# CHARACTERIZATION OF *Prunella vulgaris* L. EXTRACTS IN TERMS OF POLYPHENOL TOTAL CONTENT (TPC)

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**Abstract.** Although plants have been used since Prehistory to treat certain conditions, people did not know their chemical composition, which gave them the expected effect. By conducting studies in botany, microbiology, pharmacology, analytical chemistry, or pharmacognosy, a variety of pharmaceutical forms of a plant species were discovered and more information was discovered about the active principles contained, about their effect on the human or animal body. *Prunella vulgaris* has a wide range of therapeutic uses due to its high content in compounds such as: rosmarinic acid, oleanolic acid, betulinic acid, ursolic acid, camphor, manganese, hyperoside, vitamins A, B, C, K, delphinidin which also gives it antispasmodic, carminative, vermifuge, tonic properties or which contribute to improving intellectual capacity and implicitly to preventing diseases such as schizophrenia or Alzheimer's dementia. The plant also contains numerous polyphenols and terpenes, which gives it broad antibacterial and antimicrobial properties. Therefore, it is recommended to find new ways to extract the active substances and administer them to patients in such a way as to target the neuralgic point. The polyphenol total content analyzed in ethanolic extracts of *Prunella vulgaris* species varies from 35.12 to 39.10 mg GAE/100 g dry plant (in the case of the extract obtained by ultrasound) and from 47.70 to 142.84 mg GAE/100 g dry plant (in the case of the extract obtained by microwave) depending on the concentration of the solvent used, the extraction method applied, the extraction conditions and the amount of extract used. The research conducted confirmed the strong antioxidant activity of the extracts of *Prunella vulgaris* species obtained by microwave and ultrasound, the values obtained being 76.20% and 77.15%, respectively, which reinforces the idea that both extraction methods can be used to obtain ethanolic extract of species *Prunella vulgaris* with an increased content of polyphenols and high antioxidant activity.

**Keywords:** ethanolic extract, microwave and ultrasound extract, *Prunella vulgaris* L., antioxidant activity.

**Rezumat. Caracterizarea extractelor de *Prunella vulgaris* L. din punct de vedere al conținutului total de polifenoli (TPC).** Deși plantele au fost folosite, încă din preistorie, pentru tratarea unor afecțiuni, oamenii nu cunoșteau și compoziția lor chimică, care le imprima efectul scontat. Prin efectuarea unor studii de botanică, microbiologie, farmacologie, chimie analitică, sau farmacognozie au fost descoperite o varietate de forme farmaceutice ale unei specii de plante și s-au descoperit mai multe informații despre principiile active conținute, despre efectul acestora asupra organismului uman sau animal. *Prunella vulgaris* are o gamă largă de utilizări terapeutice datorită conținutului ridicat în compuși precum: acid rozmarinic, acid oleanoic, acid betulinic, acid ursolic, camfor, mangan, hiperozid, vitaminele A, B, C, K, delfinidină care îi atribuie și proprietăți antispamodice, carminative, vermifuge, tonice sau care contribuie la îmbunătățirea capacității intelectuale și implicit la prevenirea unor boli precum schizofrenia sau demența Alzheimer. De asemenea, planta conține și numeroși polifenoli și terpene, ceea ce îi atribuie ample proprietăți antibacteriene și antimicrobiene. De aceea, se recomandă găsirea unor noi modalități de extragere a substanțelor active și de administrare a acestora către pacienți astfel încât să fie țintit punctul nevralgic. Conținutul total de polifenoli analizat în extractele etanolice ale speciei *Prunella vulgaris* variază de la 35.12 până la 39.10 mg GAE/100 g planta uscată (în cazul extractului obținut la ultrasunete) și de la 47.70 până la 142.84 mg GAE/100 g (în cazul extractului obținut la microunde) în funcție de concentrația solventului utilizat, metoda de extracție aplicată, condițiile de extracție și cantitatea de extract utilizat. Cercetarea realizată a confirmat puternica activitate antioxidantă a extractelor de *Prunella vulgaris* obținute la microunde și la ultrasunete, valorile obținute fiind de 76,20%, respectiv 77,15%, ceea ce întărește ideea că ambele metode de extracție pot fi utilizate pentru a obține extract etanolic pe bază de *Prunella vulgaris* cu un conținut sporit de polifenoli și activitate antioxidantă înaltă.

