Determination of Antioxidant Components and Activity of *Tamarix ramosissima* Comparative with *Vaccinium myrtillus* on Streptozotocin-diabetic Mice

LUCIANA TEODORA ROTARU[1], RENATA MARIA VARUT[2]*, MIHAI BANICIOIU COVEI[3], IRINA IULIANA COSTACHE[4], MARIUS NOVAC[5], OANA NICOLAESCU[2], CRISTINA FLORESCU[6], ALINA PETRICA[7], ROXANA KOSTICI[2], DANIELA CIOBANU[8]

[1] University of Medicine and Pharmacy of Craiova, Emergency & First Aid Department, Emergency Department, Emergency County Hospital Craiova, 2-4 Petru Rares Str., 200349, Craiova, Romania

[2] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, 2-4 Petru Rares Str., 200349, Craiova, Romania

[3] University of Medicine and Pharmacy of Craiova, Emergency & First Aid Department, Emergency Compartment, Filantropia Hospital Craiova, 2-4 Petru Rares Str., 200349, Craiova, Romania

[4] Grigore T. Popa University of Medicine and Pharmacy, Faculty of Medicine, 1st Medical Department, 16 Universitatii Str., 700115, Iasi, Romania

[5] University of Medicine and Pharmacy of Craiova, Anesthesiology and Intensive Care, Emergency County Hospital Craiova, 2-4 Petru Rares Str., 200349, Craiova, Romania

[6] University of Medicine and Pharmacy of Craiova, Cardiology Department, Emergency County Hospital Craiova, 2-4 Petru Rares Str., 200349, Craiova, Romania

[7] Emergency Department, Emergency County Hospital Timisoara, 156 Liviu Rebreanu Str, Nr., 300723, Timisoara, Romania

[8] University of Medicine and Pharmacy of Craiova, Internal Medicine Department, Emergency County Hospital Craiova, 2-4 Petru Rares Str., 200349, Craiova, Romania

Tamarix ramosissima (Tamaricaceae) is a small tree that grows spontaneously in Europe and Asia, being considered an invasive species in geographical areas with warm climates. The chemical composition is partially elucidated, being empirically used for antiinflammatory, analgesic, antibacterial and antioxidant effect. Our study aimed to evaluate the total polyphenol and flavonoid content of vegetal extracts and to test in vivo antioxidant therapeutic effect of it, comparative with Vaccinium myrtillus, using streptozotocin-induced diabetic mice. After five weeks the animals were sacrificed and we determined erythrocyte activities of superoxide dismutase, glutathione peroxidase, glutathione reductase and level of lipid peroxides as thiobarbituric acid reactive substances. Antioxidant enzymes had highest activities in mice treated with T. ramosissima extract and the level of lipid peroxides was the lowest. The tested extract had higher content of polyphenols comparative with V. myrtillus. Our results sustain the efficiency of T. ramosissima extracts on normalizing the effects of oxidative stress in diabetes.

Keywords: Tamarix ramosissima, antioxidant effect, streptozotocin, vegetal extract

Diabetes is a major source of morbidity, mortality, and economic cost to society. The prevalence of diabetes is increasing worldwide due to population growth, aging, urbanization and obesity caused by physical inactivity [1]. Therapies, consumable or behavioural have the capacity to reduces the impact of oxidative stress, may be beneficial to deplete diabetic associates interventions.

Our body is exposed all the time to free radicals which can transfer their free unpaired electron producing cellular oxidation. Fortunately it has endogenous antioxidant systems or obtains exogenous antioxidants from diet and neutralizes this species, keeping the homeostasis of body. Any imbalance between the reactive species and antioxidants causes oxidative stress characterized by lipid peroxidation, impaired glutathione metabolism and decreased Vitamin C levels [2].

Increased level of MDA in diabetics suggests that peroxidative injury may be involved in the development of diabetic complications, being also an indication of decline in defence mechanisms of enzymatic and nonenzymatic antioxidants. Oxidized lipids are able to produce MDA as a decomposition product and the mechanism is thought to involve formation of prostaglandins, like endoperoxides, from polyunsaturated fatty acid (PUFA) with two or more double bonds.

