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BOTANICAL CHARACTERIZATION, PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF INDIGENOUS RED RASPBERRY (*RUBUS IDAEUS* L.) LEAVES

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ABSTRACT: In the present study, the botanical profile, chemical composition and antioxidant activity of indigenous red raspberry (*Rubus idaeus* L.) leaves were investigated. The microscopic characterization was determined by means of scanning electronic microscopy and transverse sections. Polyphenols, sterolic compounds and ascorbic acid were determined using thin layer chromatography, spectrophotometric and HPLC assays. The scavenger activity against 2,2-diphenyl-1-picrylhydrazyl free radical and the reducing power capacity were used for antioxidant activity evaluation. It was found that raspberry leaves are a source of tannins (9.88 g%), flavonoids (0.50 g%), phenolcarboxylic acids (1.13 g%) and sterols (0.89 g%). The HPLC assays reaveled a considerable amount of quercitrin (15.63 mg%), *p*-coumaric acid (17.55 mg%), ferulic acid (4.88 mg%) and ascorbic acid (4.39 g%). The antioxidant activity was weak compared to standard reference (chlorogenic acid). The herbal product is a source of natural compounds with moderate antioxidant activity.

Keywords: polyphenols, sterols, tannins, antioxidant capacity, scanning electron microscopy.

INTRODUCTION:

The *Rosaceae* family includes numerous genera (95-125) according to Pakistan's, Taiwan's and China's Flora and over 3000 species (Efloras, 2008). The family is divided into four subfamilies: *Spiroideae*, *Rosoideae*, *Maloideae* and *Prunoideae* (Ciocarlan, 2009).

Rubus genus includes over 41 species according to Romania's Flora, the most important are: *Rubus idaeus* L. (red raspberry), *Rubus fruticosus* L. (blackberry), *Rubus caesius* L. (European dewberry), *Rubus saxatilis* L. (stone bramble), *Rubus tomentosus* Borkh., *Rubus hirtus* W.K (Constantinescu, 2004; Cheers, 2003).

Red raspberry leaves (*Rubi idaei folium*) and fruits (*Rubi idaei fructus*) are used for therapeutic purposes.

Rubi idaei folium are an important source of: flavonoids (rutin – 52-234.6 mg/kg; hyperoside – 34.4-720.2 mg/kg; isorhamnetin-3-O-rutinoside – 176.4-2914.2 mg/kg; isorhamnetin-3-O-glucoside – 138.7-1000 mg/kg; kaempferol-3-O-glucoside – 266.9-1260.9 mg/kg; kaempferol-3-O-α-rhamnosil(1-2)[6-O-3hydroxi-3-methylglutaryl]-β-galactosidase; tiliroside =kaempferol–3–*O*–β–D-(6″- *O*- (*E*) – *p* - coumaroyl) glucopyranoside) (Barnes, 2007; Pelc et al., 2009; Porter et al., 2012), phenolcarboxylic acids (caffeic acid – 3.28-22.61 mg/kg; chlorogenic acid - 29.04-77.23mg/kg; rosmarinic acid – 6.57-30.82 mg/kg; ellagic acid – 20.26-127.4 mg/kg; gentisic acid and gallic acid) (Buricova et al, 2011; Pelc et al, 2009). Red raspberry leaves are also a source of tannins (2.09-15%) (sanguuin H6, lambertianin C,D) (Barnes, 2007; PDR, 2005; Patel et al., 2004; Ross et al., 2006), proanthocyanidins B1(Buricova et al., 2011), vitamins (ascorbic acid) (Fejer et. Al., 1970), mineral elements (zinc, manganese, vanadium, calcium, potassium, selenium) (Sikiric et. al., 2011; Kowalenko et al., 2005; Antal et al., 2009; Antal et al., 2010), sterols (stigmasterol, campestrol) (Patel at al., 2004) and volatile compounds (2-hexenal) (Istudor, 2009).

Regarding their therapeutic properties, red raspberry leaves have uterine relaxant effects (Holst et al., 2009; Evans, 2009), cytotoxic properties towards HL-60 leukemic cells (Skupien et al., 2006) and antibacterial effect against gram-positive (*Bacillus subtilis* and *Bacillus polymyxa)* and gram-negative microrganisms (*Azobacter* s.p., *Pseudomonas* s.p.) (Nikitina et al., 2007). *Rubi idaei folium* are also known for their antiinflamatory and antioxidant properties (Jean-Gilles et. al, 2012; Piwowarski et. al, 2011; Tosun et al., 2009).

The aim of our study was the determination of raspberry leaves botanical characteristics (through macroscopic and microscopic exams), their chemical composition and antioxidant capacity (scavenger activity of 2,2-diphenyl-1-picrylhydrazyil – DPPH free radical and reducing power activity).

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MATERIAL AND METHODS:

Material

Rubi idaei folium (red raspberry leaves) were collected in May 2012, from Jilava village (private garden), Ilfov county (44°37'N,26°07'E), Romania, before flowering. Leaves were air-dried in shade and stored in laboratory conditions. For macroscopic, microscopic exams and for ascorbic acid determination fresh leaves have been used.

Reagents and solvents

All reagents and solvents were purchased from Karl Roth (Germany) unless otherwise stated. 2,2-diphenyl-1-picrylhydrazyl (DPPH free radical) and diphenylboryloxyethylenamine (DPhBOA) were from Sigma-Aldrich (Germany). Trichloroacetic acid was acquired from Merck (Germany).

Botanical characterization (macroscopic and microscopic exams)

The identity of the herbal product was determined through macroscopic and microscopic examination. The macroscopic exam was performed on fresh leaves, using a Belphotonics Bio (ob. 3x, 4x, 4,5x) stereomicroscope. For microscopic examination we have used transverse sections, which were double-stained with iodine green and carmine alum.

