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Total Polyphenol, Flavonoid Content and Antioxidant Activity of Two Species with Ethnopharmaceutical Uses from the Spontaneous Flora of the Oltenia Region, Romania

Ștefania Eliza Tănasie [1], Valentin Nănescu [1,*], Emilia Amzoiu [1], Manuel Ovidiu Amzoiu [1], Andreea Gabriela Mocanu [1], Felicia Ciulu-Costinescu [2], Liviu Chirigiu [1]

[1] Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 200349 Craiova, Romania; eliza_tanasie@yahoo.com (Ș.E.T.); emanro2002@yahoo.com (E.A.); m_amzoiu@yahoo.com (M.O.A.); gabriela_deca@yahoo.com (A.G.M.); liviu.chirigiu@yahoo.com (L.C.)
[2] Faculty of Pharmacy, University Titu Maiorescu, 031593 Bucuresti, Romania; felicia.costinescu@yahoo.com
* Correspondence: valentin.nanescu@gmail.com; Tel.: +40-0764-373-763

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Abstract: In this study, we investigated the total phenolic content of the alcoholic extracts from various vegetative organs of two plants of Romanian wild flora, Oltenia region: *Dipsacus laciniatus* and *Armoracia rusticana*. The antioxidant activities were determined using the ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging methods. We also determined the total polyphenol and flavonoid content. The results showed significant differences among various plant vegetative organs, the highest content of polyphenols being found in the leaf extract of the *Dipsacus laciniatus* species, followed by *Armoracia rusticana* leaves. The highest antioxidant reducing power was obtained for the Armoracia leaf extract (IC50 = 101.94 µg/mL), followed by the leaves of *Dipsacus* (IC50 = 77.86 µg/mL).

Keywords: antioxidant activities; total polyphenols; *Dipsacus laciniatus*; *Armoracia rusticana*

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Introduction

As the interest in herbal remedies and traditional medicine continues to increase [1], there is potential for further research into the pharmacological properties of *Dipsacus laciniatus* (*Dip*) and the development of standardized preparations for various health applications. While there are a lot of historical uses of *Dip* in ethnomedicine, scientific validation is often lacking, with additional research being needed on this species.

Our aim was to compare a plant considered a widespread weed (*Dipsacus laciniatus*) with a plant recognized for its beneficial effects on health (*Armoracia Rusticana*) (Figure 1) from the point of view of antioxidant properties.

Figure 1. *Dipsacus laciniatus* (**left**) and *Armoracia rusticana* (**right**).

Some traditional medicinal practices involve the use of *Dip* for its potential anti-inflammatory properties. The plant contains compounds that are believed to help reduce inflammation, and in certain cultures, it has been used topically or as an infusion for ailments associated with inflammation, such as arthritis [2].

Dip has been historically recognized for its diuretic effects. In certain ethnomedical traditions, infusions or decoctions made from the roots of the plant are consumed to promote diuresis, helping the body eliminate excess fluids and potentially aiding in conditions related to water retention. The inflorescences of *Dip* have been used for their potential wound-healing properties [3].

On the other hand, *Armoracia rusticana*, (*Arm*) commonly known as horseradish, has a long history of use in traditional and folk medicine across various cultures. The plant is valued not only for its pungent flavor in culinary applications, but also for its potential health benefits [4].

Horseradish has been traditionally used as a stimulant of the digestive system, promoting the production of digestive juices and enzymes [5].

In respiratory conditions, horseradish is known for its strong, pungent aroma, and this characteristic is associated with potential respiratory benefits. In traditional medicine, horseradish has been used to help alleviate symptoms of respiratory conditions such as congestion, sinusitis and coughs. Its natural compounds may act as decongestants.

Some studies observed that horseradish contains compounds with antimicrobial properties, being suitable for treating minor infections and wounds, applied topically or ingested to support the body's defense against certain microbial threats [6].

The plant also contains compounds that may have mild anti-inflammatory effects, and as such, it has been used to address conditions associated with inflammation, including arthritis and joint pain [7].

In certain traditional practices, horseradish has been considered a detoxifying agent. It is believed to help eliminate toxins from the body, supporting the liver and kidneys in their natural detoxification processes. It has also been used as a stimulant and tonic in traditional medicine to provide a general boost to the body's vitality and energy levels. Its use in this context reflects a belief in its overall health-promoting properties [8].