Glutathione (GSH), a tripeptide, γ-L-glutamyl-L-cysteinylglycine, is present in all mammalian tissues at 1-10 mM concentrations, as the most abundant nonprotein thiol that defends against oxidative stress. It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level. There are several reports that claim reduced level of GSH in diabetes and this can be one of the factors in the oxidative DNA damage in type 2 diabetics. As a consequence of increased oxidative status, GSH showed the frequent alteration in its concentration.

Overexpression of SOD or the supplements of antioxidants including SOD mimetics, targeted to overcome oxidative stress, reduce ROS, and increase antioxidant enzymes, has been shown to prevent diabetes mellitus. Decline in the level of SOD in diabetic tissue and blood has been reported in many studies [3, 4].

The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators and reductants of ferryl hemoglobin. Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity [5].

*email:rennata_maria@yahoo.com

#All authors have equally contributed to this work

Various metabolic diseases such as diabetes, adiposity and cardiovascular complications can be treated by plant extracts, which can be affordable, easy to administer and have fewer side effects than pharmaceutical interventions [6].

Tamarix ramosissima Ledeb. sin *Tamarix pentandra Pall.* - *(Tamaricaceae)*, commonly known as tamarisk or salt cedar is cultivated as ornamental plants in gardens [7]. The plant contains polyphenolic compounds such as flavonoids, phenolic acids, hydrolyzable tannins and coumarins, but the chemical composition is not completely elucidated [8]. Leaves of *T. ramosissima* are one of the oldest herbal medicines, used for the treatment of rheumatism and jaundice, having a possible antioxidant, astringent and antimicrobial activities [9].

In this context, this study aimed to evaluate the chemical composition, *in vitro* total polyphenol and flavonoid content and *in vivo* therapeutic effect of *Tamarix ramosissima (TR)* comparative with *Vaccinium myrtillus (VM)* on streptozotocin-induced diabetes in mice.

Experimental part

Ethical considerations

The research was conducted in accordance with University of Medicine and Pharmacy Craiova (UMFCV) guidelines on the international accepted conduct of experimental research and the internationally accepted principles for laboratory research. The research was conducted in accordance with the international standards presented in the guides care and use of animals in experimental models. The preclinical study protocol was approved by the University scientific Commission for ethics and deontology of UMFCV.

Preparing the sample

The vegetal products were used as tinctures, obtained by simple percolation, in a ratio vegetal product / solvent (ethanol 70º) of 1:5 (F.R. X). The control sample of each tested tincture is found in the Collection of the Laboratory of Pharmacognosy, Faculty of Pharmacy from Craiova [10].

Thin Layer Chromatography

The stationary phase used for the determination of flavonoidic heterozis, their corresponding aglycones and polyphenol carboxylic acids was silica gel aluminum plates of 20×20 cm activated 60 min at 105 ° C (G F254 Merck); mobile phase (A) ethyl acetate-formic acid -water (80:8:12, v/v/v), (B) toluene-dioxane-glacial acetic acid (80:25:4, v /v/v). The analyzed samples was: ethanol solution (ethanol, 70º) at 20% concentration (mobile phase A) and hydrolyzed ethanol solutions extracted with apolar solvent (mobile phase B). To obtain the hydrolyzed solution: 3 mL each of the tincture was refluxed, with electric bath, with 3 mL of 10% HCl solution for 30 min. After cooling they were extracted twice with 7 mL of diethyl ether. Apolar layers were combined, filtered through anhydrous sodium sulfate and brought to the residue, which was solubilized in 3 mL of absolute methanol. The reference solutions used: methanol solution of 0.1 mg/mL of rutin, ferulic acid (Fluka), hyperoside, isoquercitrin, kaempferol, luteolin, chlorogenic acid (Roth), quercetol (Sigma), caffeic acid (Merck). The amount applied to the starting line: 10 µL of the test sample/ reference solutions; tapes applied have a width of 1 cm, with 1.5 cm between them, the migration distance of 8 cm (mobile phase A); 16 cm (mobile phase B). Revelation: DFBOA reactive sputtering 10% ethanol solution; UV Examination (λ 365 nm) before and after the revelation, at a lamp Camag Reprostar 3 with Epson Phota Z incorporated camera [11].