**Cuvinte cheie:** extract etanolic, *Prunella vulgaris* L., polifenoli, activitate antioxidantă.

## INTRODUCTION

*Prunella vulgaris L.* (Order: Lamiales; Family: Lamiaceae; Genus: Prunella) is a herbaceous, perennial, rarely annual and biennial plant, native to Eurasia, North America and northwest Africa, but which subsequently spread and adapted to all continents, preferring acidic, neutral or basic soils, with a sandy, loamy or clayey texture, respectively shady, cool and humid places in temperate zones located at an altitude between 5 and 2500 m.

The plant grows, especially, in plain, hill and plateau regions, in deciduous and coniferous forests, in gardens, pastures and orchards.Since ancient times, *Prunella vulgaris* L. has been used as a folk medicine to relieve sore throats, reduce fever and accelerate wound healing. Recent studies (BO-HOU et al., 2024; DAG et al., 2017) have shown that the methanol or water extract of this plant exhibits antihyperglycemic activity, contributes to the inhibition of systemic anaphylaxis, is used as an antioxidant and may have antiviral and antibacterial effects, anti-inflammatory, pro-apoptotic, neuroprotective and antitumor effects. In Indian medicine, the plant is used as an antipyretic, tonic, diuretic remedy. Recently, it has been shown that the species can be used as a remedy anti-HIV. As a remedy against herpes simplex virus type 1 and 2 and also has antioxidant and cardioprotective effects (LING, 2021).

## MATERIAL AND METHODS

The *Prunella vulgaris* species appeared spontaneously in the fields of Doicești, Dâmbovița county, and was harvested from the first year of growth, in the months of June-August. The samples were taken for investigations when the plant reached its maximum development period, the entire plant being harvested, from which the extracts were obtained.

In the laboratory of the Research Institute of the University of Pitești, in November 2024, ethanolic extracts of *Prunella vulgaris* were obtained by ultrasound and microwave, and in order to establish the polyphenol content of the Prunella vulgaris species, the following materials were used: Folin Ciocâlteu reagent, 99.2% absolute ethyl alcohol, 96% ethyl alcohol, distilled water, sodium carbonate powder, *P. vulgaris* extracts.

The Folin-Ciocalteu method is used to determine the total phenol content by measuring the ability of phenolic compounds to reduce the Folin-Ciocalteu reagent, which contains molybdenum and tungsten oxides. This reaction generates a blue complex that can be quantified spectrophotometrically at 765 nm (LAWAG et al, 2023).

For the quantitative determination of polyphenols, Folin-Ciocâlteu reagent of 10% concentration was used, together with a $Na_2CO_3$ solution at a concentration of 7.5%, to ensure the alkaline conditions necessary for the reaction. The method was adjusted in accordance with the international standard SR EN ISO/CEI 17025:2005. Gallic acid was used as a standard. For each sample diluted by a factor of 600, 500 µL were taken and 2.5 mL of reagent were added. The samples were kept at room temperature for 5 minutes, then 2 mL of sodium carbonate solution was added. After stirring and keeping in the dark for one hour, spectrophotometric analysis was performed at 765 nm using quartz cuvettes. The polyphenol content for each *P. vulgaris* extract was determined using the regression equation obtained from the gallic acid calibration curve. The results were expressed as gallic acid equivalents per gram of dry plant (mg GAE/100 g, dry plant), and the polyphenol total content (TPC) was estimated based on the specific calculation formula:

TPC= (CMxFD)/1000

where:

C represents the concentration measured from the calibration curve;

M represents dry plant;

FD represents the dilution factor.

**Determination of the antioxidant activity of *Prunella vulgaris* L. extracts.** The antioxidant activity capacity was determined by the DPPH radical scavenging method (1,1-diphenyl-2-picrylhydrazyl), a method frequently used due to its stability, simplicity and reproducibility.

