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The antioxidant, hemolytic and cholinesterase inhibition properties of *Galium verum* L. and *Tragopogon pratensis* subsp. *pratensis*

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The antioxidant properties of *Galium verum* L. (Lady's Bedstraw) and *Tragopogon pratensis* subsp. *pratensis* (Goat's beard) methanol extracts, from localities in Serbia were evaluated, through determination of total phenolics and flavonoids content, as well as DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, ABTS$^{\bullet+}$ (2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) radical cation decolorization and ferricyanide methods. Methanol extracts of *G. verum* showed stronger free radical scavenging activity (IC_{50} values of 26.98 μg/mL for DPPH$^{\bullet}$ decolorization assays and 125.14 mg Trolox/ g dry extract for ABTS$^{\bullet+}$ radical cation decolorization assays) and total reducing power (70.31 μg/mL) than methanol extracts of *T. pratensis* (SC_{50} values of 59.25 μg/mL for DPPH$^{\bullet}$ decolorization assays and 6.31 mg Trolox/ g dry extract for ABTS$^{\bullet+}$ radical cation decolorization assays and 15.66 μg/mL of extract for total reducing power). Observed could be connected with a much higher content of phenolic and flavonoid constituents in *G. verum* extracts. Applying modified Ellman's method, *G.verum* methanol extracts showed slight activation of humane serum cholinesterase (16.28 ± 0.09 %), while methanol extract of *T. pratensis* was proven as a weak inhibitor of the enzyme (4.65 ± 0.08 %). Also, methanol extracts of *G. verum* and *T. pratensis* were tested *in vitro* using standard Drabkin's method to evaluate whether these extracts have hemolytic activity. Extracts of *T. pratensis* showed very strong hemolytic activity (after the first hour of incubation 50% of hemolysis induced concentration of 501.97 ± 32.65 μg/mL, the second hour 499.98 ± 38.45 μg/mL, 498.656 ± 39.85 μg/mL of the third and fourth 421.33 ± 34.98 μg/mL) while *G. verum* extracts had weak hemolytic activity.

Keywords: antioxidant activity, hemolytic activity, cholinesterase inhibition, *Galium verum* L., *Tragopogon pratensis* subsp. *pratensis*

INTRODUCTION

Serbian flora represents an abundant resource for scientific investigation. Above all, the genus *Galium* (Rubiaceae) is represented by 37 species [1, 2]. *Galium verum* L. (Lady's Bedstraw) is widely distributed a perennial herbaceous plant with golden yellow flowers that are 2-3 mm in diameter and grouped in many-flowered panicles [3]. The aerial parts of *G. verum* were used as a diuretic, sedative or spasmolytic, as well as for rheumatic diseases in the traditional medicine [4]. Upper herbaceous parts of *G. verum* have beneficial effects on nervousness, phobias, cardiovascular diseases and liver disorders [5]. This herb has been shown to contain speruloside, monotropein, scandoside and geniposidic acid [4, 6, 7, 8], as well as small amounts of tannins, saponins, essential oils, waxes, pigments and vitamin C [8].

The genus *Tragopogon* L. comprises approximately 100 species with a number of widely introduced species [9]. The genus comprises annual, biennial or mostly perennial herbs, *Tragopogon pratensis* subsp. *pratensis* (Goat's beard) is a annual/perennial plant, growing to 0.6 m. *T. pratensis* is considered to be a useful remedy for the liver and gallbladder. It appears to have a detoxifying effect and may stimulate the appetite and digestion. Its high inulin content makes this herb a useful food for diabetics. The root is astringent, depurative, diuretic, expectorant, nutritive and stomachic. Experimental studies of methanol extracts of *T. pratensis* have shown that phenolic compounds have antiproliferative and tumor arresting effects [10].

Polyphenols are the major plant compounds that are characterized by antioxidant activity. This antioxidant activity of polyphenols is believed to be mainly due to their redox properties [11], in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Flavonoids are the most common and widely distributed group of plant phenolics compounds, occurring in almost all plants. Flavonoids may have an additive effect to the endogenous scavenging compounds and can prevent injury caused by free radicals in various ways. One way is the direct scavenging of free radicals.

