

# Inhibitory potential of some Romanian medicinal plants against enzymes linked to neurodegenerative diseases and their antioxidant activity

Gabriela Paun<sup>1</sup>, Elena Neagu<sup>1</sup>, Camelia Albu<sup>1</sup>, Gabriel Lucian Radu<sup>1,2</sup>

<sup>1</sup>Centre of Bioanalysis, National Institute for Research-Development of Biological Sciences, Centre of Bioanalysis, 060031 Bucharest, <sup>2</sup>Department of Analytical Chemistry, Faculty of Applied Chemistry and Materials Science, Politehnica University of Bucharest, 060042 Bucharest, Romania

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## ABSTRACT

**Context:** *Eryngium planum*, *Geum urbanum* and *Cnicus benedictus* plants are an endemic botanical from the Romanian used in folk medicine. **Objective:** The extracts from three Romanian medicinal plants were investigated for their possible neuroprotective potential. **Materials and Methods:** Within this study, *in vitro* neuroprotective activity of the extracts of *E. planum*, *G. urbanum*, and *C. benedictus* plants were investigated via inhibition of acetylcholinesterase (AChE) and tyrosinase (TYR). Total content of phenolics, flavonoids, and proanthocyanidins, high-performance liquid chromatography profile of the main phenolic compounds and antioxidant activity were also determined. **Results:** Among the tested extracts, the best inhibition of AChE ( $88.76 \pm 5.2\%$ ) and TYR ( $88.5 \pm 5.2\%$ ) was caused by *C. benedictus* ethanol (EtOH) extract. The *G. urbanum* extracts exerted remarkable scavenging effect against 2,2-diphenyl-1-picrylhydrazyl ( $IC_{50}$ ,  $7.8 \pm 0.5 \mu\text{g/mL}$  aqueous extract, and  $IC_{50}$ ,  $1.3 \pm 0.1 \mu\text{g/mL}$  EtOH extract, respectively) and reducing power, whereas the EtOH extract of *C. benedictus* showed high scavenging activity ( $IC_{50}$ ,  $0.609 \pm 0.04 \text{ mg/mL}$ ), also. **Conclusion:** According to our knowledge, this is the first study that demonstrates *in vitro* neuroprotective effects of *E. planum*, *G. urbanum* and *C. benedictus*.

**Key words:** Antioxidant, *Cnicus benedictus*, enzyme inhibition, *Eryngium planum*, *Geum urbanum*, neurodegenerative diseases

## INTRODUCTION

Herbal infusions represent a rich source of phytochemicals, many of which have potent antioxidant activities and are consumed for their health enhancing properties.<sup>[1,2]</sup> Age-related neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD) are increasing in prevalence with the rise in longevity of populations world-wide. The cholinergic hypothesis is the most accepted theory to explain the pathogenesis of AD, and, therefore, the most prescribed drugs for the treatment of AD are the cholinesterase inhibitors.<sup>[3]</sup> On the other hand, tyrosinase (TYR) is involved in the

neuromelanin-biosynthetic pathway and could be central to dopamine neurotoxicity, as well as contributing to the neurodegeneration associated with PD.<sup>[4]</sup> Thus, there is great interest in finding better acetylcholinesterase (AChE) and TYR inhibitors, particularly from edible plants sources, showing low toxicity, good brain penetration and high bioavailability.<sup>[5]</sup> Oxidative stresses a significant risk factor for age-associated cognitive decline and is widely considered to be a critical aspect in the complex pathogenesis of AD.<sup>[6]</sup> Among the dietary constituents, polyphenolics appear to play a significant role as antioxidants in the protective effect of plant derived foods.<sup>[7]</sup>

*Eryngium planum* L. (Flat Sea Holly), a species that belongs to the *Apiaceae* family is widely spread throughout the Romanian territory. *Eryngium* species are of great value for their use in traditional European medicines, due to their content of phenolic acids, flavonoids, saponins, coumarin derivatives, essential oils and acetylenes.<sup>[8-10]</sup>

*Geum urbanum* L. (Common Avens) is a herbaceous plant in the rose family (Rosaceae), widely spread in the temperate