For scanning electron microscopy (SEM) fresh leaves were fixed in formol : 70% ethanol : acetic acid = 5 : 90 : 5 (v/v/v) for 48 h, washed with distilled water and stored in 70% etahnol. After dehydration in a graded ethanol series (80%, 90% and 100%) and acetone, the material was critical point dried with CO_2 (EMS 850 critical point dryer), sputter-coated with a thin layer of gold (30 nm) (EMS 550X Sputter Coater) and examined by scanning electron microscopy (Tescan Vega II SBH) at an accelaration voltage of 22.78 kV (Gostin et al, 2011).

Thin layer chromatography analysis (TLC)

Thin layer chromatography was used for polyphenols and sterolic/triterpenic compounds identification. It was performed on aluminium-coated TLC plates (20 x 20 cm and 10 x 20 cm, Merck, Germany), which were kept for 1 h at 105°C before use. Several eluent systems were used: ethyl acetate : formic acid : water = 80:8:12 (v/v/v) (mobile phase I) (Wichtl, 2002); toluene : dioxane : acetic acid = 90:25:4 (v/v/v) (mobile phase II) (Toiu et al, 2007), chloroform: acetone = 80 : 20 (v/v) (mobile phase III) (Popescu et al, 2012). Eluent systems I and II were used for polyphenols identification, while mobile phase III was used for triterpenic/sterolic compounds determination. Plates were spotted with 10% methanolic solutions and hydrolysed methanolic solutions (prepared as described bellow). Chlorogenic acid, caffeic acid, rutin, hyperoside, quercetin, kampferol, stigmasterol, ursolic acid and oleanolic acid (0.1 mg/mL methanolic solutions) were used as standard references. Plates were developed over a path of 8 cm (for mobile phase I), 16 cm (for mobile phase II) and 13 cm (for mobile phase III), air dryed and sprayed with a 100 g/L ethanolic solution of diphenylboryloxyethylenamine (DPhBOA) (for mobile phases I, II) and acetic anhydride, mixture of sulphuric acid and ethanol (1:1 v/v) (for mobile phase III). The plate corresponding to mobile phase III was kept for 10 min at 105°C. All plates were examined in UV light (λ = 365 nm) (Camag Reprostar Lamp with Epson Photo PC850) before and after spraying with the detection reagents.

Preparation of samples: 1 g of dried herbal product was heated twice on a reflux condenser with 20 mL methanol for 15 min. (**solution A**). 10 mL of solution A was heated with 100 g/L hydrocholoric acid on a reflux condenser for 60 min. (**solution AH**). The mixture (solution AH) was cooled and shaked with 3 quantities (each of 10 mL diethyl ether) in a separating funnel. The apolar layers were combined and evaporated on a water bath and the residue was dissolved in 2 mL methanol (**solution AHE**).

Spectrophotometric determinations

Spectrophotometric assays involved the determination of total polyphenols, flavonoids, phenolcarboxylic acids (PCA), tannins and sterolic compounds.

Total polyphenols (expressed as gallic acid equivalents) were determined with Folin-Ciocalteu reagent (Singleton et al., 1965). The flavonoid content (expressed as quercetin equivalent) was estimated based on their chelating reaction with aluminium chloride (Christ et. al, 1960). Tannins (expressed as pyrogallol equivalent) were determined based on their precipitation properties with hide powder (Romanian Pharmacopoeia 10th edition, 1993).

Phenolcarboxylic acids (PCA) were assesed based on formation of oxymes in the presence of sodium nitrite/hydrochloric acid and sodium hydroxide (European Pharmacopoeia 7th edition, 2011). Sterolic compounds (free, glycosidic and esterified forms – expressed as stigmasterol equivalent) were determined based on their dehydration in the presence of perchloric acid and vanillin acetate with formation of compounds with a maximum absorption at λ = 540-560 nm (Lu et. al, 2012). Calibration curves of: gallic acid (1.22-2.44 µg/mL, R^2 = 0.9982, n = 6), caffeic acid (5.55-35.52 µg/mL, R^2 = 0.9964, n = 6), quercetin (2.06-14.42 µg/mL, R^2 = 0.9983, n = 8) and stigmasterol (3.125-21.87 µg/mL, R^2 = 0.9972, n = 7) were used to determine the content of active substances. All spectrophotometric determinations were performed using a Jasco V-530 spectrophotometer (Jasco, Japan).

Preparation of samples: for total polpyphenols and PCA analysis, 5 g of herbal dried extract were heated twice on a reflux condenser for 30 min. with 50 mL of 50% ethanol (**solution R**). The combined solutions were filtered (through a plug of absorbant cotton) in a 100 mL volumetric flask.

For flavonoids and tannins, samples were prepared according to previously mentioned methods (Christ et. al, 1960; Romanian Pharmacopoeia 10th edition, 1993).

Preparation of samples for sterolic compounds determination:

- free forms: 0.5 g dried herbal product were heated twice on a reflux condenser with 25 mL hexane.

- The solutions were brought in a 50 mL volumetric flask and evaporated on a water bath (Raypa, Spain). The residue was dissolved in ethanol and brought to 10 mL with the same solvent in a volumetric flask.
- glycosidic forms: 0.5 g dried herbal product was heated twice on a reflux condenser with 25 mL ethanol for 30 min. The ethanolic solutions were filtered in a 50 mL volumetric flask. 10 mL of this solution were treated with 20 mL 6 M HCl on a reflux condenser for 2 h. The hydrolysed solution was treated with 3 quantities (each of 15 mL) hexane; the organic layers were filtered over anhydrous sodium sulphate and evaporated on a water bath. The residue was dissolved in 10 mL ethanol (volumetric flask).
- esterified forms: 0.5 g dried herbal product was heated on a reflux condenser with 20 mL ethanol and 5 mL 33% potassium hydroxide for 1 h. The solution was brought into a separating funnel and 20 mL water were added. The mixture was extracted with 3 quantities (each of 15 mL) hexane. The organnic layers were combined, filtered over anhydrous sodium sulphate and evaporated on a water bath. The residue was dissolved in 10 mL ethanol (volumetric flask).

