

# The Study of Antioxidant Capacity in Extracts from Vegetal Sources with Hypoglycaemic Action

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*In the dental office, diabetes mellitus is a chronic affection that many patients suffer. Apart from the treatment of all diabetic patients, they use homeopathic medicines. We consider the importance of knowing these extracted hypoglycemic plants and their way of acting by the dentist. In this study we investigated some extracts obtained from vegetal sources used by hypoglycaemic action: Juglans regia L. -nut, Morus nigra L. -black mulberry, Olea europaea L. -olive. We followed the level of antioxidant compounds and the antioxidant capacity in the alcoholic extracts. Our results indicate higher antioxidant efficiency in the extracts from young plant parts, with meristematic tissues, and also a modified phytochemical profile, compared to the extract from mature plant parts.*

**Keywords:** *Juglans regia L., Morus nigra L., Olea europaea L., phenolic compounds, flavonoids, antioxidant capacity*

Type 2 diabetes mellitus consists of an array of dysfunctions characterized by hyperglycemia and resulting from the combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion, affects a growing number of children [1-3].

There is a special interest for the use of medicinal plants in the treatment of diabetes, in parallel with medical treatment in order to improve the quality of life of the patients.

It was demonstrated that the antidiabetic properties of the plants are attributed mostly to the content of antioxidant compounds [1-6].

The meristemotherapy, or its more used denomination gemmotherapy, was founded as a distinct branch of the phytotherapy at the begin of the 20th century [7].

Buds and young shoots are parts of plants with meristematic tissues, vegetative parts that are used in gemmotherapy. Gemmotherapy or meristemo-therapy is a field of phytotherapy, which uses buds, young shoots of trees and shrubs. They are harvested in spring, in their natural development cycle, and they are processed fresh, in a mixture of water, alcohol and glycerin.

Buds and young shoots are made up of growing embryonic tissues, containing the entire development power of the future plant, in which the plant's vitality is concentrated. These vegetative organs contain many active compounds that no longer will be present in the mature plant. In addition, because they contain vitamins, oligoelements and minerals, they are richer in nucleic acids and growth hormones than other vegetal tissues [7-18].

## Experimental part

In this study we investigated some extracts obtained from vegetal sources with hypoglycaemic action. We followed the composition of phenolic compounds and total flavonoid contents. Antioxidant capacity of the extracts

was evaluated by using DPPH method, FRAP method, CUPRAC assay, SNP method, and by determination of nitric oxide (NO) inhibition.

## Plant materials

There were studied 3 species known for hypoglycemic effect, *Juglans regia L.* -Nut, *Morus nigra L.* -black mulberry, *Olea europaea L.* -olive.

From these species the following vegetative parts were harvested and processed: buds in case of nut and black mulberry; young shoots in case of the olive and leaves in case of nut, black mulberry and olive;

These are processed exclusively in the fresh state, using as extraction solvent a mixture of glycerin -ethanol 96 % vol. (1:1). The extraction ratio is 1:20, vegetal material expressed as dry -extraction solvent. Buds and branches have a humidity of around 40-50 %. Extraction is carried out by cold maceration (room temperature, max. 30°C), by periodic stirring, 2 x 10 min/day for 20 days, followed by decantation, pressing the vegetal material impregnated with solvent, and mixing the extractive solutions (decantation + pressing). The solutions prepared this way, were used for the analysis in this study [7, 19-23].

The nut and black mulberry leaves were processed fresh. The moisture content of the vegetal material is about 65-70 %, and the extraction ratio is 1:5 dry vegetal material-extraction solvent. The extraction solvent was ethyl alcohol of 90 % vol.

The olive leaves were processed dry, the extraction ratio is 1:10, vegetal material - ethylic alcohol of 70 % vol. Extraction was carried out in all cases by cold maceration (room temperature, max. 30°C), by periodical stirring, 2 x 10 min /day, for 10 days, followed by decantation, pressing of the vegetal material impregnated with solvent, and mixing the extractive solvents (decantation + pressing), and after 5 days of rest, extractive solutions were filtered. These solvents were used for the analyses in this study [7, 19, 22, 23].