### Address for correspondence:

Prof. Gabriel Lucian Radu, National Institute for Research-Development of Biological Sciences, Centre of Bioanalysis, 296 Spl.Independentei, P. O. Box 17-16, Sector 6, 060031 Bucharest, Romania.  
Faculty of Applied Chemistry and Materials Science, Politehnica University of Bu-Charest, 313 Spl.Independentei, 060042 Bucharest, Romania.  
E-mail: gl\_radu@chim.upb.ro

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zone of Europe. Both, the herb and underground parts have been used in folk medicine. In these raw materials, the main biologically active compounds are tannins and phenolic acids.<sup>[11]</sup>

*Cnicus benedictus* L. (Blessed Thistle), the sole species in the genus *Cnicus*, is a thistle-like plant in the family *Asteraceae*, native to the Mediterranean region. This herb contains alkaloids, cnicin, benedictin, mucilage, polyacetylene, triterpenoids, lignans, flavonoids, tannin, phytosterines and volatile oils.<sup>[12,13]</sup> This is a plant with antidepressant, anti-inflammatory, antiseptic, cardiac and antimicrobial properties.

For the selected plants, the phytochemical analysis and biological studies are poorly explored.

However, no study or analysis of the *G. urbanum* L., *E. planum* L. and *C. benedictus* L. extracts anti-cholinesterase or anti-TYR activities have previously been published.

## MATERIALS AND METHODS

All chemicals and solvents were purchased from Sigma Chemical Company (Sigma Aldrich, Germany), Fluka (Switzerland), Roth (Carl Roth GmbH, Germany) and deionized water was used for all the performed analysis (Millipore, Bedford, MA, USA). The medicinal plants were acquired from a local processing plant (SC STEF MAR SRL) of herbal teas in the dry form.

### Preparation of the extracts

Ground plant material was separately extracted with 70% (v/v) ethanol (EtOH) and distilled water at 60°C (aqueous) by sonication for 1 h. The obtained extract was filtered under vacuum through no. 1 Whatman filter paper, stored at 4°C for further use. The herbal's mass concentration in the solvent was 100 g/L.

### Phytochemical characterization of the extracts

#### Determination of total phenolic acids, flavonoids, and proanthocyanidins

The total phenolic content in extracts was determined using Folin–Ciocalteu reagent, as earlier described.<sup>[14]</sup> Gallic acid was used as a standard to perform the calibration. Briefly, an aliquot (0.5 mL) of extract was mixed with 0.5 mL of Folin–Ciocalteu reagent. The mixture was filtered, and 4.75 mL of 200 g/L sodium carbonate was added to 0.25 mL filtrate. Methanol (MeOH) was used as the blank, and total phenolic content was calculated from a calibration curve as gallic acid equivalents (GAEs) in mg/L of extracts.

The total flavonoid content was determined according Lin method (aluminum chloride based method), with slight modifications.<sup>[15]</sup> Briefly, 5 mL of extract was mixed with

7.5 mL of MeOH and after that the mixture is filtered. Then, 1 mL of the extract sample was transferred to a 5 mL volumetric flask and 1 mL of 10% sodium acetate solution, 0.6 mL of 2.5% aluminum chloride hexahydrate solution and 0.5 mL MeOH were added. After 15 min. the absorbance of the mixture was measured at 430 nm and the flavonoids content expressed in mg rutin equivalent (RE)/L of extract was calculated using the calibration curve obtained in the 0–120 mg/L concentration range.

Total proanthocyanidin content, expressed as epicatechin equivalents (CEs), was evaluated by a modified vanillin-HCl assay method.<sup>[16,17]</sup> Catechin is commonly used to standardize the vanillin reaction. The extract solution (0.5 mL) was mixed with 2.5 mL of glacial acetic acid/hydrochloric acid (5:1 v/v) solution and 2.5 mL of 1% vanillin in glacial acetic acid (w/v). After 30 min incubation in a 30°C water bath, the absorbance of the sample and control mixtures was measured at 500 nm against a reagent blank and their difference was used to determine total proanthocyanidins of the samples, expressed as mg CE/L sample.