HPLC-MS/HPLC-DAD analysis

a. Determination of phenolic compounds

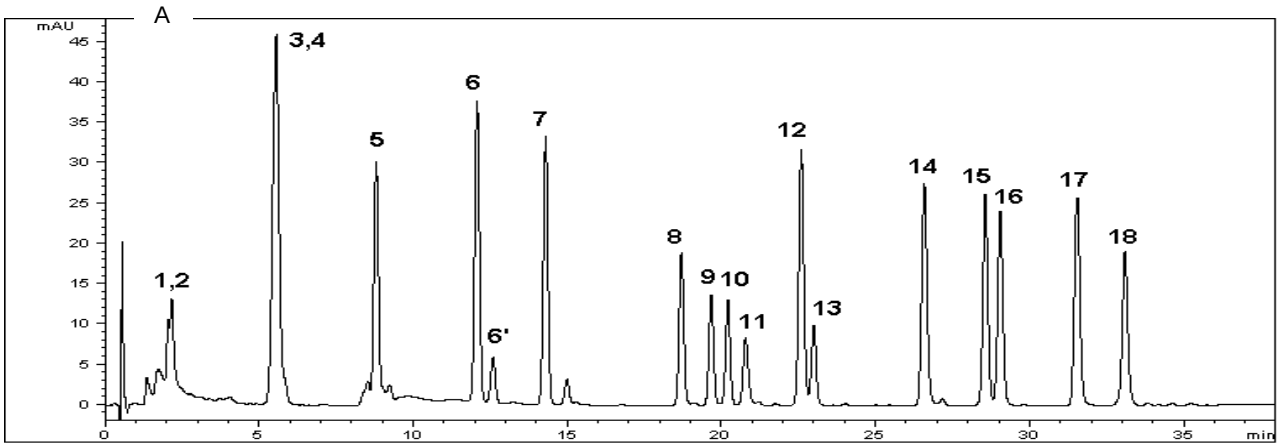
HPLC analysis was performed on Agilent 1100 HPLC Series System (Agilent, SUA) using the chromatographic conditions previously described (Vlase et. al, 2005). Calibration curves in 0.5-50 mg/mL range with good linearity (R^2 > 0.99, n = 5) have been used. Chlorogenic and caffeic acid were not quantified in the present chromatographic conditions, due to overlapping.

Preparation of samples: solution R (prepared as described above) was used. 10 mL of solution R was treated with 10 mL 2N HCl and heated on a reflux condenser at 80°C for 1h (**solution RH**).

b. Determination of ascorbic acid

HPLC analysis was carried out using a Varian HPLC (SUA) equipment. The separation was achieved on a C18, 150 x 4.6 mm i.d., 5 µm particle columm. The mobile phase consisted of methanol (A) and water (B). The gradient used was 75% A and 25% B 0-10 min. The flow rate was 1 mL/min and the injection volume 10 µL. The ascorbic acid content was determined based on a calibration curve (0.05-0.3 mg/mL, R^2 = 0.986, n=5).

Preparation of samples: 30 g of fresh leaves were mixed with 30 mL 50% ethanol (v/v) for 1 h, using a magnetic stirrer (Kartell, Italy).



Legend: 1,2 - caftaric acid, gentisic acid; 3, 4 – caffeic acid, chlorogenic acid; 5 – *p*-coumaric acid, 6 – ferulic acid; 7 – sinapic acid; 8 – hyperoside; 9 – isoquercitrin; 10 – rutin; 11 – myricetin; 12 – fisetin; 13 – quercitrin; 14 – quercetin; 15 – patuletin; 16 – luteolin; 17 – kaempferol; 18 – apigenin

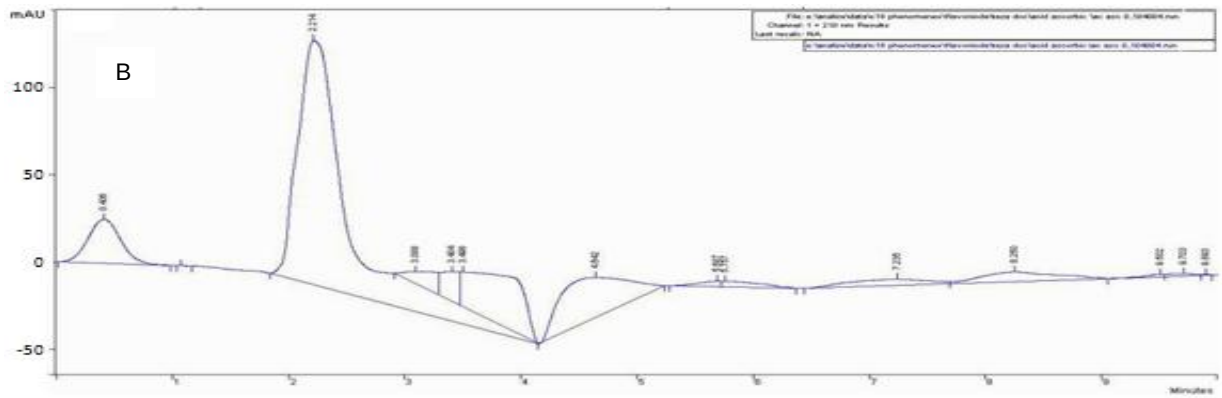


Fig. 1 HPLC chromatograms of standards: polyphenols (A) and ascorbic acid (B)

Antioxidant activity

a. DPPH radical scavenging capacity

Briefly 0.5 mL of R solution (0.05-0.8 mg/mL) were mixed with 3 mL ethanolic solution 0.1 mM of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The mixture was kept in the dark, at room temperature and the absorbance of the DPPH solution was measured at λ = 517 nm, before (A_{start}) and 30 min. after adding the extractive solutions (A_{end}). Ethanol was used as blank (Ohnishi et. al, 1994). Chlorogenic acid (dissolved in 50% ethanol) in the concentration range 0.01-0.07 mg/mL was used as positive control.