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Flavonoids are oxidized by radicals, resulting in a more stable, less-reactive radical.

For evaluations of antiradical activity of the herbs extracts the most spread DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ methods were used. Both radicals show similar bi-phase kinetic reactions with many antioxidants. Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The DPPH free radical (DPPH$^{\bullet}$) does not require any special preparation, while the ABTS radical cation (ABTS$^{\bullet+}$) must be generated by enzymes or chemical reactions [12]. Another important difference is that ABTS can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples. In contrast, DPPH can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants [13].

Radical cation ABTS$^{\bullet+}$ interacts with the extract or standard Trolox that suppressed the absorbance of the ABTS$^{\bullet+}$. Trolox [6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid], a water soluble vitamin E analog, serves as a positive control inhibiting the formation of the radical cation in a dose dependent manner. Results are expressed as trolox equivalents that cause the same reduction in absorbance as an appropriate concentration of the sample.

Reducing power is considered to be a strong indicator of the antioxidant activity and was determined using a modified iron (III) to iron (II) reduction assay. Reducing agents, react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Compounds with reducing power are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so they can act as primary and secondary antioxidants. The reducing ability is generally associated with the presence of reductants which exert antioxidant action through breaking the free radical chain by donating a hydrogen atom or preventing peroxide formation [14].

Acetylcholinesterase (AChE) plays the key role in the hydrolysis of acetylcholine at the cholinergic synapses, ending the transmission of nerve impulses. Inhibition of the acetylcholinesterase is considered as a promising strategy in confronting neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis. As potent source of AChE inhibitors is are numerous plant natural products. Studies carried out earlier, in order to find new molecules or a group of molecules that can be used in the therapy without the toxicity of the synthesized chemical compounds, included in the beginning screening of plant's extracts [15,16].

Taking into account the above mentioned characteristics of *G. verum* and *T. pratensis* we thought that it could be of relevance for their medical use to examine the antioxidant capacity, hemolytic activity and activity against cholineesterase.

Methanolic herbs extracts were characterized by total polyphenol and flavonoides content. Free radical scavenging capacity was evaluated by measuring the scavenging capacity of extracts on DPPH and ABTS$^{\bullet+}$ radicals scavenging methods, and reducing power assay Fe(III) to Fe(II). Anti-cholinesterase action of selected extracts was tested applying modified Ellman's method [17]. Hemolytic assay was done by using standard Drabkin's method to determine the percentage of hemolysis in the RBC suspension [18].

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride, gallic acid, 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), butyrylthiocholine iodide (purity > 99%), DTNB, neostygmine bromide were purchased from Sigma Co. St. Louis, Missouri, USA.

All other chemicals and reagents used (K3[Fe(CN)6], NaH2PO4-Na2HPO4, CCl3COOH, ascorbic acid, butylhydroxytoluene (BHT), AlCl3 rutin, CH3COONa, Na2CO3, potassium persulfate, Methanol, were purchased from Merck, Darmstadt, Germany. All the chemicals and reagents were of analytical grade.

Apparatus

All spectrophotometric measurements of antioxidative potential of selected plant extracts and were performed on a spectrophotometar Perkin Elmer lambda 15.

All spectrophotometric measurements of inhibiton of humane serum cholinesterase were performed on a Konelab 20 analyzer (Thermofisher'Scientific) with flow thermostatted cells, length 7 mm (at wave lennght 405 nm). The advantage of the Konelab 20 analyzer, besides a significant reduction in reagent consumption (200 μL), was the possibility of performing up to 20 analyses per hour.

Plant material

Plant samples of the selected species Galium verum L. and Tragopogon pratensis subsp pratensis were collected from Seličevica (43° 13' 58" N 21° 55' 51" E) and Lalinac (43° 20′ 19" N 21° 47′ 04" E) in June 2010, identified by dr Bojan Zlatkovic, Deparment of Biology, and voucher specimens were deposited at the same department, Faculty of Science and Mathematics, Nis, Serbia.

Samples were dried under shade for seven days. Dried samples were ground into a uniform powder using a blender and stored in polyethylene bags at room temperature.