Analysis by HPLC chromatography technique of flavonoids and polyphenol carboxylic acids of tinctures

Equipment and working conditions for HPLC analysis were as follows: HPLC Jasco MD-2015, two-pump, thermostat, UV-DAD detection system, degassing system; eluent A (acetonitrile); eluent B (0.1% phosphoric acid); working gradient: *prerun* →10% A, 90% B; 13.1 min.→ 22% A, 78% B; 14.1 min.→ 40% A, 60% B; 20.1 min.→ 40% A, 60% B; 50 mPA pressure; detection: 330 nm; retention times [min.] for flavonosids, flavonoid aglycones and polyphenol carboxylic acids: chlorogenic acid - 7.12, caffeic acid - 7.964, ferulic acid - 13.147, rutoside - 15.19, isoquercitrin - 15.68, rosmarinic acid - 17.58, apigenin-7-glucoside - 17.65, quercetol - 18.71, kaempferol -20.25 [12].

Tinctures analysis by gas chromatography coupled with mass spectrometry (GC–MS)

Tincture was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) to an apparatus Shimadzu GCMS-QP2010. The separation of the volatiles compounds was performed on a capillary column with weakly polar stationary phase, 5% phenyl, 95% metoxipoli siloxane, Alltec 15894, Ate 5 of 30 m length, internal diameter 0.32 mm and stationary phase thickness of 0.25 µm layer. The working parameters for GC-MS: injector temperature of 150°C, injection volume: 0.5 µL; 30 kPa pressure; total flow: 101.8 mL/min; column flow: 1.62 mL/min; linear velocity: 46.9 cm/s; split ratio: 60; purge flow 3 mL/min; carrier gas: helium; column temperature (temperature ramp) 80°C (1 min), 170°C (3 min), 200°C (3 min); MS detector: ion source temperature 220°C; The interface temperature 250°C; Scan speed 1666 u/s. Identification of the volatiles compounds was performed by comparing the mass spectra obtained with the data of the software from libraries (NIST 05) [13,14].

Evaluation of total polyphenols content (TPC)

TPC in plant ethanolic extracts was determined by using Folin–Ciocalteu colorimetric method based on the oxidation/reduction reaction as described by Waterhouse (2002) using gallic acid ranging between 0 and 1500 as standard for calibration. Determinations were carried out in triplicate, averaged and calculated from the calibration curve of gallic acid. An aliquot of 200 µL of each sample solution and each calibration solution or blank was pipetted into separated cuvettes and 2.5 mL Folin Ciocalteu reagent were added (pre-diluted with distilled water in a ratio of 1:10) and well mixed. Samples were left for four minutes at ambient temperature, then 2 mL of sodium carbonate solution concentration 75 g/L was added and shaked to mix. Samples were incubated for two hours at room temperature [15]. The absorbance was measured at 765 nm in a Able–Jasco V530 UV/Vis spectrophotometer. TPC in plant extracts was expressed in gallic acid equivalents (GAE)/L extracts.

Evaluation of flavonoids content

Flavonoid content was determined colorimetrically using the method of aluminum chloride. 0.5 mL tincture were added to 0.1 mL of 10% aluminum chloride solution, 0.1 mL 1 M potassium acetate, 2.8 mL of distilled water . The mixture was left at room temperature for a few minutes, and then evaluated with a spectrophotometer at 415 nm. The calibration curve was constructed with quercetol (QE) as reference, with concentrations ranging from 0 to 200 mg/L [16].

Determination of total carotenoids and chlorophylls a and b

Ethanolic solutions of plant extracts of the appropriate concentration (1.0 to 4.0 mg/mL) were analyzed in a UV/Vis spectrophotometer at 470, 653 and 666 nm. The concentrations of carotenoids and chlorophylls a and b were determined according to the equations reported by Lichtenthaler and Wellburn (1985), as follows:

Total carotenoids (mg/L) = 1000 Abs470 - 2.860 Ca - 129.2 Cb/245

Chlorophyll a (mg/L) = 15.65 Abs666 -7.340 Abs653

Chlorophyll b (mg/L) = 27.05 Abs653 -11.21 Abs666 [17].

Study design and blood sampling

Experiment was conducted on adult Swiss Albino mice distributed in four groups:

- Ist group – mice with normal pancreatic function
- IInd group – diabetic mice
- IIIrd group - diabetic mice treated with *TRFF* extract of 150 mg/kg b.w.
- IVth group – diabetic mice treated with *VM* extract, a plant product recognized for its antioxidant effect.