### High-performance liquid chromatography analysis

The experiments to adapt a method for the polyphenol's measurement were based on the previously high-performance liquid chromatography (HPLC) method for the measurement of these compounds in extracts.<sup>[18]</sup> The chromatographic measurements were performed using a complete HPLC Shimadzu system, by means of a Nucleosil 100–3.5 C18 column, Kromasil, 100 × 2.1 mm. The system was coupled to a mass spectrometry (MS) detector, liquid chromatograph mass spectrometer-2010 detector, equipped with an ESI interface. The mobile phase consists of formic acid in water (pH = 3.0) as solvent A and formic acid in acetonitrile (pH = 3.0) as solvent B. The polyphenolic compounds separation was performed using binary gradient elution: 0 min 5% solvent B; 0.01–20 min 5–30% solvent B; 20–40 min 30% solvent B; 40.01–50 min 30–50% solvent B; 50.01–52 min 50–5% solvent B. The flow rate was: 0–5 min 0.1 mL/min; 5.01–15 min 0.2 mL/min; 15.01–35 min 0.1 mL/min; 35.01–50 min 0.2 mL/min; 50–52 min 0.1 mL/min. ESI source and negative ionisation mode have been used. The rutin, ellagic acid, sinapic acid, epicatechin, chlorogenic acid, rosmarinic acid, quercetin 3-β-D-glucoside, genistein, daidzein, myricetin, quercetin, luteolin, kaempferol, gallic acid, caffeic acid, ferulic acid and p-coumaric acid were used as reference standard.

### Enzyme inhibition assays

#### Acetylcholinesterase inhibitory activity

The AChE inhibitory activity was measured using an adaptation of the method described of Ingkaninan *et al.*<sup>[19]</sup> Acetylthiocholine iodide (AChI) as substrate of the reaction and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were used

for the measurement of the anti-AChE activity. Briefly, 3000  $\mu\text{L}$  of 50 mM Tris-HCl buffer (pH 8.0), 100  $\mu\text{L}$  of sample solution at different concentrations (3 mg/mL, 1.5 mg/mL and 0.75 mg/mL) and 20  $\mu\text{L}$  AChE (6 U/mL) solution were mixed and incubated for 15 min at 30°C, and 50  $\mu\text{L}$  of 3 mM DTNB were added. The reaction was then initiated with the addition of 50  $\mu\text{L}$  of AChI (15 mM). The hydrolysis of these substrates was monitored by the quantity of yellow 5-thio-2-nitrobenzoate anion formed as the result of the reaction of DTNB with thiocholine, at a wavelength of 405 nm.

Enzyme activity was calculated as a percentage of the average velocities compared to that of the assay using buffer instead of inhibitor (extract), based on the formula  $(E - S)/E \times 100$ , where E is the activity of enzyme without test sample, and S is the activity of enzyme with test sample. The experiments were carried out in triplicate.

#### Tyrosinase inhibition assay

The TYR activity was measured spectrophotometrically using l-DOPA as substrate.<sup>[20]</sup> TYR aqueous solution (100  $\mu\text{L}$ , 0.5 mg/mL), plant extract (100  $\mu\text{L}$ ) and 1850  $\mu\text{L}$  of 0.2M phosphate buffer (pH 7.0) were mixed and preincubated at 30°C for 15 min. and then l-DOPA solution (50  $\mu\text{L}$ , 10 mM) was added and the absorbance at 475 nm was measured after 3 min. The same reaction mixture without the plant extract but the equivalent amount of phosphate buffer served as the blank. Kojic acid (Sigma) was used as the reference. The percentage inhibition of TYR activity was calculated as follows:

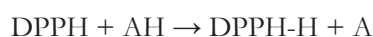
$$\% \text{ TYR inhibition} = \frac{\Delta A_{\text{control}} - \Delta A_{\text{sample}}}{\Delta A_{\text{control}}} \times 100$$

where  $\Delta A_{\text{control}}$  is the change of absorbance at 475 nm without a test sample, and  $\Delta A_{\text{sample}}$  is the change of absorbance at 475 nm with a test sample.