Preparation of extracts - ultrasonic extraction

Grounded air-dried plant material (10 g) was extracted with 100 mL 80 % methanol in the presence of low-frequency ultrasound. Sonication was performed 2 x 30 minutes using an ultrasonic cleaning bath (Sonic, Niš, Serbia; internal dimensions: 30, 15, 20 cm; total nominal power: 350 W; and frequency: 40 kHz). The temperature was maintained at 25 0C. At the end of the extraction process, the combined MeOH extracts were evaporated under vacuum to give crude MeOH extracts that were subject to subsequent analysis.

Determination of total phenolic content

The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method [19] with slight modifications. Methanol extract solution (0.1 mL), 1 mL of Folin-Ciocalteau reagent, were mixed into a 20 mL calibrated flask. After 1 min, 4 mL of sodium carbonate (20 %, v/v) was added and the volume was made to 20 mL with distilled water; finally, the mixture was allowed to stand at room temperature in dark for 30 min and the absorbance of the solution at 750 nm was measured with a Perkin-Elmer Lambda 15 UV-VIS spectrophotometer. If the sample absorbance exceeded, the sample was appropriately diluted to give reading less than total phenolics were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions (15-350 μg/mL in 80 % methanol). Since ascorbic acid also contributes to the formation of the blue molybdenum-tungsten complex, it is important to correct for the absorbance originating from it. An ascorbic acid calibration curve was therefore prepared. The total phenol compounds reported in this paper have all been corrected for ascorbic acid. Data were expressed as mg of gallic acid equivalents (GAE)/g of extract [20].

Determination of total flavonoid content

The amount of total flavonoids was determined with aluminium chloride (AlCl3) colorimetric assay according to a known method [13]. Briefly, 0.5 mL of each extract was made up to a final volume of 1 mL with reaction medium (MeO/H2O/CH3COOH=14:5:1). Prepared solution was mixed with AlCl3 reagent (4 mL, 133 mg of AlCl3x6H2O and 400 mg of CH3COONa dissolved in 100 mL H2O). After 5 min, the absorbance level was measured versus prepared reagent blank (containing the same chemicals, except for the sample) at 430 nm (Perkin-Elmer Lambda 15 UV-VIS spectrophotometer). Total flavonoid content was calculated on the basis of the calibration curve of rutin and expressed by mg rutin/g dry extract. [19].

Antioxidant assay

The free radical-scavenging activity was determined by different in-vitro methods such as the DPPH• free radical scavenging assay, the ABTS•+ radical cation decolorization assay and reducing power methods. All the assays were carried out in triplicate and average value was considered.

DPPH• radical scavenging activity

DPPH• scavenging activity of the plant extract was carried out according to the method [13]. Briefly, 10 μL of each extract was mixed with 90 μmol/L DPPH• in methanol (1.0 mL) and made up with methanol to a final volume of 4.0 mL. The mixtures were shaken vigorously and incubated in dark for 60 min at room temperature. Absorbance of the resulting solution was measured at 517 nm (Perkin-Elmer Lambda 15 UV-VIS spectrophotometer). All reactions were carried out with BHA (Butylated Hydroxy Anisole) as a positive control. The DPPH• scavenging activity was expressed by radical scavenging capacity using the following equation:

DPPH• RSC (%) = 100 (A0 - A 1 / A0)

where A0 was the absorbance of the control reaction (full reaction, without the tested extract or BHT) and A1 was the absorbance in the presence of the sample. DPPH• stock solution was stored at 4 0C until it was used. The SC50 value, which represented the concentrations of the extracts in the reaction mixture that caused 50 % inhibition, was determined by the linear regression analysis from the obtained. Decreased absorbance of the reaction

mixture indicates stronger DPPH• radical-scavenging activity.

ABTS•+ radical cation decolorization assay

Antioxidant capacity was measured based on the scavenging of ABTS•+ radical cation method. Concentration of ABTS solution was 7mM and it mixed with 2.45 mM potassium persulfate and incubated for 12-16 h in dark to generate ABTS•+ radical cation. The ABTS•+ solution was diluted with ethanol, to give an absorbance of 0.700 ± 0.050 at 734 nm. All samples were diluted appropriately to give absorbance values 20-80 % of that of the blank. Then fifty microlitres of diluted sample were mixed with 1.9 mL of diluted ABTS•+ solution.