Diabetes mellitus inducing protocol in order to induce diabetes mellitus we used healthy, male Swiss Albino mice, weighing between 35-45 g aged between 6-8 weeks, which were provided by the Biobasis of the Faculty of Medicine and Pharmacy, Craiova. The mice acclimated to laboratory the diet and to the laboratory environment for one week prior to streptozotocin injection. The experiment was performed according to the standards set out in the guides to the care and use of experimental animals. The protocol of the study was approved by the Ethical and University and Scientific Commission of the Faculty of Medicine and Pharmacy, Craiova.

The animals were kept fasting (with free access to water in order to maintain an optimal hydration) 12 hours before the streptozotocin injection and three hours after the injection. In order to induce diabetes mellitus, streptozotocin was intraperitoneally injected, in an single dose of 180 mg/kg, the amounts injected being determined according to the animal body weight. All doses of streptozotocin were administered in volumes not exceeding 1mL/100 g body weight/mouse [18]. Mice weight and blood glucose were monitored before streptozotocin injection, and then 72 h after and one week after, the animals being kept fasting for 12 h before blood sampling for biochemical determinations. Animals with fasting blood glucose over 300 mg / dL, confirmed by two measurements (3 days and one week after the streptozotocin injection) were considered diabetic and were placed in research groups [19]. Mice glycaemia was measured with a glucometer eBsensor, taking blood from the tail vein.

Determination of oxidative stress biomarkers in blood

After five weeks, animals were sacrificed, blood samples were drawn in heparinised tubes, processed for plasma separation and erythrocytes hemolysis to determine level of lipid peroxides and activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR). Colorimetric method of measurement is carried out using a UV-Vis spectrophotometer Beckman model DU-65.

Erythrocyte hemolysis

0.5 mL of whole blood was centifuged for 10 min at 3000 rpm, plasma was aspirated and the cells were washed four times with 3 mL of physiologic solution, centrifuged for 10 min at 3000 rpm after each wash. The washed centrifuged erythrocytes were made up to 2 mL with cold redistilled water, mixed and left at 4°C for 15 minutes. Lysate was then diluted with 0.01M PB (phosphate buffer) solution *p*H 7 and used to assess enzymes activities.

Assessment of superoxide dismutase activity

SOD activity in erythrocytes was measured by the rate of inhibition of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium (INT) transformation by the superoxide generated from the reaction catalyzed by xanthine oxidase (RanSOD kit).

Assessment of glutathione peroxidase activity

GPx activity in erythrocytes was measured according variation of absorbance at 340 nm following the oxidation of GSH by cumene hydroperoxide and the reduction of GSSG with GR and NADPH, H+ (Ransel kit).

Assessment of glutathione reductase activity

GR activity was measured from the decrease in absorbance at 340 nm after the oxidation of NADPH,H+. when GSSG in reduced to GSH (Randox GR kit).

Assessment of lipid peroxidation

Malondialdehyde, marker of lipid peroxidation, was estimated as a thiobarbituric acid reactive substance (TBARS), using a solution of thiobarbituric acid (TBA) in TCA 20% [20].

Statistical analysis

The data were statistically evaluated with a two-sample t-test. The differences between the measured parameters were interpreted as important from a statistical point of view if the significance level $p < 0.05$. P-values much lower than 0.0001 indicate highly significant differences between groups. If $p > 0.05$, no significant differences of the measured parameters between the treated groups and the untreated (control) one exist, so the hypothesis is not tested.

Results and discussions

When analyzing polyphenol carboxylic acids, flavonoids and corresponding aglycons were identified caffeic acid, lutheol and kaempferol in *TRFF*. In *M-fr* extracts we identified chlorogenic acid, caffeic acid and rutoside.

Fig. 1.Chromatogram obtained before revelation (mobile phase A)1.*TRFF*; 2. *M-fr* 3.rutin, hyperoside, isoquercitrin (bottom-up); 4.chlorogenic acid, caffeic acid (bottom-up); 5.ferulic acid.

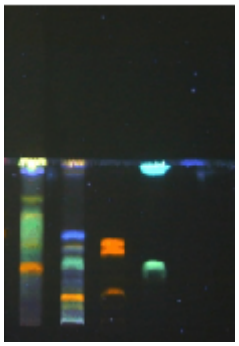

Fig. 2. Chromatogram obtained after revelation (mobile phase A) *1.TRFF* 2. *M-fr* 3.rutin, hyperoside, isoquercitrin (bottom-up); 4.chlorogenic acid, caffeic acid (bottom-up); 5.ferulic acid.