The ability to scavenge the DPPH free radical was calculated using the following formula:

DPPH radical scavenging activity (%) = ($A_{start} – A_{end}$) / A_{start} x 100.

The concentration of raspberry leaves/positive control that inhibited 50% of the DPPH free radical (EC_{50}, mg/mL) was determined graphically from the linear regression curve plotted between percent (%) of inhibition and herbal product/positive control concentrations. All measurements were done in triplicate.

b. Reducing power assay

Briefly, 2.5 mL of R solution (0.05-0.8 mg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Samples were kept at 50°C in a water bath (Raypa, Spain) for 20 min. After, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 2500 rpm for 5 min. (Universal 16 centrifuge). The upper layer (2.5 mL) was mixed with 2.5 mL water and 0.5 mL of a 0.1% ferric chloride solution. The absorbance was measured at λ = 700 nm, after 10 min., against a blank that contained all reagents except for herbal sample/positive controls. A higher absorbance indicates stronger reducing power (Oyaizu, 1986).

Chlorogenic acid (dissolved in 50% ethanol) (0.01-0.1 mg/mL) was used as positive control. The herbal product/ positive control concentration providing 0.5 of absorbance (EC_{50} mg/mL) was determined graphically from the linear regression curve plotted between absorbance and herbal product/positive control concentration (mg/mL). All determinations were performed in triplicate.

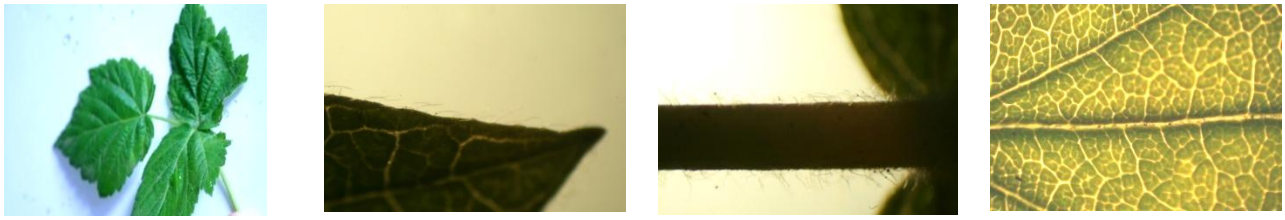
Statistical analysis

Results for spectrophotometric and antioxidant determinations represent the average ± standard deviation (SD) of three independent determinations and were calculated in Microsoft Office Excel 2007 and GraphPad Prism v.5 for Windows (www. graphpad.com)

RESULTS AND DISCUSSIONS:

The macroscopic exam (fig. 2) revealed that raspberry leaves are imparipenate with 3-7 alternate pinnate leaflets, with a hairy surface. Leaves have numerous stipules and the rhachis is pubescent (fig. 2). All aspects are similar to the ones mentioned by scientific literature (Ciocârlan, 2009).

The microscopic exam of raspeberry leaves (on transverse sections) (fig. 3) has outlined the presence of numerous non-glandular unicellular trichomes (that are mainly observed on the lower epidermis) and also glandular trichomes (mainly on the upper epidermis) (fig 3).



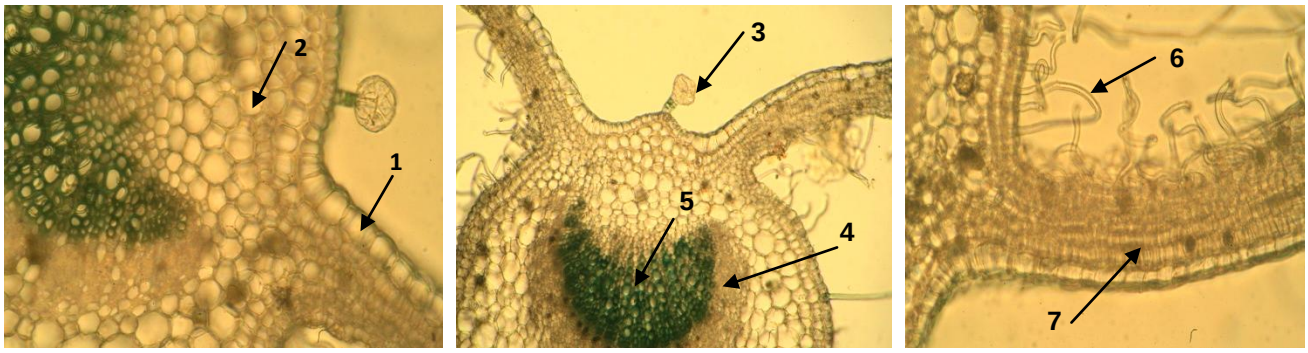
A B (ob. 4,5x) C (ob. 3x) D (ob. 4x)

Fig. 2 Morphological characteristics of raspberry leaves (A – fresh herbal product, B- non-glandular trichomes, C – rhachis details, D – veins details)

According to scanning electron microscopy (fig. 4) red raspberry leaves' lower and upper epidermis cells are isodiametric and their walls have sinuous cells, which are covered with a smooth cuticle. Anomocytic stomata can be seen in the lower epidermis (fig 4A). The microscopic exam revealed several types of trichomes: a. short non-glandular trichomes, that can be seen mainly in the lower epidermis (fig 4B); 2. non glandular massive, straight and sharp trichomes (which were observed only on the lower epidermis near the veins) (fig. 4C); 3. glandular pluricellular trichomes that can be seen both on the lower and upper epidermis (fig. 4D).