Materials and Methods

Chemicals

All reagents and chemicals used are of analytical grade, purchased from Merck or Sigma Aldrich.

Plant Material

The plant material was harvested from the region of Oltenia, from spontaneous flora, during the optimal period considering extractive components (flowering period for *Dip* and plants in the second year of life, in September, for *Arm*, respectively). The specimens of each plant were identified and kept.

Plant Extracts

Of the sample, 0.2 g was dried and passed through a 0.2 micron sieve, and the plant powder was weighed. Then, 5 mL of 70% methanol was added. In order to extract the active components, the samples were stirred constantly at 200 rpm. Then, the extracts were subjected to an ultrasonic process for 10 min. The samples were subsequently centrifuged at 5000 rpm for 10 min, and the supernatant was collected and made up to 10 mL with the solvent.

Total Phenolic Content

The total polyphenol content was determined in accordance with the methods described by Aryal et al. [9] and Basu et al. [10], with slight modifications.

Thus, we used the reagent Folin–Ciocîlteu 2 N with a 1:10 dilution and sodium carbonate 2%. As the standard, we used gallic acid in concentrations ranging from 0 to 250 µg/mL. Of each alcoholic extract diluted at a ratio of 1:10, and each solution from the calibration curve, 2.5 mL was pipetted (in triplicate). The Folin–Ciocâlteu diluted reagent (1 mL) and 1 mL of Na2CO3 2% were pipetted over the samples and the solutions from the calibration curve. The solutions were left for 30 min in the dark and the resulting blue complex was read at 765 nm.

The following samples were obtained:

- *Armoracia*—root (ArmR);
- *Armoracia*—leaf (ArmL);
- *Dipsacus root* (DipR);
- *Dipsacus stem* (DipS);
- *Dipsacus leaves* (DipL);
- *Dipsacus flowers* (DipF).

Total Flavonoid Content

The quantitative determination of flavonoids was carried out according to the method described by Orsavova et al. Thus, 1 mL of alcoholic extract (diluted 1:10) was stirred with 0.3 mL of 5% $NaNO_2$ solution and 5 mL distilled water for 5 min. Then, 0.3 mL of 10% $AlCl_3$ and 2 mL of 1 M NaOH were added. The final volume of 10 mL was adjusted with distilled water. After 20 min, the absorbance was read at 510 nm (DLAB SP-UV1000). The total flavonoid content was calculated as mg QE equivalents/g dry plant [11].

FRAP Method

A Sigma Aldrich FRAP assay kit was used to determine the antioxidant activity, which measures the ion reduction capacity of Fe^{3+} ions to Fe^{2+} at pH = 3.7–4. To carry out the analyses, plant extracts were diluted at 1:30. The absorbance was measured after 60 min at 595 nm (BMG Labtech, FLUOstar OPTIMA). The amount in the samples (mM Fe^{2+} equivalents) was calculated with the following relation :

$$\frac{\text{mmol}Fe^{2+} \times F_D}{V}$$

where mmol Fe^{2+} = Fe^{2+} amount from calibration curve (y = 0.9568x + 0.0152, R^2 = 0.9974); F_D = dilution factor.

DPPH Assay

The DPPH assay was conducted by applying the spectrophotometric method. The antioxidant activity based on the scavenging activity of the stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described by Bondet et al., with slight modifications. The following concentrations of extracts were prepared: 20 μg mL^{-1}, 40 μg mL^{-1}, 60 μg mL^{-1}, 80 μg mL^{-1} and 100 μg mL^{-1}. All the solutions were prepared in 70% methanol. Five mL of each prepared concentration was mixed with 0.5 mL of 1 mM DPPH solution in 70% methanol. The test tubes were incubated for 30 min at room temperature and the absorbance was measured at 517 nm. A lower absorbance of the reaction mixture indicates a higher free radical scavenging activity. Ascorbic acid was used as the standard and the following concentrations were prepared as the test solutions: 1 μg mL^{-1}, 2.5 μg mL^{-1}, 5 μg mL^{-1} and 7.5 μg mL^{-1}. The difference in absorbance between the test and the control (DPPH in 70% methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ Antioxidant activity} = (1 - As/Ac) \times 100$$

where As is the absorbance of the sample at 0 min and Ac is the absorbance of the control at 30 min.