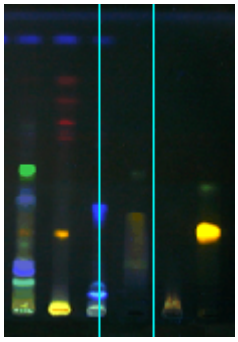

Fig. 3.Chromatogram obtained before revelation (mobile phase B)*1.TRFF* 2. *M-fr* 3.quercetol, kaempferol (bottom-up); 4.myricetin; 5.luteol

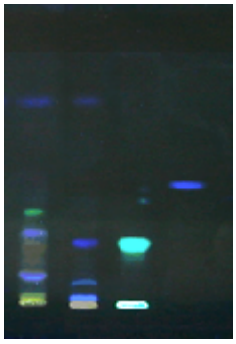

Fig. 4.Chromatogram obtained after DFBOA revelation, (mobile phase B) 1. *TRFF* 2. *M-fr* 3.chlorogenic acid; 4.ferulic acid.

Ferulic acid was absent in both extracts and we observed the presence of chlorophylls.

Retention times for the main flavonoside components, polyphenol carboxylic acids and flavonoid aglycone are shown in table 1. From the analysis of experimental results, we noticed that *TRFF* tincture contains quercetol 280.57 [µg/mL] and chlorogenic acid, kaempferol, apigenin-7-glucoside, apigenin isoquercitrin in traces. In *M-fr* tinctures we found chlorogenic acid, rutin and apigenin in traces.

GC-MS analysis carried out after the tinctures *TRFF* and *M-fr* library of spectra allowed the identification of a few of volatile compounds. The results of the GC-MS analysis of the volatile compounds from the tincture obtained by relating the retention time of the gas chromatograms of the data provided by the mass spectrometer, led to the observation that the extract *TRFF* contains 5 volatile compounds. For tincture *M-fr.*, in the mass spectra is characteristic the presence of ethyl-methyl carbonate (C4H8O3). Ethanimidic acid ethyl ester has been identified in both samples (table 2).

The extract *TRFF* had higher content of polyphenols than *VM* fructus and lower content of flavonoids and carotenoids.

At the end of the experiment, untreated diabetic group II had the lowest mean glutathione reductase value 46.3 ± 2.12 U / mL. *M-fr* tincture has antioxidant properties noted by the mean glutathione reductase enzyme of 52.5 ± 0.70 U / mL, higher than that of II group, 46.3 ± 2.12 U / mL. The highest glutathione reductase activity was obtained in group III (89.6 ± 0 U / mL), treated with 20% plant extract, 150 mg / kg body, from *Tamarix ramosissima*. The plasma level of the enzyme is much higher compared to group I ($p < 0.002108996$), group II ($p < 0.001197914$), group IV ($p < 0.000181582$).

At the end of the experiment, untreated diabetic group II had the lowest mean glutathione peroxidase 3415.5 ±

Table 1

HPLC CHROMATOGRAM RESULTS OF PLANT EXTRACTS

Tinctures	Chlorogenic acid [µg/mL]	Rosmarinic acid [µg/mL]	Kaempferol [µg/mL]	Quercetol [µg/mL]	Apigenin-7-glucoside [µg/mL]	Apigenin	Isoquercitrin [µg/L]
TRFF	+	-	+	280,57	+	+	+
M-fr.	-	-	-	-	-	-	-

Table 2

GAS CHROMATOGRAPHY AND IDENTIFIED BY MASS SPECTROMETRY PAINTINGS FOR VOLATILE COMPOUNDS

		TRFF	***M-fr***
Compound	**Chemical formula**	**RT (min)**	**%**	**%**
Ethyl-methyl carbonate	$C_4H_8O_3$	1.069	-	60.22
Ethoxyacetate acid	$C_4H_8O_3$	1.066	63.3	-
2-propen-1-ol acetate	$C_5H_7O_2$	1.125	12,46	-
methyl ester of 2-hydroxy-2-methyl-butanoic acid	$C_6H_{12}O_3$	1.162	1.88	-
1,1-diethoxy-ethane	$C_6H_{14}O$	1.506	1.44	-