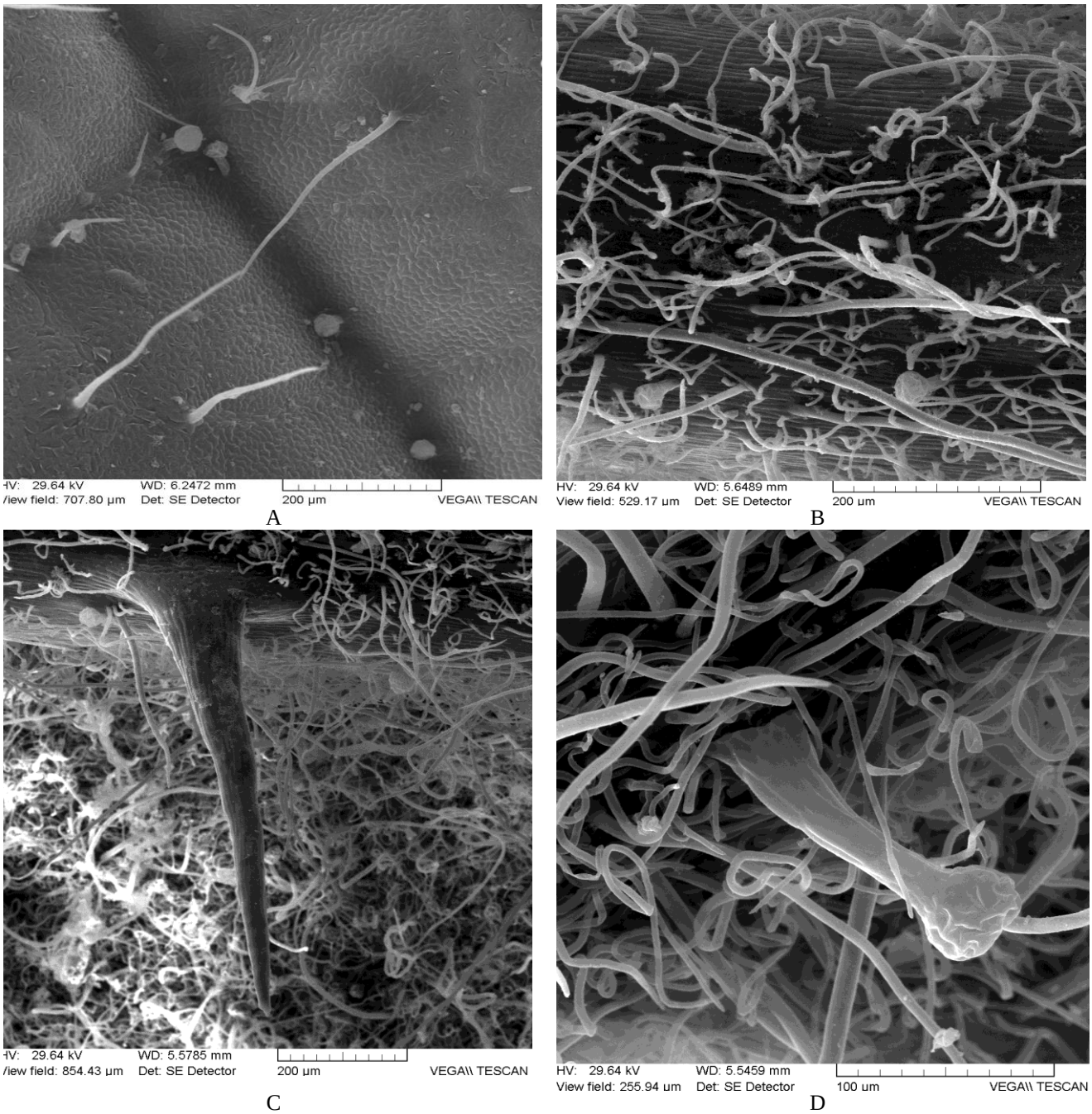
TLC analysis of phenolic compounds (mobile phases I and II) showed the presence of several compounds, that are also mentioned by scientific literature: chlorogenic acid (R_f = 0.31), kaempferol (R_f = 0.34), quercetin (R_f = 0.21) and probably rutin (a pale spot with yellow fluorescence) (R_f = 0.17) (fig 5A, 5B). Analysing the chromatograms one can note the presence of other spots corresponding to compounds with flavonoidic behaviour (yellow fluorescent zones after spraying with DPhBOA reagent) or to phenolcarboxylic acids (blue fluorescent zones after spraying with DPhBOA reagent), that were not identified due to lack of standards.

TLC analysis of triterpenic/sterolic compounds (fig. 5C) showed the presence of stigmasterol (R_f = 0.76) and ursolic acid/oleanolic acid (R_f = 0.70).



A (ob. 20x) B (ob. 10x) C (ob. 20x)

Fig.3. Leaf structure (fig. A, B, C – transverse sections): 1- upper epidermis, 2 – collenchyma cells, 3 – glandular trichome, 4 – phloem, 5 – xylem, 6 – non-glandular trichomes, 7 – pallisade mesophyll



