ± 59.88[a,c] |

Note: [*] Results are expressed as mean ± standard deviation (n = 3), calculated on the basis of dry plant material weight (dw); GAE - gallic acid equivalents; RE - rutin equivalents; CE - catechin equivalents; CG - cyanidin-3-glucoside chloride. In each column different letters mean significant differences (p < 0.01).

*Tyrosinase inhibitory and antioxidant activity of extracts*

The assessment of tyrosinase inhibitory potential of investigated fruits provided valuable information about the possible use of the extracts as a source of anti-tyrosinase agents, for application against different disorders accompanied with melanin overproduction. Table 3 shows the TIA (for the first time, to the best of our knowledge) and one part of the antioxidant activity of ultrasonic fruit extracts obtained using different extraction solvents. The second part of antioxidant activity evaluation was presented in Table 4. As indicated in Table 3, all samples proved to have anti-tyrosinase activity, being the most significant for sample PSPE (the lowest $IC_{50,TIA}$ values), followed by PSME. Sample PSWE demonstrated approximately two times lower anti-tyrosinase activity.

To evaluate antioxidant activity, several *in vitro* techniques were applied (some of them for the first time in this paper), such as radical scavenging assays against DPPH, nitric oxide and hydroxyl radicals, FRAP assay, assay of inhibition of β-carotene bleaching and metal-chelating activity. The scavenging of free radicals by the extracts was evaluated by measuring scavenging of different types of radicals: DPPH radicals, nitric oxide and hydroxyl radicals and compared to reference compounds. The PEs of *P. spinosa* fruits demonstrated the highest free-radical-scavenging activity evaluated by DPPH, NOS and HRS_NS assays (Table 3), while MEs showed better HRS_SS and FIC activity (Table 4). A moderate FIC activity of all extracts was observed in comparison to the standard Na₂EDTA. The other extracts were less effective in scavenging free radicals. It is worth to mention that HRS_NS and HRS_SS activities of extracts were higher than those reported for positive control. However, little evidence is available about radical scavenging capacity of investigated extracts. $IC_{50,DPPH}$ values obtained in previous

studies for *P. spinosa* phenolic extracts were from 64.98 to 597.5 µg mL⁻¹ (Barros *et al.*, 2010; Guimarães *et al.*, 2014). Pinacho *et al.* (2015) reported higher DPPH scavenging activity of ethanolic *P. spinosa* extracts compared to extracts with water, which is in line with our study, although extracts were obtained by different extraction technique (maceration). Egea *et al.* (2010) investigated scavenging hydroxyl radical capacity of wild *P. spinosa* fruits from Spain which was 52.25%. Measurements showed that the PSPE sample had the highest BCB and FRAP activity (Table 4), which is consistent with the amount of polyphenols reported in present study. The $IC_{50,BCB}$ values reported in the literature for *P. spinosa* fruit phenolic extracts ranged from 641.11 to 986.9 µg mL⁻¹ (Barros *et al.*, 2010; Guimarães *et al.*, 2014), while FRAP value was 141.7 µmol Fe²⁺ g⁻¹ (Jabłońska-Ryś *et al.*, 2009), which is in line with our results.

We can speculate that *P. spinosa* extracts have high tyrosinase inhibitory and antioxidant potential. As can be seen, the tyrosinase inhibitory and antioxidant activity of extracts is closely related to the content of the polyphenolic components. Results suggest that PE of *P. spinosa* fruits is the best source of antioxidants and polyphenolic substances that may inhibit the oxidative stress damaging effects on cells and aging process, as well. *P. spinosa* fruit extracts have high potential for prevention of oxidant/antioxidant disbalance, melanin-related disorders and could be considered in the formulation of dietary supplements and pharmaceutical formulations. Our study results show that the 45% propylene glycolic extracts from *Prunus spinosa* L. fruits obtained by ultrasonic extraction could be incorporated in some pharmaceutical formulas for obtaining tyrosinase inhibitory and antioxidant effects.