#### 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity and ferric reducing antioxidant power assay

##### 2,2-diphenyl-1-picrylhydrazyl –radical scavenging assay

The free radical scavenging activity of the extracts was studied by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method-based on the decrease of the DPPH (Sigma-Aldrich) maximum absorbance in the antioxidant presence, with slight modification.<sup>[21]</sup> It can readily undergo reduction by an antioxidant (AH), which runs as the following reaction:



100  $\mu\text{L}$  of different concentrations of extract (0.3–3 mg/mL), were mixed with 1000  $\mu\text{L}$  of the freshly prepared 0.25 mM

DPPH in MeOH and 1900  $\mu\text{L}$  MeOH. Absorbance at 516 nm was determined after 3 min. The decreasing of the DPPH radical absorption by the action of antioxidants could be used for measuring the antioxidative activity. The percentage of DPPH radical scavenging activity of the samples was calculated as follows:

$$\text{DPPH radical scavenging activity (\%)} = \frac{([A_0] - A_s) / A_0}{\times 100}$$

Where

$A_0$  = blank absorbance;  $A_s$  = sample absorbance. Trolox and vitamin C were used as standard antioxidant.

#### Reducing power

The reductive potential of the extracts was determined according to the method of Oyaizu with some few changes.<sup>[22]</sup> Briefly, 0.1 mL of extract was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ; 1%). The mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid were added, and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. Finally, the absorbance was measured at 700 nm against a blank, by ultra violet spectrophotometer (Thermo Scientific Evolution 260 Bio). Vitamin C was used as a control.

#### Statistical analysis

The measurements were performed in triplicate and for statistical processing Excel 2007 was used, standard deviation was <10%.

## RESULTS AND DISCUSSION

During the over studies on medicinal plants, in order to discover new enzyme inhibitors relevant to neurodegenerative diseases, we investigated enzyme inhibitory potential of *E. planum*, *G. urbanum* and *C. benedictus* extracts.

As neurodegenerative disease is also associated with inflammation, free radicals being one of the causes of the inflammatory process, the antioxidant activity of the extracts were also investigated.<sup>[23,24]</sup> In the last several years herbs rich in phenolic and flavonoids contents were given the prime importance due to their free radical scavenging capability.

As can be observed in Table 1, the total phenolic acid contents of the different extracts as GAEs were found to be highest in EtOH extracts.

The *G. urbanum* EtOH extract showed the highest total phenolics ( $1261.5 \pm 31.3$  mg GAE/L), whereas the phenolic contents of *E. planum* aqueous extract were much smaller ( $342.5 \pm 16.2$  mg GAE/L). The highest flavonoid content was confined to the *C. benedictus* EtOH extract ( $205.9 \pm 9.5$  mg RE/L) followed by the *E. planum* EtOH extract ( $116.1 \pm 6.1$  mg RE/L). The most abundant proanthocyanidins amount was determined in the *G. urbanum* EtOH extract ( $60.1 \pm 2.1$  mg CE/L), followed by the *C. benedictus* EtOH extract ( $58.5 \pm 1.9$  mg CE/L).

The HPLC-MS method has been applied for the evaluation of the sample's polyphenolic profile. The contents of polyphenols and flavones in analyzed extracts are shown in Table 2.

**Table 1: Bioactive compounds quantified in *Eryngium planum*, *Geum urbanum* and *Cnicus benedictus* extracts**

Plant	Extract type	Total phenolic acid (mg GAE/L)	Total flavonoid (mg RE/L)	Proanthocyanidins (mg CE/L)
<i>Eryngium planum</i>	H <sub>2</sub> O	342.5±16.2	57.6±2.4	34.3±1.2
	EtOH	660.1±22.4	116.1±6.1	44.9±1.8
<i>Geum urbanum</i>	H <sub>2</sub> O	768.2±25.9	14.7±0.9	37.5±1.4
	EtOH	1261.5±31.3	49.9±2.2	60.1±2.1
<i>Cnicus benedictus</i>	H <sub>2</sub> O	437.2±17.1	78.5±5.3	28.9±0.8
	EtOH	572.3±18.6	205.9±9.5	58.5±1.9

Results are mean±SD of three parallel measurements. SD: Standard deviation; GAE: Gallic acid equivalent; RE: Rutin equivalent; CE: Catechin equivalent; EtOH: Ethanol

Ellagic acid and epicatechin were the major identified compounds in *G. urbanum* ethanolic extract while rutin was the major compounds in an aqueous extract.

Rutin, rosmarinic acid, chlorogenic acid and quercetin 3-β-D-glucoside were the major identified compounds in *E. planum* ethanolic extract while ellagic acid was present in higher amounts in the aqueous extracts.