The steps for determining the antioxidant activity of *Prunella vulgaris* extracts are:

- in the presence of antioxidants, DPPH (violet compound) is reduced to a pale yellow compound, and the variation in DPPH absorbance was measured at a wavelength of 517 nm.
- stock solutions (1 mg/ml) were diluted to final concentrations of 200, 100, 75, 50, 25, 10 and 1 µg/ml in 96% ethyl alcohol.
- solutions of different concentrations in a volume of 1.5 ml were added to the ethanolic solution of DPPH (3 ml, 20 mg/l).
- after 20 minutes of incubation in a place protected from light and at room temperature, the absorbance of the solutions was measured at a wavelength of 517 nm.
- the DPPH solution (3 ml, 20mg/l) in ethyl alcohol (1.5 ml) was used as a negative control.
- trolox (1 mg/ml) served as a standard solution (Fig. 1).

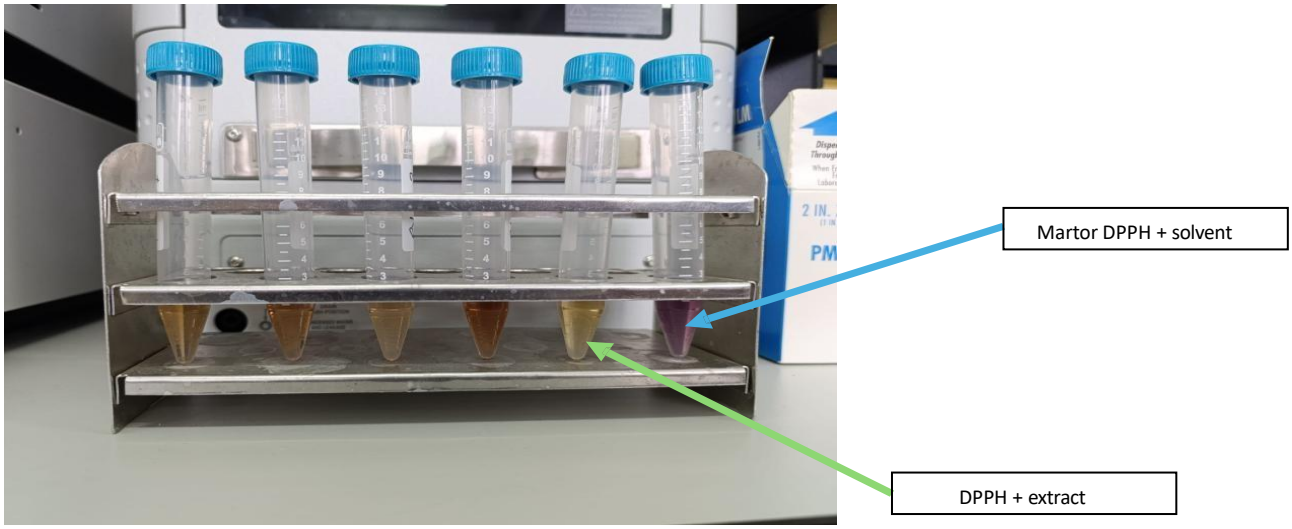


Figure 1. Reduction of DPPH free radicals in the presence of P. vulgaris extracts
Source: Research Institute, University of Pitești, November 2024.

## RESULTS

The polyphenols in the EEP obtained in advance react with the Folin-Ciocâlteu reagent in the presence of sodium carbonate and form a blue complex. The intensity of the blue color is proportional to the amount of reactive phenolic compounds in the sample. The method is based on electron transfer in alkaline medium with the reduction of the phosphomolybdenum/phosphotungstic acid complex with the formation of the chromogen which was determined spectrophotometrically at a wavelength of 765 nm. Gallic acid was used as a reference standard.

The polyphenol content is determined by measuring the absorbance of the solution to be analyzed at 765 nm (Table 1).

Table 1. Polyphenol total content of *Prunella vulgaris* extracts.

| Sample cod | Dilution factor | TPC mg gallic acid/100 g dry plant |
|---|---|---|
| PV_U_1 | 600 | 36.74 |
| PV_U_2 | 600 | 39.10 |
| PV_U_3 | 600 | 36.13 |
| PV_U_4 | 600 | 35.12 |
| PV_M_1 | 600 | 47.70 |
| PV_M_2 | 600 | 48.73 |
| PV_M_3 | 600 | 52.72 |
| PV_M_4 | 600 | 62.89 |
| PV_M_5 | 600 | 76.85 |
| PV_M_6 | 1200 | 142.84 |

Source: Research Institute, University of Pitești, November 2024

The total polyphenol content analyzed in ethanolic extracts of *Prunella vulgaris* species varies from 35.12 to 39.10 mg GAE/100 g (in the case of the extract obtained by ultrasound) and from 47.70 to 142.84 mg GAE/100 g (in the case of the extract obtained by microwave) depending on the concentration of the solvent used, the extraction method applied, the extraction conditions and the amount of extract used, as shown in figure 1.