Results

For the total polyphenol dosage, a calibration curve was obtained with the equation y = 0.0028x + 0.0264 and the regression coefficient R^2 = 0.9967. For the flavonoid content, the equation for the calibration curve was y = 0.0065x − 0.0105 (R^2 = 0.9917).

A surprising result was the finding that the leaves especially, but also the flowers of *Dip* had a high content of polyphenols and flavonoids, surpassing *Arm*, so they constitute a valuable resource from spontaneous flora, uncharacterized until now (Figures 2 and 3).

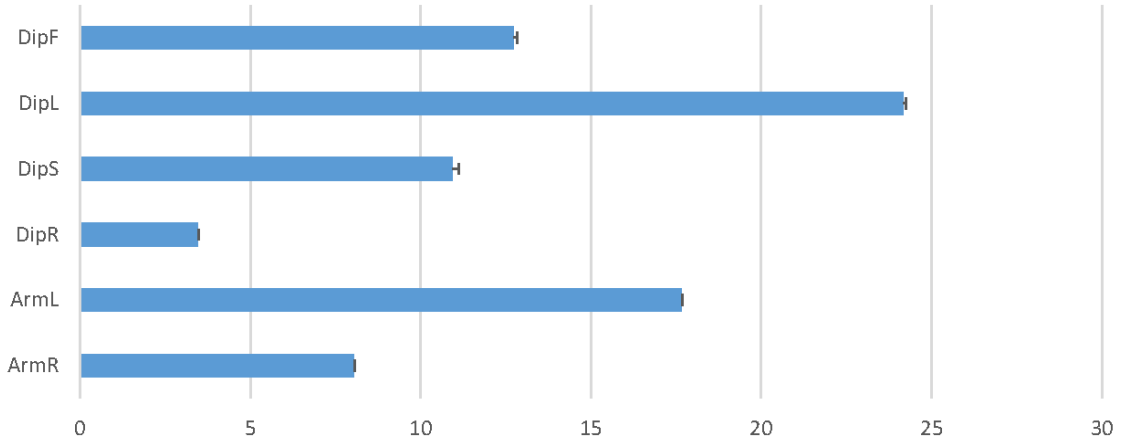

Figure 2. Total polyphenol content expressed as gallic acid equivalents.

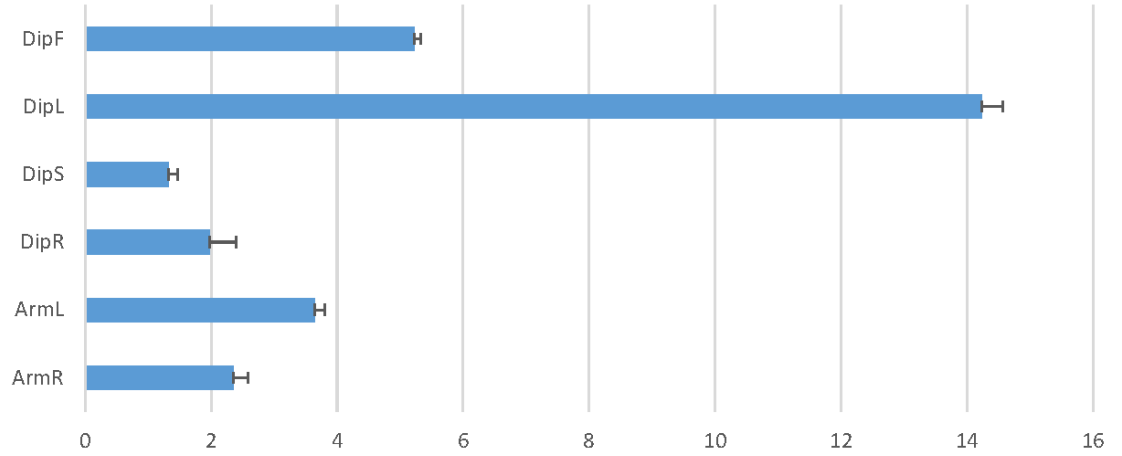

Figure 3. Total flavonoid content expressed as quercetin equivalents.