Tincture	Total polyphenols (g GAE/L)	Flavonoids (g QE/L)	Carotenoids (g/L)	Chlorophyll *a* (g/L)	Chlorophyll *b* (g/L)
TRFF	1.352 ± 0.161	0.502 ± 0.030	2.453928794	0.0188857	0.0228656
M-fr.	0.634 ± 0.020	0.791 ± 0.015	4.881734066	0.0282459	0.0710778

Table 3
DETERMINATION OF ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS (DATA AS MEAN ± SD)

Groups	GR (U/ml)	GPx (U/L)	SOD (U/ml)	TBARS (mmol MDA/l)
Ist group - C	60.25 ± 1.91	4251.5 ± 28.99	203.8 ± 1.84	0.5350 ± 0.06
IInd group - D	46.3 ± 2.12	3415.5 ± 98.29	191.7 ± 10.75	2.6937 ± 0.007
IIIrd group -*TRFF*	89.6 ± 0	7124 ± 246.07	227.65 ± 10.39	0.3690 ± 0.014
IVth - *VM*	52.5 ± 0.70	4301 ± 11.31	194.15 ± 3.18	0.5904 ± 0.028

Table 4
OXIDATIVE STRESS BIOMARKERS IN BLOOD (DATA AS MEAN ± SD)

98.29 U / L value. *M-fr* tincture has antioxidant properties, resulting from the comparison of the mean value of the glutathione peroxidase group IV (4301 ± 11.31 U / L, with the average values of the group II (3415.5 ± 98.29 U / L) and group I (4251.5 ± 28.99 U / L).

Glutathione peroxidase had the highest activity in diabetic mice treated with *TRFF* tincture (7124 ± 246.07 U / L), with high levels of plasma compared to group I ($p < 0.003699559$), group II ($p < 0.002542898$), lot IV (p Â 0.003785492).

The highest superoxide dismutase level was obtained in group III, treated with 20% vegetable extract, 150 mg / kg body, derived from *TRFF*, having higher values than healthy group I, ($p < 0.001467685$), group II ($p < 0.00135763$), group IV ($p < 0.021000394$).

Recently, a clinical trial conducted in 2012 notes a correlation between lipid peroxidation, hyperglycemia, HbA1c level, and oxidative stress in diabetes [21].

The untreated diabetic group II had the highest lipid peroxide values of 2.6937 ± 0.007 mmol / L. The plasma level of lipid peroxides was lower in diabetic mice treated with *TRFF* group, compared to group I ($p < 0.324462$), untreated diabetic group II ($p < 0.000558$), respectively group IV ($p < 0.019979$).

Conclusions

Both plant extracts contains polyphenol carboxylates with antioxidant potential effect. Using CSS technique we identified in *TRFF* caffeic acid, lutheol, kaempferol and in *M-fr* extract chlorogenic acid, caffeic acid and rutoside. Ferulic acid was absent in both extracts and we observed the presence of chlorophylls. *TRFF* extract had higher content of polyphenols comparative with *V. myrtillus*.

Using HPLC we noticed that *TRFF* tincture contains quercetol 280,57 [µg/mL] and chlorogenic acid, kaempferol, apigenin-7-glucoside, apigenin isoquercitrin in traces and in *M-fr* tinctures we found chlorogenic acid, rutin and apigenin in traces. *TRFF* extract had higher content of polyphenols comparative with *V. myrtillus*.

Antioxidant status is unbalanced in diabetes, as demonstrated by using streptozotocin-induced diabetic mice.

Antioxidant enzymes had highest activities in mice treated with *T. ramosissima* extract and the level of lipid peroxides was the lowest, comparative with all the other groups. The tested extract had higher content of polyphenols comparative with *V. myrtillus*.

Our results sustain the efficiency of *T. ramosissima* extracts on normalizing the effects of oxidative stress in diabetes.

References

1.*** National Diabetes Statistics Report, 2014.

2. ULLAH, A., KHAN, A., KHAN, I., Diabetes mellitus and oxidative stress-A concise review, Saudi Pharm J, **24**, nr. 5, 2016, p. 547–553.

3.TIWARI, B. K., PANDEY, K. B., ABIDI, A. B., Markers of Oxidative Stress during Diabetes Mellitus, Journal of Biomarkers, 2013.