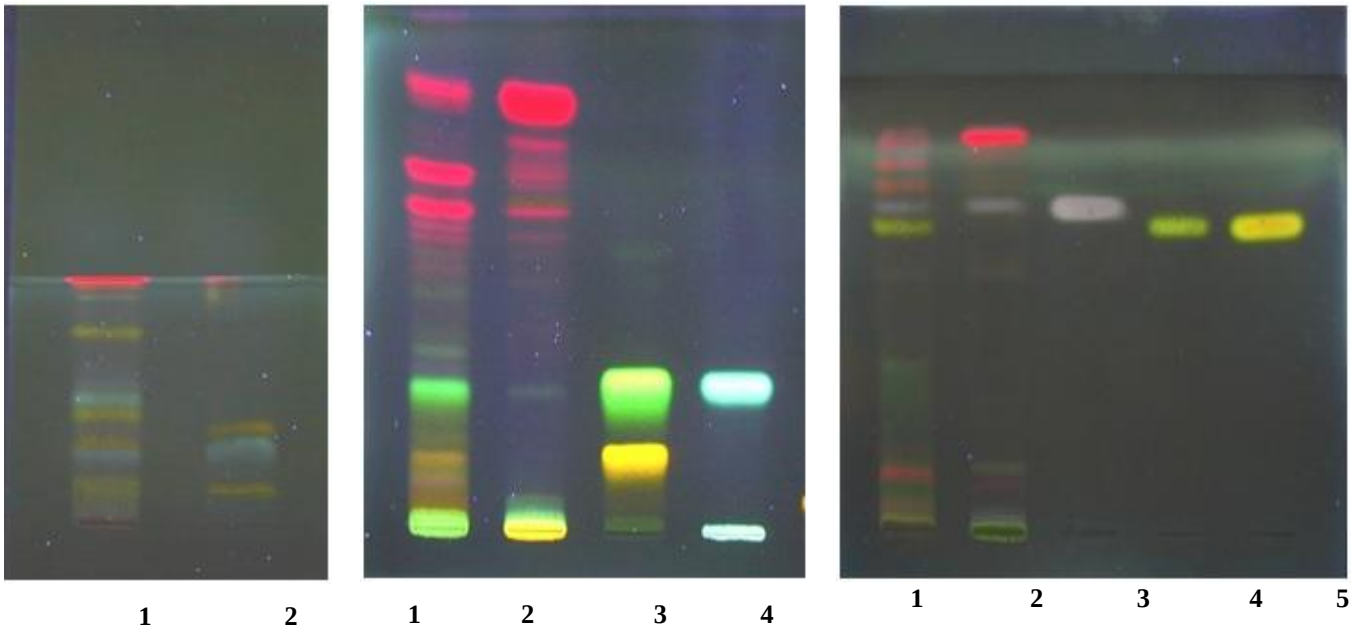
A B C D

Fig.4. Scanning electron microscopy : A – epidermis with anomocytic stomata B – short non-glandular trichomes , C – non glandular massive, straight and sharp trichomes, D – glandular pluricellular trichomes

According to our spectrophotometric results (table I) raspberry leaves have a considerable amount of flavonoids, tannins and phenolcarboxylic acids. The total phenolic content is similar to results found by other authors (47.2-129.2 mg gallic acid/g of herbal product) (Wang et al, 2000) or 4.8-12 mg gallic acid/g (Venskutonis et al, 2007). Tannins and flavonids content is similar to the one reported by scientific literature (Barnes, 2007; PDR, 2005), while glycosidic forms of sterolic compounds prevail.

Using HPLC-MS analysis (table II, fig 6 A, B) we have identified several phenolic compounds, that are also mentioned by scientific literature (Pelc et al, 2009, Barnes, 2007). Qurcetin content in R solution is similar to the one found by Pelc et al, 2009, while kaempferol content is much lower. Based on our results quercetin comes from quercitrin, isoquercitrin or other heterosides (quercetin-3-O-galactoside) hydrolysis, which are also mentioned by scientifc literature (Barnes, 2007). The flavonoids aglycons content increased significantly after hydrolysis. In contrast to scientific literature (Pelc et al, 2009) rutin and hyperoside were not identified. Due to lack of standards we couldn't establish if kaempferol comes from tiliroside or kaempferol-3-O-α-rhamnosyl-[6-O-3-hydroxy-3-methylglutaryl]-β-galactoside (Barnes, 2007, Pelc et al, 2009, Porter et al, 2012). According to scientific literature (PDR, 2005; Pelc et. al 2009), isorhamnetin-3-O-rutinoside and isorhamnetin-3-O-glucoside are also found in red raspberry leaves, but these compounds were not identified, due to lack of standards.

Regarding phenolcarboxylic acids, chlorogenic acid, caffeic acid, gentisic acid, ferulic acid and *p*-coumaric acid were identified in R and RH solutions, so we concluded that they exist in both free, esterified and glycosidic forms.



A (1 – solution A, 2 – mixture of rutin, hyperoside and chlorogenic acid from downwards to top)

B (1- solution AHE, 2- solution AH, 3 – mixture of quercetin and kaempferol from downwards to top, 4 – chlorogenic acid)

C (1- solution AHE, 2 – solution AH, 3-stigmasterol, 4 –ursolic acid, 5 – oleanolic acid)

Fig.5 . TLC chromatograms (examined in UV light λ = 365 nm) for polyphenols (A, B) and triterpenic/sterolic compounds (C) after spraying with detection reagents

Table 1.
Results for spectrophotometric determinations

Red raspberry leaves		
Total polyphenols (g% gallic acid)		6.2433 ± 0.5073
Flavonoids (g% quercetin)		0.5040 ± 0.2164
PCA (g% caffeic acid)		1.1341± 0.2069
Tannins (g% pyrogallol)		9.882 ± 0.6049
Sterolic compounds (g% stigmasterol)	free forms	0.3213 ± 0.0264
	esterified forms	0.5791 ± 0.1675
	glycosidic forms	0.8964 ± 0.0609

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Ascorbic acid was found only in red raspberry fresh leaves (fig. 6 C) and the content (4.3953 g%) is higher compared to results found by other authors (Fejer et al, 1970). However the comparison with scientific data was difficult, since there are various sorts of red raspberry.