Figure 4 shows the results of the free radical scavenging activity in % inhibition. The results for *Dip* revealed that the flower extract had the highest radical scavenging activity, at 87.43% followed by the leaf extract at 86.63%, the stem extract at 83.54% and the root extract at 78.17%.

The results for *Armoracia rusticana* show that the root extract has the highest DPPH activity, at 82.12%, followed by the leaf extract at 80.03% (Figure 5).

Table 1 presents the IC_{50} values for various plant extracts. The data shows that DipS extract has the lowest IC50, indicating the highest potency.The highest antioxidant value (FRAP method) was obtained for the leaf extract (*Dip*) or root extract (*Arm*), as shown in Table 2.

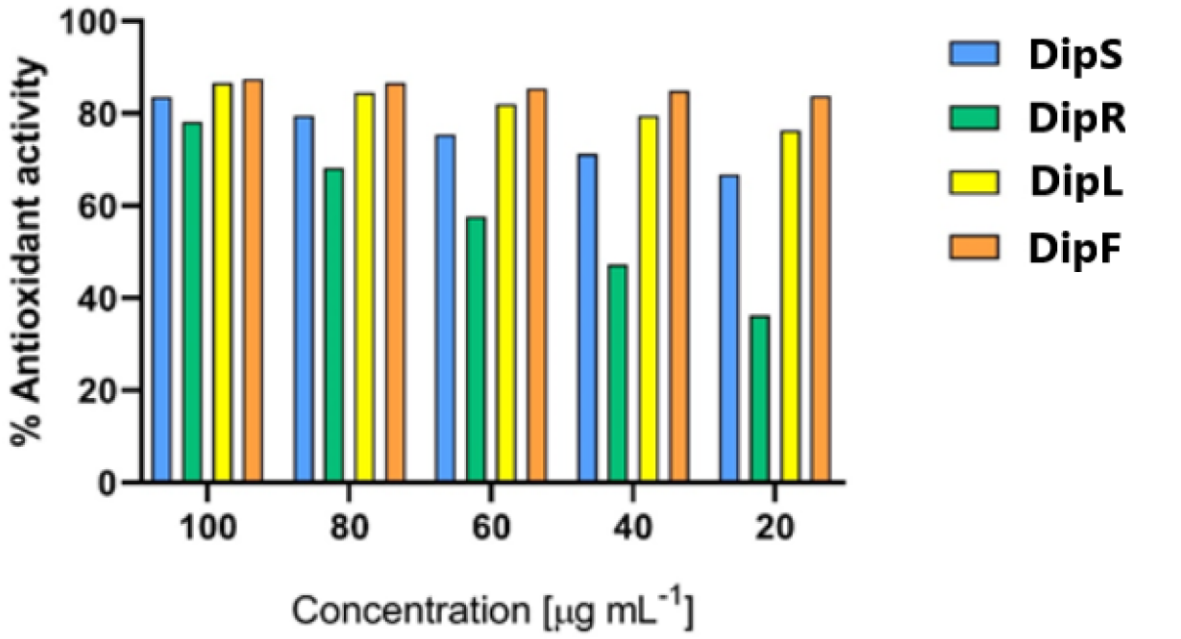

Figure 4. Antioxidant activity of *Dipsacus laciniatus*.