The mixture was allowed to stand for 6 min at room temperature and the absorbance was immediately recorded at 734 nm. Trolox solution (final concentration 0-15 μM) was used as a reference standard. The results were expressed as mg Trolox/ g dry extract [21].

Reducing power assay Fe(III) to Fe(II)

Each prepared extract (10 μL) was mixed with K3[Fe(CN)6] (1 mL, 1 %) and NaH2PO4-Na2HPO4 buffer (1 mL, 0.2 mol/L, pH 6.6). These mixtures were incubated at 50 0C for 30 min, then trichloroacetic acid (1 mL, 10 %) was added and mixtures were centrifuged at 3000 rpm for 10 min. Finally, the supernatant fractions (1 mL) were mixed with distilled water (1 mL) and FeCl3 (0.2 mL, 0.1 %). The absorbances of resulting solutions were measured at 700 nm. For each sample three replicates were carried out. Reducing power assay Fe(III) to Fe(II) was calculated by the following equation:

$$AEAC = C_A(A_S / A_A)$$

where CA- final concentration of ascorbic acid in μg/mL, AS- absorbance of the sample, AA- absorbance of ascorbic acid [19]. Reducing power was expressed as Ascorbate Equivalent Antioxidant Capacity, AEAC, (μg of ascorbic acid/mL of extract). Increased absorbance of the reaction mixture indicates stronger reducing power.

CHOLINESTERASE INHIBITION PROPERTIES

Cholinesterase source for the assay

A total of 10 healthy volunteers (18-65 years old from both sexes), from the Pirot General Hospital, donated blood with written consent. According to the questionnaire, none of them had serious medical disorders, nor are or have been drug, cigarette, or alcohol abusers. At least a month before the blood donation, none of them had been taking any medication. From all participants, a 5 mL blood sample was collected in a Vacutainer tube, centrifuged at 3000 rpm for 10 min and the serum supernatant was collected and used as the source of the enzyme for the assay and for the spiked sample preparations.

PROCEDURE

Serum cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine, which reacts with chromogen DTNB. The reaction rate is determined from the rate of 5-thio-2-nitro benzoic acid formation, measured at 405 nm, in six cycles of 28 sec. Analysed solutions (10 μL) were mixed with 10 μL of the pooled serum (diluted with the phosphate buffer in ratio 1:9, v/v), and the phosphate buffer solution (160 μL). These were preincubated for 10 minutes (at 310 K) when a DTNB solution (10 μL) was added. After being allowed to stand 60 sec, finally the substrate solution (BuTC, 10 μL) was added.

RBC HEMOLYSIS ASSAY

Erythrocyte preparation

For the study healthy male Wistar rats 16 weeks of age were used. Animals were kept in group cages with 12 h light and 12 h dark cycle. Body weight was in the range of 200-250 g and food and water were supplied ad libitum. Experiment was done with the approval of ethic committee of Faculty of Medicine, University of Niš. Blood was obtained by cardiatic puncture and collected in heparinized tubes. Whole blood was centrifuged at 2200 rpm for 10 minutes at 4 0C. The buffy coat and plasma were removed from the tube and the equal amount of PBS (pH 7.4) was aded to the packed erythrocytes at the bottom. The procedure was repeated three times to obtain washed erythrocytes. At the end erythrocytes were diluted with PBS to obtain 4 % suspension. Erythrocytes suspension was used the same day for the experiment.

Sample preparation and hemolytic assay

Plant extracts of both species were dissolved in PBS (pH 7.4) and the final concentractions were made up to give 750 μg/mL, 500 μg/mL, 250 μg/mL and 100 μg/mL.

Hemolytic assay was done as previously described with some modifications [18]. Briefly, 200 μL of different concentractions of both plant extracts were added to 200 μL of erythrocytes suspension in a microtube. The positive control consisted of 200 μL of distilled water and 200 μL of

erythrocytes suspension. The negative control consisted of 200 μL of phosphate-buffered saline (PBS, pH 7.4) and 200 μL of erythrocytes suspension. The mixtures were incubated at room temperature and the percentage of hemolysis was determinated after first, second, third and fourth hour of incubation. Drabkin's method was used to measure the absorbance of hemoglobin and to calculate the percentage of hemolysis, as previously described [22].