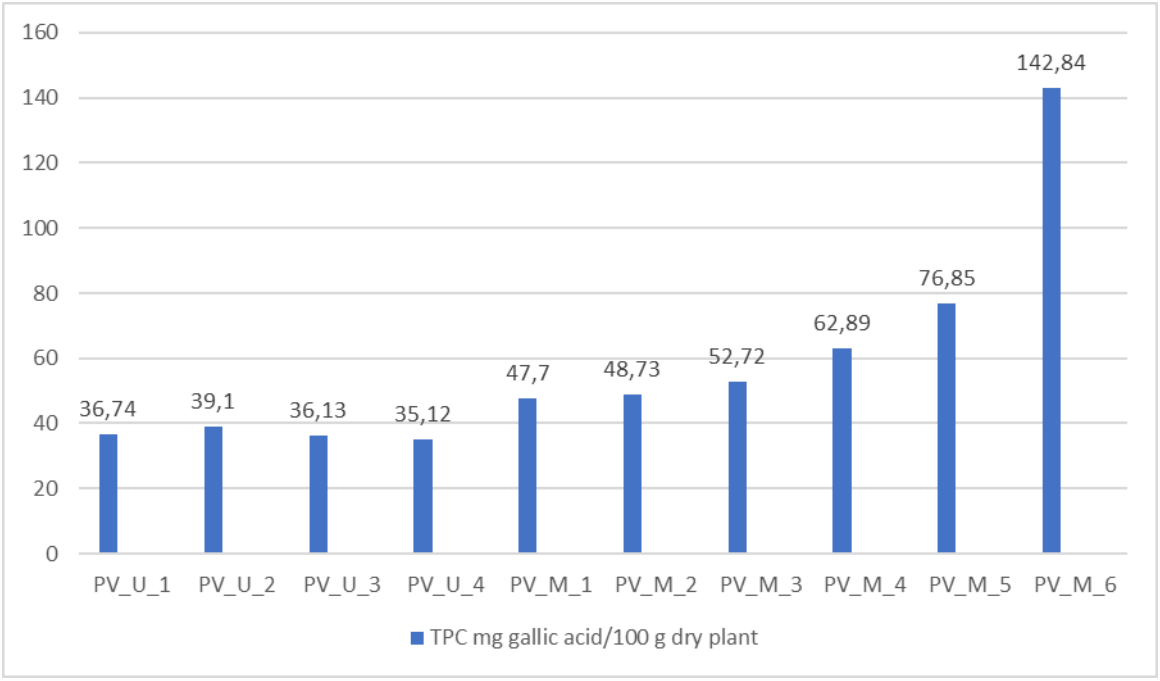


Figure 1. Polyphenol total content of *Prunella vulgaris* extracts. Source: Research Institute, University of Pitești, November 2024.

Data analyzed reveals that the EtOH concentration of 96% is the most optimal for the extraction of polyphenolic compounds for both extraction methods. Although the total polyphenol content in the case of extraction with 99.20% EtOH, at a dilution factor of 1200 is higher and is 142.84 mg GAE/100 g, this difference is insignificant compared to the total polyphenol content in the case of extracts with 96% EtOH, at a dilution factor of 600 obtained by

both methods. At the same time, the lowest total polyphenol content (35.12 mg GAE/100 g) was identified in the *Prunella vulgaris* extract with 96% EtOH at ultrasonic extraction, which can be explained by the fact that the extraction conditions and the concentration of the solvent used led to the degradation of certain polyphenolic compounds.

The results were expressed in mg gallic acid equivalent (GAE) dry mass of *Prunella vulgaris* species and demonstrate that the dry extract of this plant is rich in polyphenolic compounds, which has also been demonstrated by other studies conducted in the literature and in which the total amount of phenolic compounds of all fractions of *P. vulgaris* species ranged from 24.75 to 36.42 mg GAE/100 g in the case of extracts obtained by ultrasound and from 41.20 to 80.25 mg GAE/100 g in the case of the extract obtained by microwave (YU-JIN et al., 2013).