Sinapic acid and chlorogenic acid were the major identified compounds in *C. benedictus* ethanolic extract.

This is the first report of daidzein compound in *G. urbanum*, *E. planum* and *C. benedictus* extracts.

Rosmarinic acid and chlorogenic acid, which are known for their antioxidant activity, have been described for many *Eryngium* species.<sup>[25]</sup>

Inhibition of the cholinesterase enzyme family is vital in the fight against AD, while TYR inhibition is an important drug target for PD. The AChE inhibitory activity results of *E. planum*, *G. urbanum*, and *C. benedictus* extracts are presented in Table 3. According to our findings; all extracts displayed a concentration-dependent inhibitory factor against AChE and they especially showed stronger inhibition at concentration of 3 mg/mL ( $79.11 \pm 3.0 \div 88.76 \pm 5.2\%$ ). The *E. planum* and *C. benedictus* EtOH extracts were the most potent among all the extracts against AChE [Table 3]. Although this effect is significantly lower than pharmacological drugs used in neurological diseases, such as galantamine ( $IC_{50} = 0.14 \mu\text{g/mL}$ ),<sup>[26]</sup> this is a natural

**Table 2: Content of polyphenolic compounds determined by HPLC-MS**

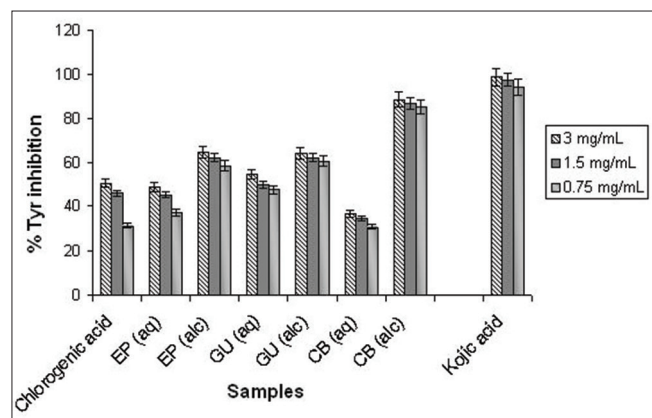
Class	Compound [M/z] <sup>-</sup>	<i>Geum urbanum</i> (μg/mL)		<i>Eryngium planum</i> (μg/mL)		<i>Cnicus benedictus</i> (μg/mL)	
		Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract
Flavanols	Epicatechin [289]	91.6	-	-	+	-	-
	Quercetin [301]	+	+	+	+	+	-
Flavonols	Rutin [609]	3.9	122.0	368.3	8.6	106.5	-
	Myricetin [317]	1.9	-	-	+	-	-
	Kaempferol [285]	+	+	+	+	+	-
	Ellagitannins	1013.5	-	7.6	120.7	18.2	9.8
Phenolic acids	Ellagic acid [301]	8.0	+	-	+	-	-
	Gallic acid [169]	8.0	+	-	+	-	-
Hydroxy cinnamic acids	p-Coumaric acid [163]	+	+	2.4	+	9.5	-
	Sinapic acid [223]	6.4	-	-	7.8	415.5	6.5
	Caffeic acid [179]	+	-	1.0	+	1.8	-
	Ferulic acid [193]	+	0.7	0.6	-	-	+
Hydroxy cinnamic acid esters	Rosmarinic acid [359]	+	+	15.2	+	+	-
	Chlorogenic acid [353]	+	3.6	46.4	6.2	294.5	+
Flavonoid glucosides	Quercetin 3-β-D-glucoside [463]	+	+	22.2	+	28.9	+
Flavone	Luteolin [285]	+	+	+	+	+	-
Isoflavones	Genistein [269]	-	3.9	2.8	3.5	2.2	2.2
	Daidzein [253]	2.6	2.3	7.1	10.5	2.3	1.5

-: Under limit of detection; +: Under limit of quantification; HPLC-MS: High-performance liquid chromatography mass spectrometry; EtOH: Ethanol

product with potential for daily consumption, without any side effects.

As shown in Figure 1, the extracts showed inhibition against the TYR enzyme ranging between  $36.4 \pm 2.8\%$  and  $88.5 \pm 5.2\%$ . Among the tested extracts, the best inhibition against AChE ( $88.76 \pm 5.2\%$ ) and TYR ( $88.5 \pm 5.2\%$ ) was found to be *C. benedictus* EtOH extract, since it is richer in flavonoids compounds than the other studied extracts [Table 1].