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### Study of the bioactive compounds of plant extracts

The determination of total flavonoid contents expressed in rutoside

Total flavonoid dosing was performed spectrophotometrically in the visible domain, based on the aluminum ion coordination to the hydroxyl and ketone groups of the flavonoid structure, and the formed complex has a yellow color. Quantitative expression was performed in rutoside, building for this purpose a calibration curve in the rutoside, under similar conditions to the determination performed on the samples. To each 0.1 mL of the described extracts is added 5 mL of 10 % sodium acetate and 3 mL of 2.5 % aluminum chloride. Each solution is brought to 25 mL with methanol. As standards were used metallogenic solutions of rutoside, having concentrations between 4 and 20  $\mu\text{g/mL}$ , processed similarly to the samples. Reading is done at 430 nm, read after 30 min of rest, in triplicate [19, 24-28].

The calibration curve (fig. 1) was obtained with a solution of known concentration of rutoside.

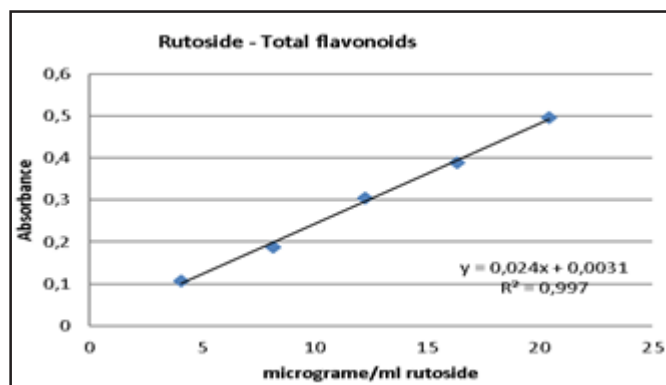


Fig. 1. The calibration curve made with rutoside - total flavonoid contents determination

### The determination of total phenolic contents expressed in caffeic acid

Total phenolic acid dosing was performed spectrophotometrically in the visible domain, based on the formation of a blue colored complex between wolfram ions and hydroxyl groups. Quantitative expression was performed in caffeic acid, building for this purpose a calibration curve in the rutoside, under similar conditions to the determination performed on the samples. To each 0.1 mL of the described extracts is added 0.5 mL phosphotungstic reagent. Each solution is brought to 25 ml with sodium carbonate of 15 %. As standards were used metallogenic solutions of caffeic acid, having concentrations between 1 and 3  $\mu\text{g/mL}$ , processed similarly to the samples. Absorbance was measured at 715 nm, read after 2 minutes of rest, in triplicate [19, 24-29]. The calibration curve (fig. 2) was obtained with a solution of known concentration of caffeic acid.

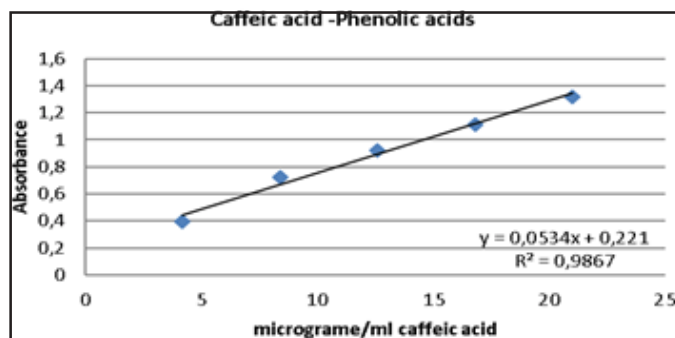


Fig. 2. The calibration curve made with caffeic acid - total phenolic contents determination