In the end of incubation period, samples were centrifuged at 2000 rpm for 10 minutes at 40C. Supernatant volume of 200 μL was added to 3 mL of Drabkin's reagent. Mixture was shaken vigorously and the absorbance was measured at 540 nm (Shimadzu UV-1650PC). Hemolysis percentage was calculated by the equation:

% hemolysis = [(Ab of sample - Ab of negative control) / Ab of positive control] x 100.

Statistical evaluation of data

The evaluation of the obtained analytical data was performed by statistical means. All analyses were run in triplicate. The elimination of outliers was done by Grubb's test, for each method the arithmetic mean and the standard deviation were calculated by Statistica 7 program. Significant differences ($p \leq 0.05$) between the means were determined using Student`s t- test.

RESULTS AND DISCUSSION

The results of investigation of antioxidant capacity, activity toward cholineesterase and hemolytic activity are given in Tables 1 and 2, and Fig. 1 and 2.

From the results it can be seen that:

- The *G. verum* methanol extract has much higher antioxidant capacity compared to T. pratensis methanol extract. Total flavonoid content was fivefold higher, total phenol content two times higher, and reducing power toward Fe3+ approximately four and half times higher. In DPPH• assay the lower IC50 (extract concentration required for 50% inhibition of the DPPH radical absorbance), means a better radical scavenging ability [23]. G. verum extract has 2.2 time lower IC50 than T. pratensis extract, and 1.5 times lower than commercial antioxidant BHT, which means that its antioxidant capacity was 2.2 and 1.5 higher than capacity of *T. pratensis* and BHT, respectively.

- Almost twenty times higher concentration of Trolox equivalent (125.14 mg Trolox/ g dry extract) was found in G. verum extracts than in T. pratensis extracts (6.31 mg Trolox/ g dry extract).

Table 1. *In vitro* antioxidant activity, total phenols content, total flavonoids content and inhibiton of humane serum cholinesterase of *T. pratensis* subsp. *pratensis* and *G. verum* L. methanol extracts

	T. pratensis	*G. verum*
Total flavonoids content (mg RE/g dry extract)	4.48 ±0.38	9.05 ±0.94
Total phenols content (mg GAE/g dry extract)	21.97±1.08	118.13 ±9.64
Reducing power assay Fe(III) to Fe(II) (μg of ascorbic acid/mL of extract)	15.66±0.67	70.31±2.95
DPPH• decolorization assays, IC_{50} (μg/mL of extract)	59.25±2.12	26.97±1.21
ABTS•+ radical cation decolorization assays (mg Trolox/ g dry extract)	6.31 ±0.66	125.14 ±9.98
Inhibiton of humane serum cholinesterase (%) *	-4.65 ± 0.08	+16.28 ± 0.09

Results are average ±SD for three independent observations

* Methanol extract of *T. pratensis and G. Verum* in concentration of 994.0 μg/mL GAE - gallic acid equivalents

RE - rutin equivalents

Table 2. Hemolytic activity (shown as percentage) of methanol exctracts *T. pratensis* subsp. *pratensis* and *G. verum* L. after first, second, third and fourth hour of incubation

Conc. (μg/ml)	1h			2h			3h			4h		
	T.pratensis	*G.verum*	*p-value*	*T.pratensis*	*G.verum*	*p-value*	*T. pratensis*	*G.verum*	*p-value*	*T. pratensis*	*G.verum*	*p-value*
100	1.389±0.45	0.967±0.30	0.124	2.043±0.66	1.042 ±0.65	0.067	1.699±0.08	0.445 ±0.25	0.000*	3.127±0.19	0.445 ±0.26	0.000*
250	2.908±0.27	0.989±0.22	0.000*	11.291 ±0.85	0.416 ±0.11	0.000*	19.443 ±2.21	0.224 ±0.19	0.000*	27.063 ±2.42	0.277 ±0.09	0.000*
500	26.259±2.40	1.200±0.30	0.000*	51.716 ±1.52	1.599 ±0.13	0.000*	56.547 ±3.16	0.396 ±0.27	0.000*	57.182 ±3.22	0.717 ±0.24	0.000*
750	89.063±13.60	1.981±0.20	0.000*	85.701 ±6.12	2.126 ±0.19	0.000*	85.651 ±9.84	1.123 ±0.22	0.000*	85.244 ±3.22	1.275 ±0.21	0.000*

Results are average ±SD for three independent observations

*$p \leq 0.05$ is significantly different by comparing methanol extracts *T. pratensis* subsp. *pratensis* and *G. verum* L.