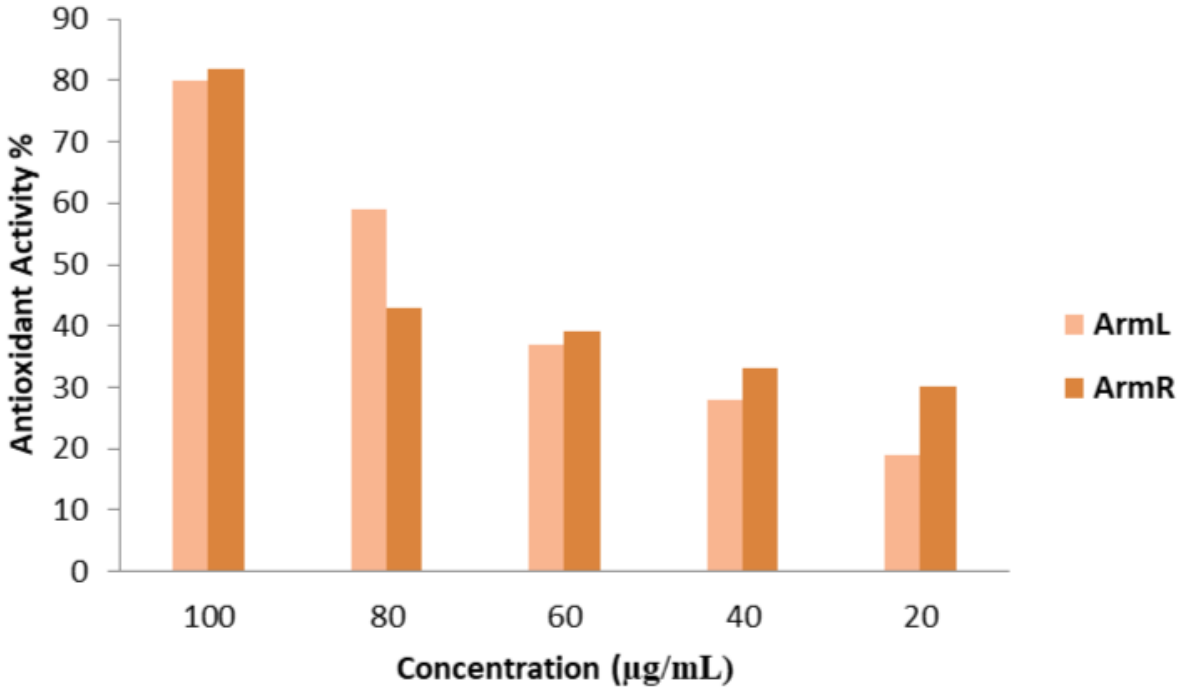

Figure 5. Antioxidant activity of each plant extract for *Armoracia rusticana*.

Table 1. IC_{50} values for each plant extract.

Plant Part Extract	IC_{50} [µg mL^{-1}]
DipR	64.67
DipS	64.52
DipL	77.86
DipF	67.13
ArmR	101.94
ArmL	60.46

Table 2. Total antioxidant capacity expressed in mM Fe(II) equivalents.

Sample	mM Fe(II) Echivalents	SD
DipR	12.32	0.15
DipS	10.86	0.05
DipL	21.14	0.23
DipF	16.18	0.09
ArmR	27.11	0.12
ArmL	24.43	0.21

Discussion

The plant biodiversity in the world comprises almost 550,000 species, but just about 100,000 are used in industry (food, pharmaceutical, textile) in order to product bioactive compounds. Several metabolites were found to be very active, with important medical properties such as salicin (*Salix* spp.) [12], the natural precursor to salicylic acid which has anti-inflammatory and pain-relieving properties, boswellic acid (*Boswellia serrata*) [13] which showed high anti-inflammatory effects, particularly beneficial for conditions like osteoarthritis, gingerol [14] (*Zingiber officinale*) with anti-nausea properties, allicin (*Allium sativum*) [15] with antimicrobial and anti-inflammatory properties, artemisinin (*Artemisia annua*) [16] with antimalarial properties, or capsaicin (*Capsicum* spp.) [17] with analgesic and anti-inflammatory effects.

Of the approximately 4000 species of plants in the Romanian flora [18], only a small percentage is exploited for its ethnopharmaceutical potential. That is why it is important to characterize other species with possible pharmaceutical properties, of which the potential must be exploited.

From the *Dipsacus* genus, other species have been better studied, such as *Dipsacus fullonum* with surprising results (antiborrelia activity) [19]. Our previous studies on the *Dipsacus laciniatus* species unexpectedly showed an extremely high concentration of chlorogenic acid in the flowers and leaves [20], with this species showing the potential to serve as a raw material for its extraction in the future. The many effects of chlorogenic acid on health are already known (reduces inflammatory stress [21], neuroprotective effects [22], oral healthcare agent against periodontitis [23]), with it already being extracted from various plant sources [24] in the food industry and used as a nutraceutical [25].

Armoracia rusticana is already a reference species regarding its medicinal properties, with its characterization as an antioxidant, alongside *Dipsacus laciniatus*, an unstudied species serving as comparative study.

Conclusions

Our study characterized a plant recognized for its therapeutic value and one considered a weed, finding that the leaves and flowers of *Dipsacus laciniatus* constitute a serious resource of polyphenols, with high antioxidant activity, comparable with that of horseradish root and leaves.

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