Table 3. Tyrosinase inhibitory activity (TIA) and antioxidant activity of *P. spinosa* extracts and positive controls: antioxidant activity against DPPH radicals, scavenging of nitric oxide radicals (NOS), nonsite-specific hydroxyl radical scavenging activity (HRS_NS)*

| Extract/standard | $IC_{50}$ (µg mL⁻¹) | | | |
| --- | --- | --- | --- | --- |
| | TIA | DPPH | NOS | HRS_NS |
| PSME | 636.51 ± 1.61[a] | 525.28 ± 1.98[a] | 797.29 ± 4.14[a] | 763.54 ± 3.45[a] |
| PSEE | 865.68 ± 1.41[b] | 262.94 ± 1.01[b] | 1,041.45 ± 4.98[b] | 652.88 ± 2.98[b] |
| PSPE | 526.65 ± 1.06[c] | 181.74 ± 0.32[c] | 648.93 ± 3.19[c] | 450.26 ± 1.73[c] |
| PSWE | 982.40 ± 1.85[d] | 493.28 ± 1.30[d] | 1597.09 ± 3.99[d] | 637.72 ± 2.38[d] |
| Kojic acid | 101.90 ± 1.08 | nd | nd | nd |
| L-ascorbic acid | 164.63 ± 1.97 | nd | 164.54 ± 2.09 | nd |
| Mannitol | nd | nd | nd | 580.79 ± 4.37 |
| BHT | nd | 8.20 ± 0.04 | nd | nd |
| Trolox | nd | 1.75 ± 0.01 | nd | nd |

Note: * Results are expressed as mean ± standard deviation (n = 3), calculated on the basis of dry plant material weight (dw). In each column different letters mean significant differences (p < 0.01); nd - not determined.

Table 4. Antioxidant activity of *P. spinosa* extracts and positive controls: site-specific hydroxyl radical scavenging activity (HRS_SS), β-carotene bleaching assay (BCB), ferrous ion chelating activity (FIC) and ferric reducing antioxidant power (FRAP)*

| Extract/standard | $IC_{50}$ (µg mL⁻¹) | | | µmol Fe²⁺ g⁻¹ |
| --- | --- | --- | --- | --- |
| | HRS_SS | BCB | FIC | FRAP |
| PSME | 364.33 ± 1.49[a] | 675.43 ± 2.51[a] | 226.14 ± 1.75[a] | 242.75 ± 0.15[a] |
| PSEE | 576.01 ± 4.57[b] | 613.68 ± 4.50[b] | 558.75 ± 4.34[b] | 229.36 ± 1.39[b] |
| PSPE | 590.92 ± 3.94[c] | 607.83 ± 6.38[b] | 585.62 ± 3.87[c] | 281.07 ± 0.98[c] |
| PSWE | 421.79 ± 2.02[d] | 863.61 ± 8.05[c] | 807.56 ± 4.69[d] | 206.74 ± 1.17[d] |
| L-ascorbic acid | nd | nd | nd | 27.90 ± 0.38[#] |
| Mannitol | 563.46 ± 3.58 | nd | nd | nd |
| BHT | nd | 10.80 ± 0.40 | nd | 18.10 ± 0.43[#] |
| Na₂EDTA | nd | nd | 82.03 ± 1.45 | nd |

Note: * Results are expressed as mean ± standard deviation (n = 3), calculated on the basis of dry plant material weight (dw). In each column different letters mean significant differences (p < 0.01); # values expressed as mmol Fe²⁺ g⁻¹; nd - not determined.

## Conclusions

In summary, the natural ultrasonic extract from *P. spinosa* fruits obtained using 45% propylene glycol as a solvent was particularly rich in polyphenolic compounds, which provided high tyrosinase inhibitory and antioxidant activity demonstrated using *in vitro* assays. Therefore, it has a great potential to be developed as natural tyrosinase inhibitor and antioxidant. Further research concerning *in vivo* investigations should be done in order to consolidate protective effects and potential benefits of extracts to human health.

## Acknowledgements

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