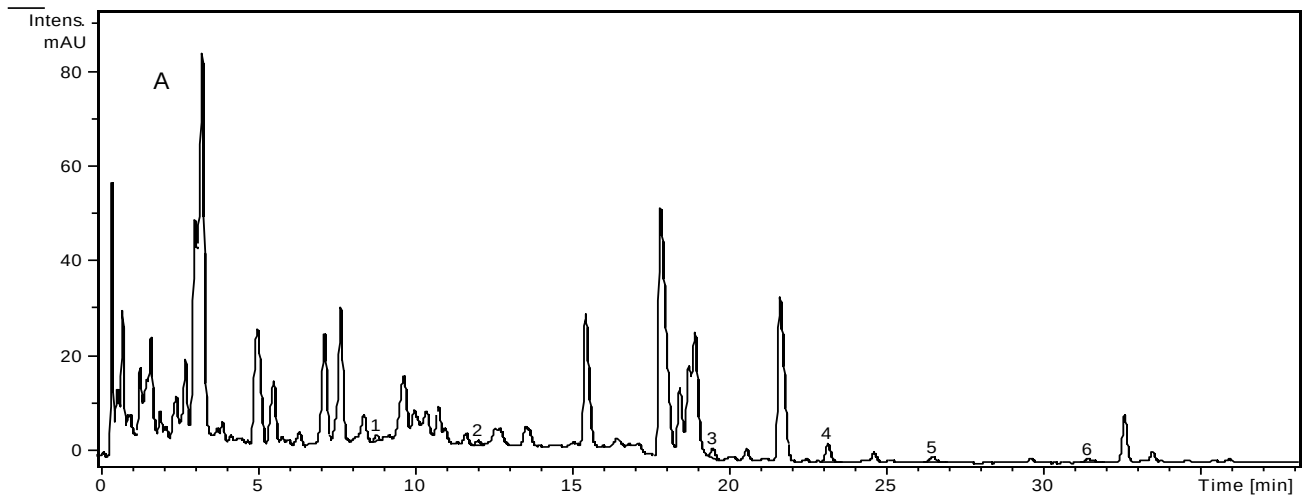
For evaluating the antioxidant activity of raspberry leaves we have used different methods: the scavenger capacity against DPPH free radical and the reducing power activity, which are commonly used for determination of antioxidant capacity of herbal drugs (Dudonné S et al, 2009).

The antioxidant activity of red raspberry leaves is low compared to standard reference, chlorogenic acid (table 3, fig. 7, 8), since EC_{50} values (mg/mL) are higher. At 0.1 mg/mL, the highest concentration common for both standard reference and red raspberry leaves the DPPH free radical inhibition was 93.09% for chlorogenic acid and 25.59% for red raspberry leaves. For reducing power activity at 0.1 mg/mL the absorbance at λ = 700 nm was 0.1913 for red raspberry leaves and 1.1350 for chlorogenic acid.

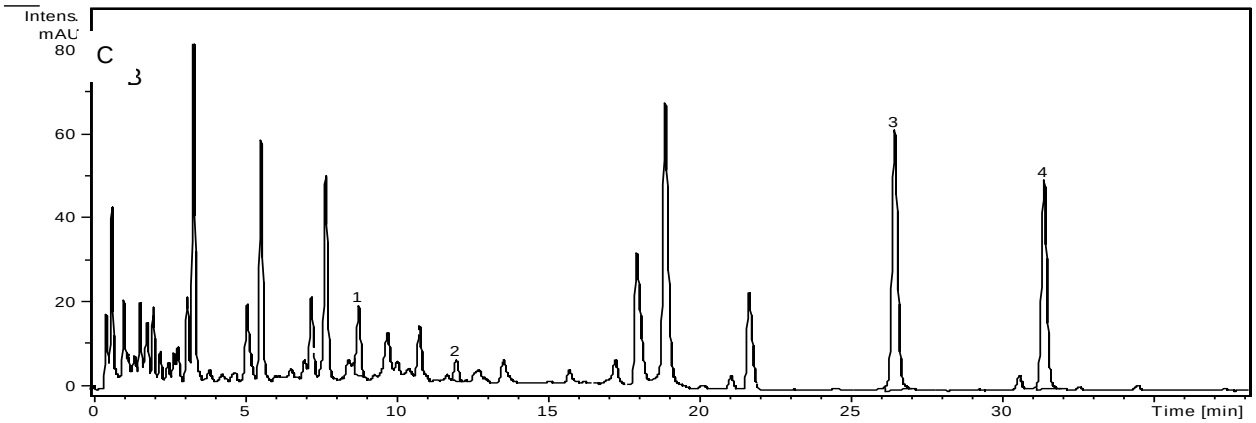
The scavenging activity against DPPH free radical is similar to results found by author authors (Venskutonis et al, 2007). We assume that polyphenols, mineral elements and glycosidic forms of sterols are responsible for the antioxidant activity. It is well known that polyphenols have free radical quenching activity, metal chelating activity and the ability to stimulate the endogenous antioxidant system (Amic et al, 2014; Rodrigo et al, 2014).

Table 2. HPLC results for polyphenols

Compound	UV identification	MS identification	Concentration (mg/100 g dried herbal product)	
			R	RH
chlorogenic acid	NO	YES	-	-
gentisic acid	NO	YES	-	-
caffeic acid	NO	YES	-	-
ferulic acid	YES	YES	0.9090	4.8401
p-coumaric acid	YES	YES	1.3595	17.55
quercitrin	YES	YES	15.63	-
isoquercitrin	YES	YES	5.92	-
quercetin	YES	YES	1.88	88.58
kaempferol	YES	YES	1.88	82.28



Legend: 1- *p*-coumaric acid, 2 – ferulic acid, 3 - isoquercitrin, 4 - quercitrin, 5- quercetin, 6 – kaempferol