The studied herbs are rich in polyphenolic compounds. As one of the major constituent of *C. benedictus*, sinapic acid seems to be responsible in a large amount for this herb's neuroprotective effect. For instance, Lee et al. reported that sinapic acid improved, via its anti-oxidative and anti-inflammatory activities, the  $A\beta_{1-42}$  protein-related pathology, including neuronal cell death and cognitive dysfunction.<sup>[27]</sup> Therefore, preparations based on this substance could be very efficient in the AD treatment. Although memory enhancing effect of the *C. benedictus* is mainly attributed to sinapic acid, other polyphenolic compounds found in these plants have been also stated to exert neuroprotective effect. In fact, rutin, ellagic acid, genistein and daidzein identified in these species have shown to exhibit neuroprotection.<sup>[28-30]</sup>

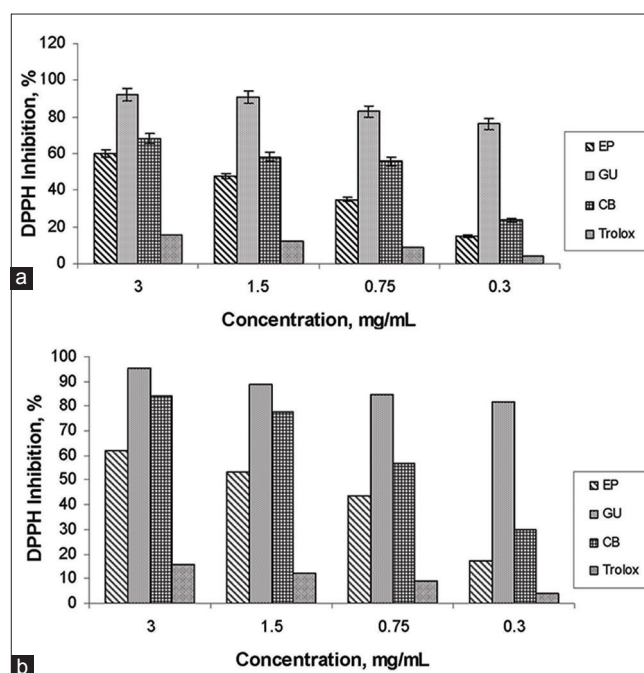


**Figure 1:** Tyrosinase inhibitory activity of the aqueous and ethanol extracts. EP: *Eryngium planum*; GU: *Geum urbanum*; CB: *Cnicus benedictus*

The inhibitory potential of TYR and AChE might depend on the hydroxyl groups of the phenolic compounds extracts that could form a hydrogen bond to the active site of the enzyme, leading to lower enzymatic activity. Phenolic compounds such as ellagic acid, tannic acid and quercetin act as potent TYR and AChE inhibitors, as reported by other authors.<sup>[31-33]</sup>

Until now, no AChE and TYR inhibitory activity of *G. urbanum* and *C. benedictus* has been reported.

In this work, two methods were used to evaluate total antioxidant capacity of the different extracts: DPPH free radical scavenging assay [Figure 2a and b] and ferric-reducing antioxidant power assay [Figure 3]. In the present investigation, extracts of *G. urbanum* showed excellent inhibition rate of DPPH:  $92 \pm 3.7\%$  aqueous extract ( $IC_{50}$ ,  $7.8 \pm 0.5 \mu\text{g/mL}$ ) and  $95.2 \pm 4.2\%$  alcoholic

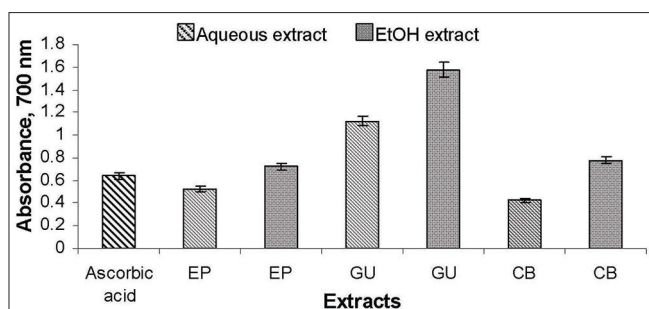


**Figure 2:** Free radical scavenging activity of the aqueous (a) and ethanol (b) extracts and standard (Trolox) by the 2,2-diphenyl-1-picrylhydrazyl method. EP: *Eryngium planum*; GU: *Geum urbanum*; CB: *Cnicus benedictus*