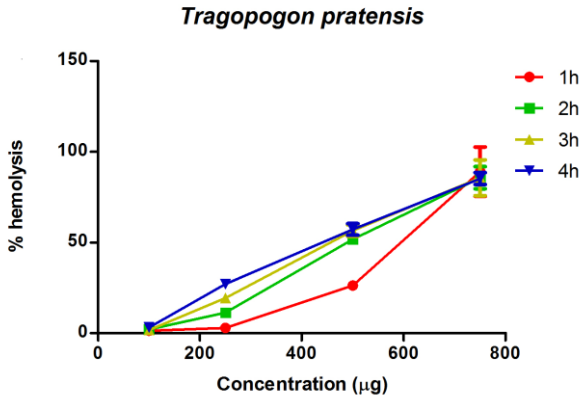

Fig. 1. Percentage of hemolysis MeOH exctracts *T. pratensis* subsp. *pratensis* after first, second, third and fourth hour of incubation.

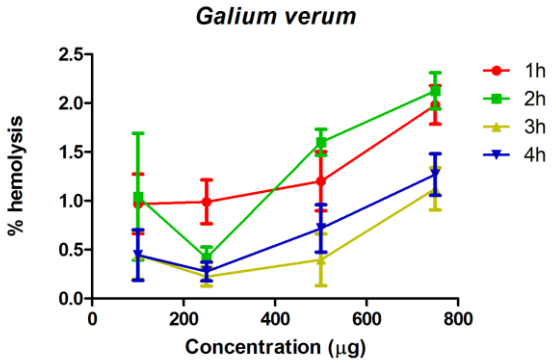

Fig. 2. Percentage of hemolysis MeOH exctracts *G. verum* L. after first, second, third and fourth hour of incubation.

- Methanol extract of *T. pratensis* in concentration of 994.0 µg/mL showed only 4.65 ± 0.08 % inhibiton of humane serum cholinesterase, while G. verum in the same concentration, showed slight activation of the enzyme (+16.28 ± 0.09 %) in comparison to neostigmine bromide 30 µg/mL solution, applied as refrent standard, that showed ihibition of -35.26 ± 0.12 %.Also, extract of T. pratensis could be considered as very weak inhibiotor of human serum cholinesterase, but G. verum didn't exibit inhibition capacity at all. On the contrary, it acted as an activator of cholinestrase and in that way could be treated as potentially harmful, concerning Alzheimer disease treatment. Though in our experiments pooled human serum was used as source of cholinesterase, obtained data are trustworthy in estimating potentias of the studied plants as a source of neurological valuable compounds.

- Methanol extract of *T. pratensis* shows hemolytic activity for each test exposure period (Table 2 and Fig. 1) with the highest percentage of 89% while the highest percentage for G. verum extract was 2.126%.

For *T. pratensis* extract hemolytic activity depends on the concentration and length of exposure proportionally. For G. verum extract (Table 2 and Fig. 2) applies the same, except for the concentration of 250 t µg/mL. After the first hour of incubation 50% of hemolysis induced concentration of 501.97 ± 32.65 µg/mL, the second hour 499.98 ± 38.45 µg/mL, 498.656 ± 39.85 µg/mL of the third and fourth 421.33 ± 34.98 µg/mL.

Observed results can be explained by the chemical composition of the extracts. The phytochemical screening of G. verum extracts and T. pratensis extracts has shown the presence of saponins (tragopogonosides) in T. pratensis extracts [24].