### Study of the antioxidant capacity

#### DPPH method

Radical scavenging activity of plant extracts against stable 2,2-diphenyl-2-picryl-hydrazyl-hydrate (DPPH) was determined by the slightly modified method of Brand-Williams *et al* 1995 [25]. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The change in color (from deep violet to light yellow) was measured at 517 nm on a UV visible light spectrophotometer. The solution of DPPH in methanol  $6 \cdot 10^{-5}$  M was prepared fresh daily before UV measurements. The samples were kept in the dark for 15 min at room temperature and the decrease in absorbance was measured. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = [(AB - AA)/AB] \times 100$$

where AB = absorption of blank sample (t= 0 min), AA = absorption of test extract solution (t=15 min) [24-29].

#### FRAP method (ferric reducing antioxidant power)

FRAP method is a simple spectrophotometric method that assesses the antioxidant power of the studied samples, being based on the reduction of ferric tripyridyltriazine complex [Fe(III)-TPTZ] by a reductant, at an acid pH. The stock solutions included: 300 mM acetate buffer; 270 mg  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$  dissolved in 50 mL distilled water; 150 mg TPTZ and 150  $\mu\text{L}$  HCl, dissolved in 50 mL distilled water. The working FRAP solution was freshly prepared by mixing 50 mL acetate buffer, 5 mL  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$  solution and 5 mL TPTZ solution. Trolox was used as a standard solution, the calibration curve was made for concentrations between 0-300  $\mu\text{M}$ , having the correlation coefficient  $R^2=0.9956$  and the regression equation ( $y=0.0017x+0.0848$ ), where y represents the absorbance detected at 595 nm. The results are expressed as  $\mu\text{mol}$  Trolox equivalents (TE) / 100  $\mu\text{L}$  extract [30-36].

#### CUPRAC assay (Cupric ions ( $\text{Cu}^{2+}$ ) reducing power)

In order to determine the cupric ions ( $\text{Cu}^{2+}$ ) reducing antioxidant capacity the method proposed by Karaman *et al.* (2010) was used with slight modifications [30]. To this end, 0.25 mL  $\text{CuCl}_2$  solution (0.01 M), 0.25 mL ethanolic neocuproine solution ( $7.5 \cdot 10^{-3}$  M) and 0.25 mL  $\text{CH}_3\text{COONH}_4$  buffer solution (1 M) were added to a test tube, followed by mixing with the plants extracts. Then, total volume was adjusted to 2 mL with distilled water, and thoroughly mixed. The tubes were stoppered and kept at room temperature. Absorbance was measured at 450 nm against a reagent blank 30 min later. Increased absorbance of the reaction mixture indicates increased reduction capability [36-38].

#### SNP Method (silver nanoparticles)

Determination of antioxidant capacity by the SNP method was performed spectrophotometrically in the visible domain, based on the reduction of silver ion to colloidal silver, with the formation of fine nanoparticles, the color changing from colorless to yellow or even brown. Quantitative expression was performed in  $\mu\text{M}$  trolox equivalent/100 ml extract. The extracts were diluted with purified water, so as to reach the concentrations provided in Table 2. 0.8 ml of each sample is mixed with 2 mL of SNP reagent, obtained from silver nitrate 10mM and sodium citrate of 1 % at boiling. The solutions are kept for 30 min at room temperature. As standards were used trolox metallogenic solutions, having concentrations between 20

and 180 µg/0,8 mL, processed similarly to the samples. Absorbance was measured at 423 nm, performed in triplicate [39].

#### Determination of nitric oxide (NO) inhibition

Determination of the NO radical was performed spectrophotometrically in the visible domain, based on the generation of NO radical from sodium nitroprusside at pH 7.4, followed by a diazotization reaction with Griess reagent. Quantitative expression was performed as a percentage of inhibition. Inhibition was calculated with the following formula:

$$I\% = (A_{ref} - A_p) * 100 / A_{ref}$$

Extracts were diluted with methanol so that to reach the concentrations provided in table 2. 0.5 mL of each sample is mixed with 3 mL of sodium nitroprusside solution in phosphate buffer 0.2 M of pH 7.4. Solutions are kept at 30°C for 150 min. After cooling, 0.5 mL of Griess reagent is added. As standards was used 0.5 mL ethanol of 70 % or glycerin mixture -ethyl alcohol of 96 % vol. (1:1) processed similarly to the samples. Absorbance was measured at 546 nm, performed in triplicate [40].