**Table 3: AChE inhibitory activities of the extracts**

Plant	Extract type	Inhibitory activity against AChE (%)			
		0.75 mg/mL	1.5 mg/mL	3 mg/mL	IC <sub>50</sub> , mg/mL
<i>Eryngium planum</i>	H <sub>2</sub> O	27.49±1.6	28.77±1.8	81.85±5.9	2.405±0.21
	EtOH	59.62±2.4	55.79±3.9	88.66±6.5	0.176±0.01
<i>Geum urbanum</i>	H <sub>2</sub> O	27.03±1.5	36.48±1.7	79.11±3.9	2.293±0.14
	EtOH	54.74±2.7	73.53±5.1	86.77±5.1	0.513±0.03
<i>Cnicus benedictus</i>	H <sub>2</sub> O	41.19±2.2	55.63±3.1	82.54±4.6	1.603±0.09
	EtOH	65.61±4.1	74.17±5.2	88.76±5.2	0.084±0.002

Results are mean±SD of three parallel measurements. SD: Standard deviation; AChE: Acetylcholinesterase; EtOH: Ethanol



**Figure 3:** Antioxidant capacities of the aqueous and ethanol extracts and standard (ascorbic acid), using ferric reducing power method. EP: *Eryngium planum*; GU: *Geum urbanum*; CB: *Cnicus benedictus*

extract ( $IC_{50}$ ,  $1.3 \pm 0.1 \mu\text{g/mL}$ ), respectively. The EtOH extract of *C. benedictus* showed high scavenging activity (84.1% DPPH inhibition;  $IC_{50}$ ,  $0.609 \pm 0.04 \text{ mg/mL}$ ) and the aqueous extract also showed a high scavenging activity (68.3% DPPH inhibition;  $IC_{50}$ ,  $0.715 \pm 0.05 \text{ mg/mL}$ ). The anti-radical activity of *E. planum* aqueous ( $IC_{50}$ ,  $1.731 \pm 0.12 \text{ mg/mL}$ ) and EtOH ( $IC_{50}$ ,  $1.362 \pm 0.11 \text{ mg/mL}$ ) extracts were reduced in comparison with the *G. urbanum* and *C. benedictus* extracts, but were comparable to the previously investigated antioxidant activity of other species of this genus.<sup>[34,35]</sup> The results indicate that the antioxidant activity of all concentrated extracts is higher than that of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the obtained results showed anti-oxidant activity depending on dosage. Significant antiradical activity of *G. urbanum* is obviously due to its high polyphenolic phytochemicals. Polyphenolic compounds are well known as effective free radical scavengers and antioxidants, resulting in a close correlation between the content of phenolic compounds and antioxidant activity.<sup>[36]</sup>

The reducing power of aqueous and EtOH extracts of the three herbs are presented in Figure 3, in that, the graphs show, that the highest reducing potential is obtained from the aqueous and EtOH of *G. urbanum*, significantly higher than that of the standard (ascorbic acid). The reducing power of *E. planum* and *C. benedictus* extracts at the same concentration were lower than of *G. urbanum* extracts but were comparable to the activities of the ascorbic acid.

The results of the study demonstrate that the studied extracts may possess memory-enhancing effect by inhibiting AChE and TYR enzymes, also showing a great antioxidant activity.

## CONCLUSION

Our findings revealed that, among the tested extracts, the EtOH extract of *C. benedictus* and *G. urbanum* showed high AChE (over 86%) and TYR inhibitory effects (over 63%).

In addition, *G. urbanum* extracts appear to have significant antioxidant properties, which might be possibly associated with the polyphenols high content of the plant. Thus, these extracts could be beneficial in the therapy of degenerative diseases especially where the oxidative stress and cholinergic hypothesis are involved. To the best of our knowledge, we report our study on AChE and TYR inhibitory activity of *E. planum*, *G. urbanum* and *C. benedictus* as being the first of this sort.

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