Legend: 1 - *p*-coumaric acid, 2 – ferulic acid, 3 - quercetin, 4 - kaempferol

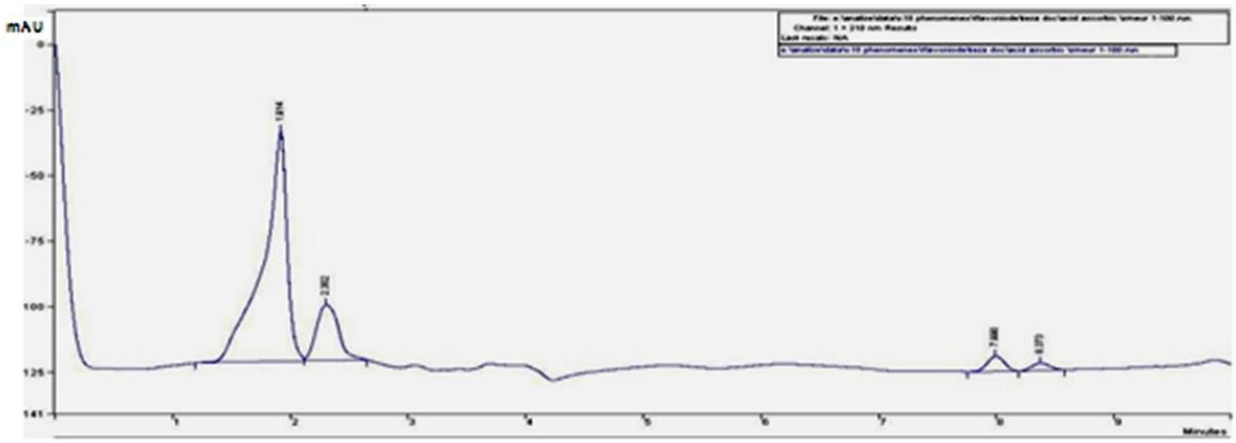
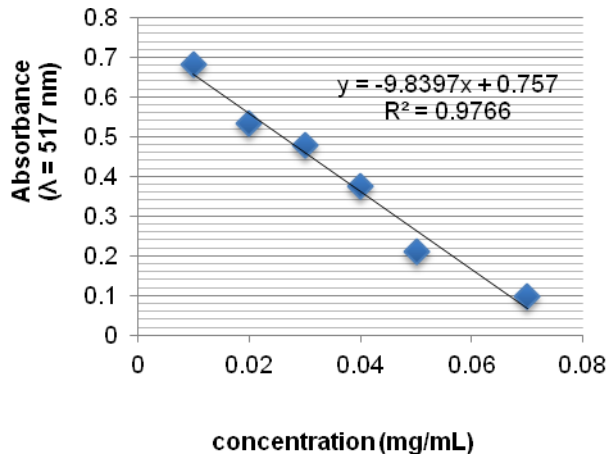


Fig. 6. HPLC-MS/HPLC-DAD chromatograms for red raspberry leaves: polyphenols (A – solution R; B – solution RH) and ascorbic acid (C)

A



B

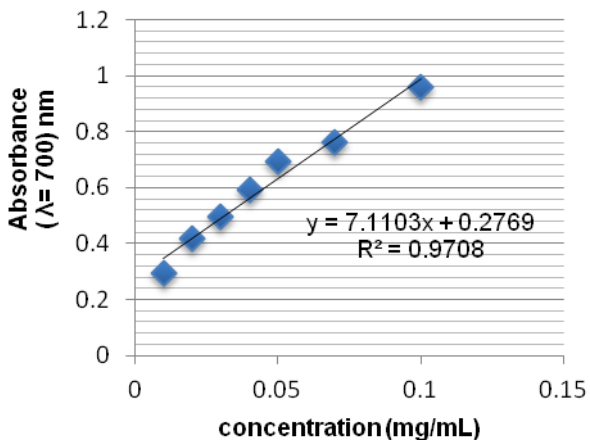


Fig.7.Calibration curves for chlorogenic acid (A - DPPH method, B – reducing power activity)

Table 3.
Antioxidant activity

Sample	Method			
	DPPH		Reducing power	
	EC_{50} (mg/mL)	mg chlorogenic acid /g dried herbal product	EC_{50} (mg/mL)	mg chlorogenic acid /g dried herbal product
Rubi idaei folium	0.2886 ± 0.2901	90.5733 ± 8.1969	0.3754 ± 0.0689	77.7066 ± 10.1346
Chlorogenic acid	0.0227 ± 0.0019	-	0.0271 ± 0.0060	-

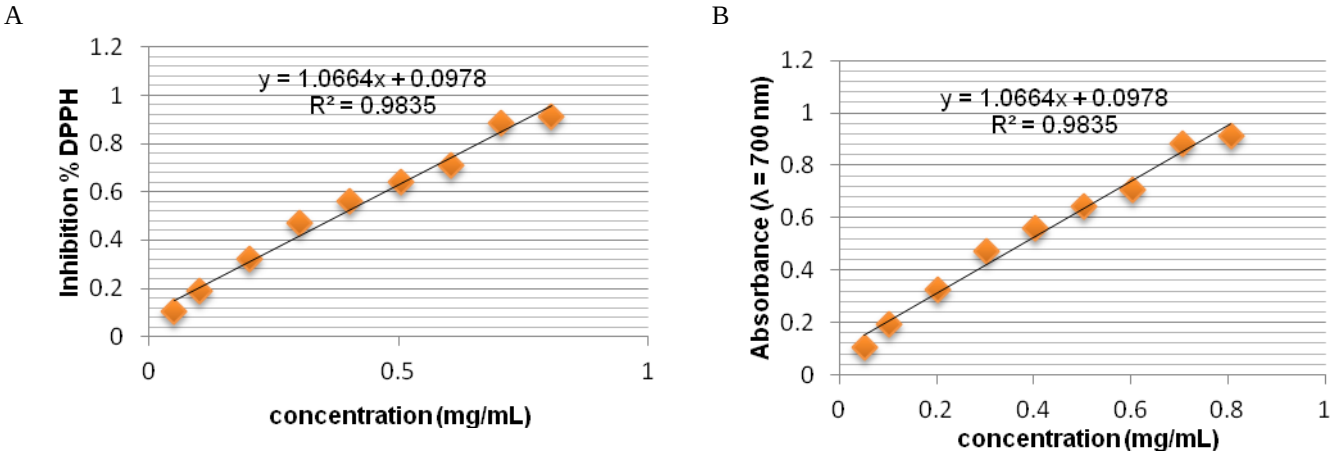


Fig.8 Antioxidant activity of red raspberry leaves (A – DPPH method, B – reducing power activity)

CONCLUSIONS:

Red raspberry leaves are an important source of natural compounds, with moderate antioxidant activity.

To our knowledge no spectrophotometric determinations regarding free, esterified and glycosidic forms of sterolic compounds have been made. Moreover scanning electron microscopy revealed the main characteristics of different type of trichomes, representing an important tool for red raspberry leaves identification.

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