The quantitative analysis demonstrated the presence of flavonoids in glycosidic form in large quantities (hyperoside, rutoside, isoquercitrin), on the other hand, smaller quantities of aglycones (quercetol, kaempferol, apigenin) were identified and quantified. Furthermore, phenol-carboxylic acids were quantified, such as caftaric, ferulic, p-coumaric acid (GROȘAN et al., 2020 ).

In other study, the total phenolic content of *Prunella vulgaris* species. was 137±17 mg GAE/ 100 g), the flavonoid content was 24±7 mg QE/100g, and the quercetin content was 2.58±0.32 mg/ 100g and catechin content was 51.2±5.5 mg/ 100 (TIAN et al., 2021,). The total amount of plant sterols recorded was 3.14±1.04 ppm. Also, the *Prunella vulgaris* extract has a higher content of flavonoids, such as catechin and quercetin, which supports the strong antioxidant capacity of this extract. Also, in the study, the extract reduced F2IP and LPO to a greater extent than other plant extracts used for comparison, and significantly increased the antioxidant enzyme activities, SOD and PO due to the high flavonoid content. The conclusion is that the *Prunella vulgaris* extract recorded a high antioxidant and anti-inflammatory activity due to the higher content of this plant in phenols and flavonoids (LAWAG et al., 2023).

In other study, extracts prepared with innovative green solvents, such as SUPRAS (IC50 = 2.061 mg·mL-1) and DES (IC50 = 2.001 mg·mL-1), recorded higher antioxidant capacities than those prepared with conventional organic solvents (IC50 = 2.386 mg·mL-1 for 30% ethanol and 3.262 mg·mL-1 for methanol).

Thus, in the case of the *Prunella vulgaris* extract obtained using SUPRAS, the amount of caffeic acid, salviaflaside and rosmarinic acid recorded was 0.240 mg, 1.443 mg and 5.254 mg, which suggests that there is a directly proportional relationship between the content of polyphenolic compounds and the antioxidant activity of *P. vulgaris* extracts (BO-HOU et al., 2024).

The antioxidant activity was determined according to the formula:

I(%) = [(Abs0 −Absl )/Abs0]×100, where Abs0 is the absorbance of the control sample and Abs1 is the absorbance of the tested samples.

Next, the IC50 (concentration of the extract that determines the inhibition of 50% of free radicals) was determined for each sample and expressed in mcg/ml.

The antioxidant activity was plotted as a function of concentration and the IC50 value, calculated from the slope by linear regression analysis. The results obtained demonstrate a linear dependence between the concentration of *Prunella vulgaris* extract and discoloration (r < 1) (Table 2).

Table 2. Inhibition ratio values regarding the antioxidant activity of *P. vulgaris* extracts.

| Sample cod | Sample concentration | Inhibitio ratio (%) |
|---|---|---|
| PV_U_1 | 33mg/mL | 71.01 |
| PV_U_2 | 33mg/mL | 62.48 |
| PV_U_3 | 33mg/mL | 75.43 |
| PV_U_4 | 33mg/mL | 77.15 |
| PV_M_1 | 33mg/mL | 64.02 |
| PV_M_2 | 33mg/mL | 38.65 |
| PV_M_3 | 33mg/mL | 72.27 |
| PV_M_4 | 33mg/mL | 76.20 |
| PV_M_5 | 33mg/mL | 75.65 |
| PV_M_6 | 33mg/mL | 71.96 |
| Trolox | 0.07mg/mL | 95.27 |

Source: ibidem

## DISCUSSIONS

The maximum inhibition ratio was obtained in the case of the *Prunella vulgaris* extract obtained by ultrasound (77.15%), and the minimum ratio (38.65%) was recorded in the case of the extract obtained by microwave, as shown in figure 2, values similar to those obtained in the specialized literature (73.05 ± 10.32, in the case of the ethanol extract(YU-JIN et al., 2013), 89.25 ± 10, in the case of the ethanol extract (LING et al., 2021) or 68.40, in the case of the ethanol extract (PIERONI et al., 2023).