#### Results and discussions

Figures 3-6 show the inhibition and trolox curves for antioxidant capacity determinations.

In table 1 are presented the equations of the curves mentioned above, and in figures 7, 8, 9, 10, 11, 12, 13 the results of the spectrophotometric determinations.

The nut leave extract has the highest concentration of flavonoids, and the buds of phenolic acids. The maximum antioxidant activity may be found in the extract of nut buds by DPPH, FRAP, SNP methods and inhibits the most powerful NO radicals, while with CUPRAC method, the extract from black mulberry shoots is indicated to have the strongest antioxidant effect (figs. 9, 10, 11, 12, 13).

The results do not show a linear or direct correlation between the antioxidant effect and the content of flavonoids or phenolic acids (figs. 7, 8).

In the series of DPPH determinations may be observed, that Nut and Olive has even a more powerful antioxidant effect than 50.4 µg of trolox, antioxidant standard. In this series may be observed correlations between the higher content of polyphenols and the stronger antioxidant effect. But in case of Olive branches it can be concluded clearly that other active compounds besides polyphenols contribute to significant antioxidant activity.

Regardless the used method, the most powerful antioxidant is the extract from nut shoots, which also has a higher concentration of polyphenols, especially flavonoids, which will determine this effect. But the type of these polyphenols also determines a high inhibition of NO radicals, as a result of the effect on the cardiovascular system and endothelium will be decreased in case of the Nut, regardless the part of plan used.

Taking into account the extraction ratio, especially the extracts from buds and branches have a higher antioxidant effect than hydroalcoholic extracts from mature plant parts.