4. BUBULICA, M.V., CHIRIGIU L., Screening of antioxidant potential of Lonicera tatarica, Viburnum opulus and Sambucus ebulus L. by multiple in vitro assays, Journal of Medicinal Plants Research 6 (3), 2012, p. 544-552

5. SHARMA, S.K., SINGH, L., SINGH, S., A review on medicinal plants having antioxidant potential, Indian Journal of Research in Pharmacy and Biotechnology, **1**, nr. 3, 2013 p. 404.

6. SHORI, A. B., Screening of antidiabetic and antioxidant activities of medicinal plants, Journal of Integrative Medicine, 13, nr. 5, 2015.

7. ZOUHAR, K., Tamarix spp. Fire Effects Information System, Rocky Mountain Research Station, Fire Sciences Laboratory, 2014.

8. MAO, Z.J., Chemical Composition of T. Ramosissima in the activity of anti-inflammatory, analgesic and antioxidant and the effects on wound healing in mice skin, 2012.

9. SULTANOVA, N., MAKHMOOR, T., ABILOV, Z.A., Antioxidant and antimicrobial activities of Tamarix ramosissima, J Ethnopharmacol, 78, nr. 2-3, 2001, p. 201-205.

10. MAJEKODUNMI, S.O., Review of extraction of medicinal plants for pharmaceutical research, Merit Research Journal of Medicine and Medical Sciences, 3, nr. 11, 2015, p. 521-527.

11. VARUT, R.M., ROTARU, L.T., VARUT M.C., QSPR correlation of physic-chemical descriptors with the molecular surface area and Rf of ten polyphenolic compounds, separated from vegetal extracts by TLC, Rev.Chim.(Bucharest), **68**, no. 8, 2017, p. 1776-1779.

12. VARUT, R.M., ROTARU, L.T., GC-MS, HPLC, TLC of Dorycnium herbaceum Tincture Species and Synergistic / Antagonist Effect Testing in Combination with Antibiotics, Rev. Chim. (Bucharest), **68**, nr. 2, 2017, p.228-231.

13 VARUT, R.M., ROTARU, L.T., Determination of polyphenol and flavonoid profiles and testing the antibacterial effect of Acanthus longifolius comparative with Vaccinium myrtillus, Rev. Chim. (Bucharest), **68**, no. 7, 2017, p. 1419- 1422.

14. CHIRIGIU, L., CHIRIGIU, R.G., TIRCOMNICU, V., BUBULICA, M.V., GC-MS analysis of chemical composition of Sambucus ebulus leaves, Chemistry of natural compounds 47 (1), 2011, p. 126-127
15. BORNEO, R., LEON, A.E., AGUIRRE, A., et al., Antioxidant capacity of medicinal plants from the Province of Cordoba (Argentina) and their in vitro testing in a model food system, Food Chemistry, 112, nr. 3, 2009, p.664–670.
16. LAYZON, A., PEZZINI B., SOARES, L., Spectrophotometric determination of the total flavonoid content in Ocimum basilicum L. (Lamiaceae) leaves, Pharmacogn Mag., 11, nr. 41, 2015, p. 96–101.
17. LICHTENTHALER, K.H., WELLBURN, A.R., Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents, Biochemical Society Transactions, 11, nr. 5, 1983, p. 591-592.
18. GOUD, B.J., DWARAKANATH, V., CHIKKA, B.K., Streptozotocin - A Diabetogenic Agent in Animal Models, Human Journals, 3, nr. 1, 2015, p. 253-269.
19. DEEDS, M.C., ANDERSON, J.M., ARMSTRONG, A.S., GASTINEAU, D.A., HIDDINGA, H.J., JAHANGIR, A., EBERHARDT, N.L., Single Dose Streptozotocin Induced Diabetes: Considerations for Study Design in Islet Transplantation Models, 2011, 45, nr. 3, p. 131–140.
20. VARUT R.M., GIRD C.E., ROTARU L.T., VARUT M.C., PISOSCHI C.G.,Pharmaceutical Chemistry, vol 51, no. 12, martie, 2018
21. ARULSELVAN, P., UMAMAHESWARI, A., FAKURAZI, S., Therapeutic approaches for diabetes with natural antioxidants, Medicinal Plants as Antioxidant Agents: Understanding Their Mechanism of Action and Therapeutic, 11, 2012, p. 243-245.

Manuscript received: 16.01.2018