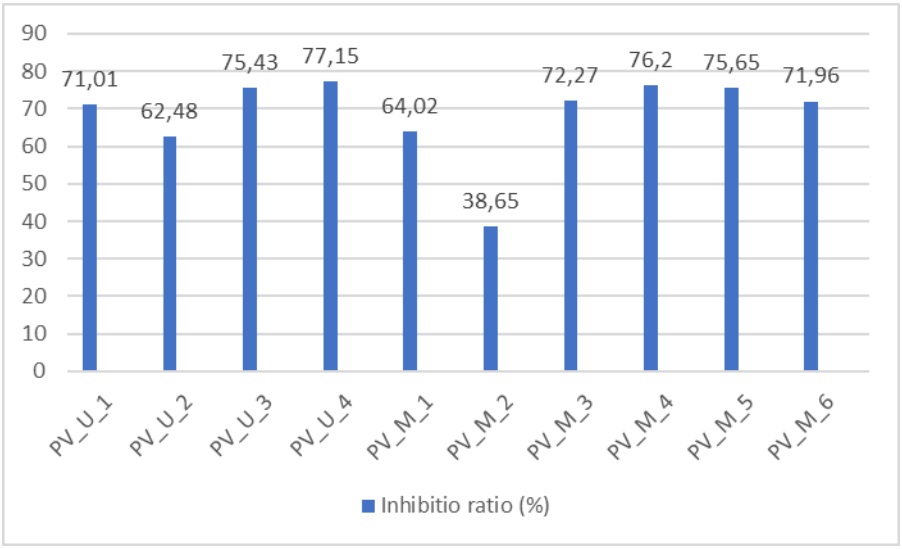


Figure 2. Inhibition ratio values regarding the antioxidant activity *of P. vulgaris* extracts. Source: ibidem.

The antioxidant activity achieved by the DPPH test denotes that the *Prunella vulgaris* extract has a well-pronounced antioxidant action that correlates proportionally with the total phenolic and that the redox effect is proportional to the content of polyphenolic compounds.

The application of the extraction, maceration and concentration technology of phenolic principles from Prunella vulgaris resulted in the amplification of the antioxidant activity of the extract, the IC50 value (38.65-77.15 mcg/ml) being inversely proportional to the free radical scavenging capacity. Thus, the collected plant presents a source of medicines and food supplements that are very competitive nationally.

The analysis of the total polyphenol content data expressed in mg GAE/100 g reveals that ethyl alcohol with a concentration of 96% is the optimal solvent used to extract polyphenolic compounds by both extraction methods.

The antioxidant activity is more pronounced in the case of the *Prunella vulgaris* extract with an ethyl alcohol concentration of 96% obtained by ultrasound (77.15%), and for the extract obtained by microwave, a maximum inhibition ratio of 76.20% was obtained, which reinforces the idea that both extraction methods can be used to obtain ethanolic extract of *Prunella vulgaris* species with an increased content of polyphenols and high antioxidant activity.

For the subsequent use of the *Prunella vulgaris* extract as an active ingredient of a cosmetic or pharmaceutical product, its standardization is recommended, especially since *Prunella vulgaris* seems to be a good choice, since the extracts obtained from this medicinal plant are rich in phenolic compounds, which are mainly responsible for the antioxidant effect.

## CONCLUSIONS

The analysis of the total polyphenol content data expressed in mg GAE/100 g revealed that ethyl alcohol with a concentration of 96% is the optimal solvent used to extract polyphenolic compounds by both extraction methods.

The total polyphenol content analyzed in ethanolic extracts of *Prunella vulgaris* species varies from 35.12 to 39.10 mg GAE/100 g (in the case of the extract obtained by ultrasound) and from 47.70 to 142.84 mg GAE/100 g (in the case of the extract obtained by microwave) depending on the concentration of the solvent used, the extraction method applied, the extraction conditions and the amount of extract used.

The antioxidant activity is more pronounced in the case of the *Prunella vulgaris* extract with an ethyl alcohol concentration of 96% obtained by ultrasound (77.15%), and for the extract obtained by microwave, a maximum inhibition ratio of 76.20% was obtained, which reinforces the idea that both extraction methods can be used to obtain ethanolic extract of *Prunella vulgaris* with an increased content of polyphenols and high antioxidant activity.

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