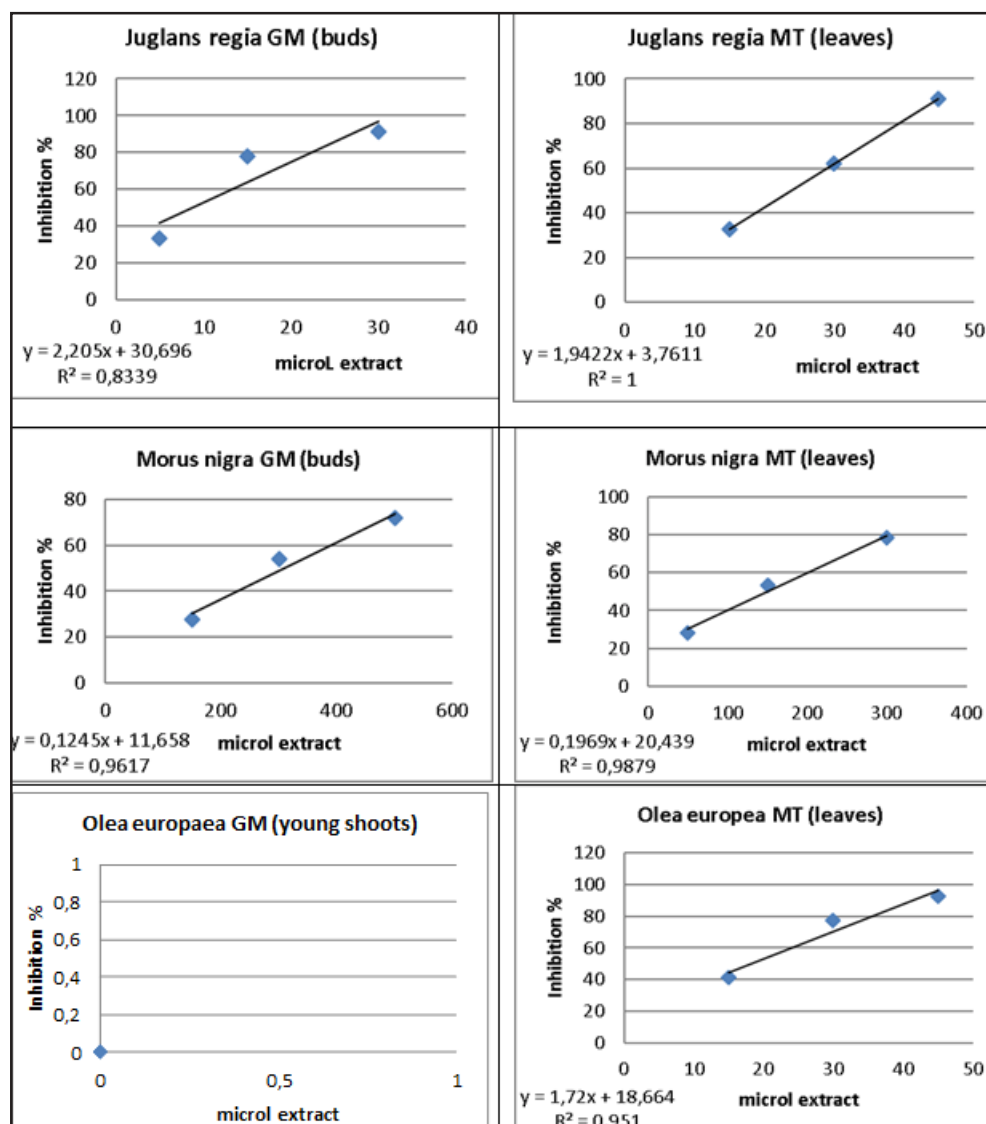


Fig. 3. The inhibition curves by DPPH method

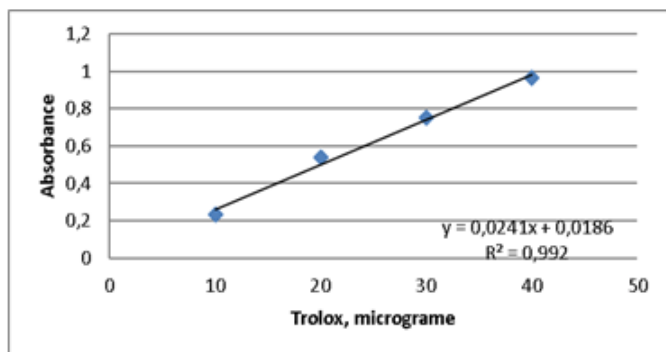


Fig.4. The Trolox curves by the FRAP method - for antioxidant capacity determinations

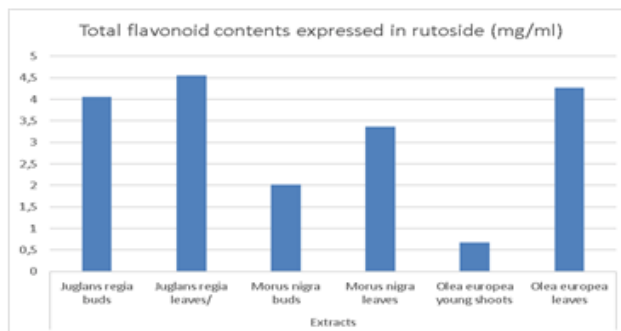


Fig.7. Total flavonoid contents expressed in rutoside (mg/mL)