Saponins are natural surfactants. Increasing the hemolytic activity of methanol extracts of *T. pratensis* in a dose-dependent manner may be explained by Fick's law, diffusion flux from a membrane is proportional to concentration difference of both sides. So the increase in the concentration of saponins in contact with the cell membrane leads to its diffusion in the membrane itself, which leads to membrane rupture and hemolysis of erythrocytes [25].

-As far as we know there are no data concerning the antioxidant, hemolytic and cholinesterase inhibition properties of Tragopogon pratensis subsp. pratensis methanol extracts as well as for G.verum hemolytic and cholinesterase activities.

Water and methanol extracts of G. verum in the range 50-500 mg/L have been the subject of research of Ahmet M. et all [26]. Methanol extract of G. verum has higher antioxidant activity than it's water extract. The values of published data can not be compared with our results, because of the different experimental protocols and expression of results.

Our results are comparable with the results published by Lakić et al. [5]. The level of G. verum phenolics (2.44–4.65 mg and 4.57–5.16 mg GAE/g dry extract) was less than we found (118.13 mg GAE/g dry extract). Also, our IC50 value for G. verum extracts (26.98 µg/mL), was higher than privously published (3.1-8.04 mg/L).

CONCLUSIONS

The results of exhibited antioxidant activities are a good reference for performing it's in vivo tests, and qualify it as a promising natural source of antioxidants. On the other hand T. pratensis subsp. pratensis methanol extract might be a good

hemolytic agent. T. pratensis could be considered as very weak inhibiotor of human serum cholinesterase, but G. verum didn't exibit inhibition capacity at all. On the contrary, it acted as an activator of cholinestrase.

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АНТИОКСИДАНТНИ И ХЕМОЛИТИЧНИ СВОЙСТВА И ИНХИБИРАНЕ НА ХОЛИНЕСТЕРАЗА ЧРЕЗ *Galium verum* L. И *Tragopogon pratensis* subsp. *pratensis*

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(Резюме)

Определени са антиоксидантните свойства на метанолови екстракти от *Galium verum* L. (еньовче) и *Tragopogon pratensis* subsp. *pratensis* (полска козя брада), брани от различни места в Сърбия. Определяно е общото съдържание на феноли и флавоноиди, както и DPPH$^{\bullet}$ (1,1-дифенил-2-пикрилхидразил)-радикал-отстраняваща активност, ABTS$^{\bullet+}$ (2,2-азинобис-(3-етилбензтиазолин-6-сулфонова киселина) радикал-катионово обезцветяване и по ферицианиден метод. Метаноловите екстракти от *G. verum* показват по-силна активност за отстраняване на свободни радикали (IC_{50}-стойности при 26.98 μg/mL за обезцветяване на DPPH$^{\bullet}$ и 125.14 mg Trolox/g сух екстракт за ABTS$^{\bullet+}$-радикал-катионово обезцветяване) и обща редукционна способност (70.31 μg/mL), отколкото метаноловите екстракти of *T. pratensis* (SC_{50}-стойности при 59.25 μg/mL for DPPH$^{\bullet}$ - обезцветяване и 6.31 mg Trolox/g сух екстракт за ABTS$^{\bullet+}$-радикал-катионово обезцветяване и 15.66 μg/mL за обща редукционна способност). Наблюдаваните резултати може да се обяснят с много по-високото съдържание на феноли и флавоноиди в екстрактите от *G. verum*s. С помощта на модифицирания метод на Ellman's method се покзва, че метаноловите екстракти от *G. Verum* показват слаба активност на човешката серумна холинестераза (16.28 ± 0.09 %), докато екстрактите от *T. pratensis* имат слабо инхибиращо действие спрямо същия ензим (4.65 ± 0.08 %). Също така, метаноловите екстракти от *G. verum* и *T. pratensis* са изпитани *in vitro* с помощта на стандартния метод на Drabkin's за да се оцени тяхната хемолитична активност. Екстрактите от *T. pratensis* показват много силна хемолитична активност (след първия час на инкубиране 50% или of 501.97 ± 32.65 μg/mL, след втория час - 499.98 ± 38.45 μg/mL, 498.656 ± 39.85 μg/mL след третия и 421.33 ± 34.98 μg/mLслед четвъртия), докато екстрактите от *G. verum* имат слаба хемолитична активност.