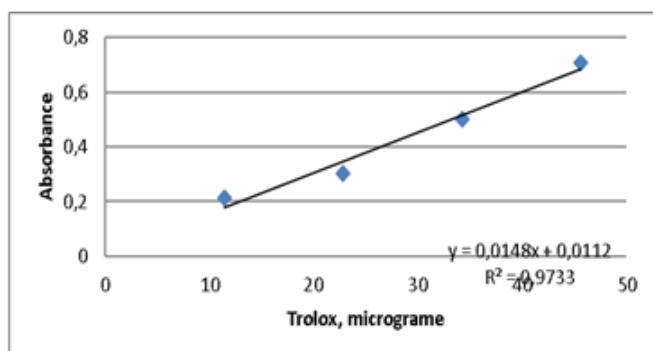


Fig.5. The Trolox curves by the CUPRAC method - for the antioxidant capacity determinations

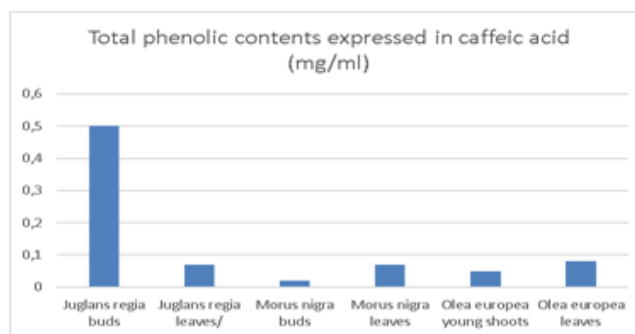


Fig.8. Total phenolic contents expressed in caffeic acid (mg/mL)

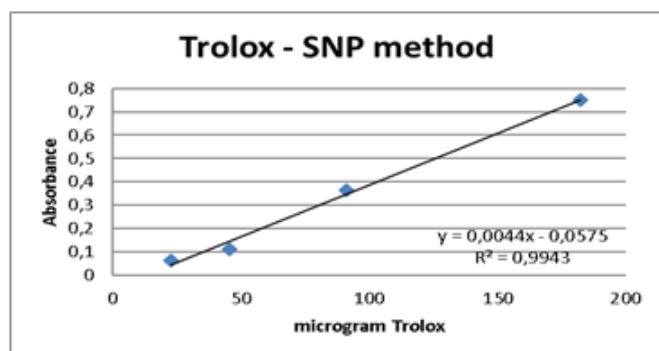


Fig.6. The Trolox curves by the SNP method - for the antioxidant capacity determinations

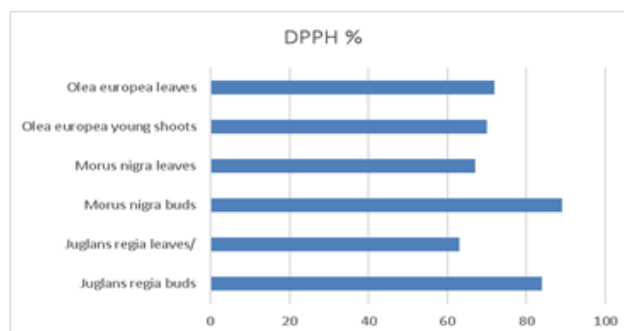


Fig.9. Antioxidant capacity - DPPH (%)

These indicate the higher therapeutic potential of the gemmotherapy extracts.

The inhibition potential of NO radical is low in case of Black Mulberry and indicates even lower values in case of Olive. This indicates a cardiovascular effect also on vascular endothelium, more significant in case of these species, especially in Olive, this being known for the hypotensive effect.

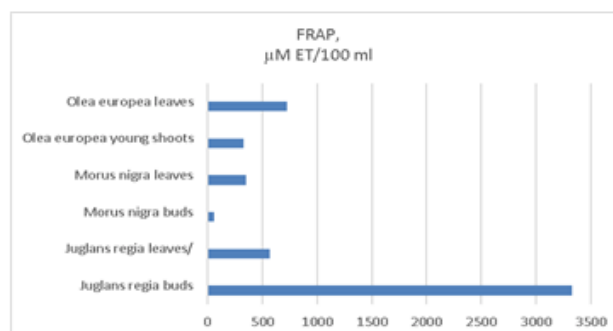


Fig 10. Antioxidant capacity - FRAP ( $\mu\text{M ET}/100 \text{ mL}$ )

Extracts/Method	Equation	Correlation factor, $R^2$
<i>Juglans regia</i> buds/ (DPPH)	$A = 2,205xc + 30,696$	0,8339
<i>Juglans regia</i> leaves/ (DPPH)	$A = 1,9422xc + 3,7611$	1,0000
<i>Morus nigra</i> buds /DPPH)	$A = 0,1245xc + 11,658$	0,9617
<i>Morus nigra</i> leaves /DPPH)	$A = 0,1969xc + 20,439$	0,9879
<i>Olea europea</i> young shoots / (DPPH)	$A = 1,394xc - 1,4007$	0,9197
<i>Olea europea</i> leaves/ (DPPH)	$A = 1,72xc + 18,644$	0,9510
Trolox (DPPH)	$A = 0,8014xc + 9,6235$	0,9972
Trolox (FRAP)	$A = 0,0241xc + 0,0186$	0,9920
Trolox (CUPRAC)	$A = 0,0148xc + 0,0112$	0,9733
Trolox (SNP)	$A = 0,0044xc - 0,0575$	0,9943

**Table 1**  
EQUATIONS OF THE CURVES



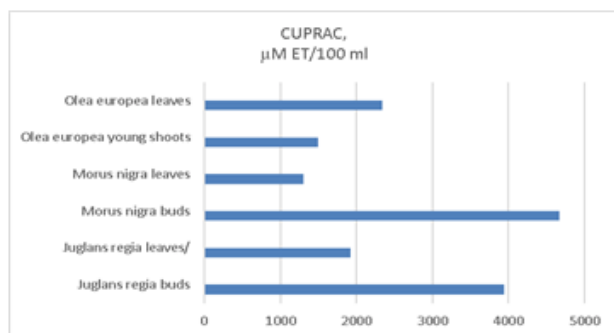


Fig 11. Antioxidant capacity -CUPRAC (µM ET/100 mL)

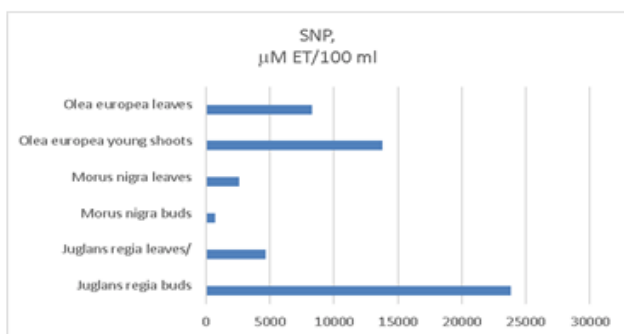


Fig 12. Antioxidant capacity -SNP (µM ET/100 mL)

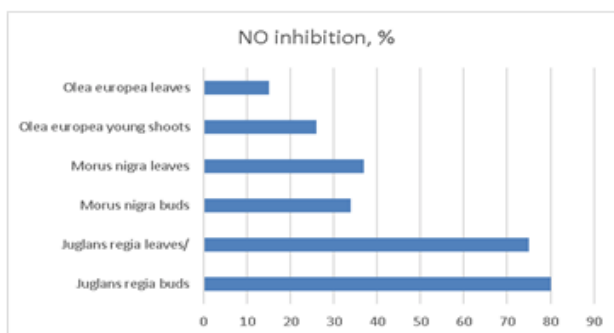


Fig 13. Antioxidant capacity -NO inhibition (%)

## Conclusions

In this paper we have conducted a study of some extracts from vegetal sources with hypoglycemic action.

The results of this study indicate higher antioxidant efficiency in case of extracts from young plant parts, with meristematic tissues, and also a modified phytochemical profile compared to the extract from mature plant parts.

The results also indicate a potential benefit of Black Mulberry and Olive on cardiovascular system and vascular endothelium, due to the weaker inhibition of NO radicals.

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