Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 27 issue 4, 2017, pp 231-238

RAGGED ROBIN (*LYCHNIS FLOS CUCULI* L.) AERIAL PARTS – BOTANICAL CHARACTERIZATION, PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY

Teodora Costea[1*], Ioana Nencu[1], Cerasela Elena Gîrd[1], Maria Lidia Popescu[1]
[1] Pharmacognosy, Phytochemistry, Phytotherapy Department, Faculty of Pharmacy, "Carol Davila University" of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT: The aim of our study was the botanical characterization, phytochemical screening and antioxidant activity of ragged robin (*Lychnis flos cuculi* L.) aerial parts. Qualitative (specific chemical reactions, thin layer chromatography, HPLC) and quantitative (HPLC and spectrophotometric determinations) assays were performed on hydroethanolic (50% ethanol) and aqueous freeze-dried extracts. Ferric reducing power and scavenger activity towards $ABTS^{\bullet}$+ free radical have been used for antioxidant capacity evalution. Both dry extracts are a source of phenolic compounds, coumarines and saponins. Hydroethanolic dry extract has the highest content of polyphenols (5.09 g%), phenolcarboxylic acids (1.68 g%) and flavones (0.35 g%). By means of HPLC analysis, rutin and apigenin-7-O-glucoside were identified in both extracts. Analysed extracts have a moderate antioxidant activity; future research is needed in order to establish the pharmao-toxicological profile and the exact antioxidant activity mechanism.

Keywords: phenols, rutin, antioxidant activity, flavones, ursolic acid

INTRODUCTION:

The discovery and valorification of new herbal products, with potential antioxidant activity represents an area of great interest in the scientific world. *Caryophyllaceae* is a major family of the *Angiosperms* and it is represented by nearly 2.600 species, distributed all over the world (Chandra et al, 2015). The genus *Lychnis*, which belongs to the *Silenoideae* subfamily comprises species such as *Lychnis coronaria* Desr., *Lychnis viscaria* Linn., *Lychnis chalcedonica* Linn., *Lychnis haageana* Hort. ex Vilm. and *Lychnis flos-cuculi* L. (Guerrero et al, 2004).

Among them, aerial parts of ragged robin (*Lychnis flos-cuculi* L.) are an important source of phytoecdysteroids (polypodine B, 20-hydroxyecdysone, makisterone, rubrosterone, taxisterone, viticosterone E) (Malinski et al, 2014), fatty acids, volatile compounds (benzaldeyde, propylbenzene, methylsalicylate, linalool, α-pinene), flavones (vitexin, orientin), phenolcarboxylic acids (ferulic acid, caffeic acid, protocatehuic acid) and triterpenic saponins (Jurgens et al, 2004; Tomczyk et al, 2008; Bottger et al, 2011).

Ragged robin aerial parts have a wide spectrum of pharmacological properties – antibacterian upon gram positive and gram negative bacteria (*Klebsiella oxytoca*, *Escherichia coli*, *Proteus rettgeri*, *Staphylococus aureus*, *Pseudomonas aeruginosa*), antifungic effects (upon *Candida sp*.), *in vitro* wound healing properties and cytotoxic effects upon MCF-7 (breast), HepG2 (liver) and HeLa (cervix) cancer cell lines (Malinski et al, 2014).

The aim of our research was the botanical characterization, phytochemical screening and *in vitro* antioxidant activity evaluation (ferric reducing power and scavenger activity towards 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) - $ABTS^{\bullet}$+ free radical) of indigenous ragged robin aerial parts.

MATERIAL AND METHODS:

Material

Lychnis flos cuculi aerial parts (ragged robin) were collected in June 2016, from Moraresti village, Arges county, Romania. Aerial parts were freeze-dried using a Christ Alpha 1-2/B Braun, Biotech-International lyophilizator.

Reagents and solvents

All reagents and solvents were purchased from Karl Roth (Germany) unless otherwise stated. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet}$+ 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 exams. The macroscopic exam was performed on both fresh and freeze-dried aerial parts. For microscopic examination we have used 800 g/L chloral hydrate as a clearing agent and 10g/L fluoroglucin as a colour agent, according to European Pharmacopeia method (European Pharmacopeia 7th edition, 2011). A Carl Zeiss Imager D1 microscope with Canon PC 1145 camera was used for microscopic examination.

Phytochemical screening

In order to determine the solvent's influence on both chemical composition and antioxidant activity, experiments were carried on, using aqueous and 50% ethanolic dry extracts.

Preparation of dry extracts: 15 g of freeze-dried herbal product were heated twice with 150 ml water or

Correspondence*: *Teodora Costea, "Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmacognosy, Phytochemistry, Phytoterapy Department, no.6, Traian Vuia Street, Bucharest, Romania, telephone: 0040722554665, email: teodoracostea85@yahoo.com*

50% ethanol (v/v) on a reflux condenser for 30 min. The combined filtrates were evaporated under reduce pressure, at 40°C (Buchi R210-215 rotary evaporator) and then, freeze-dried (Christ Alpha 1-2/B Braun, Biotech-International lyophilizator). The extracts were encoded as follows: **LE** (50% ethanolic dry extract) and **LA** (aqueous dry extract). The extraction yield was expressed as the percentage of the total mass of dry extract with respect to the mass of the herbal product loaded for the initial extraction (Yang et al, 2012).

Qualitative assays consisted in specific chemical reactions for phenolcarboxylic acids, flavones, tannins, saponins, coumarines (Gird et al, 2010) and thin layer chromatography for phenolic compounds (flavones, phenolcarboxylic acids) and sterols/triterpenes.

Preparation of samples for qualitative/ quantitative determinations and antioxidant activity: 0.5 g of LA and LE dry extracts were dissolved in 50 mL water and 50% ethanol, respectively. The obtained solutions were encoded LAS and LES.

Thin layer chromatography analysis (TLC)

Thin layer chromatography was used for phenols and sterolic/triterpenic compounds identification. It was performed on aluminium - coated TLC plates (20x20 cm and 10x20cm, 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) – for phenolic compounds identification; chloroform: acetone = 80 : 20 (v/v) (mobile phase II) (Popescu et al, 2012) – for sterols/triterpenes identification. Plates were spotted with LAS and LES solutions (for mobile phase I) and hydrolysed solutions (LEM and LAM) extracted with apolar solvent (prepared as described bellow for mobile phase II). Chlorogenic acid, rutin, ursolic acid and β- sitosterol (0.1 mg/mL methanolic solutions) were used as standard references. Plates were developed over a path of 8 cm (for mobile phase I) and 16 cm (for mobile phase II), air dryed and sprayed with a 100 g/L ethanolic solution of diphenylboryloxyethylenamine (DPhBOA) (for mobile phase I) and acetic anhydride/mixture of sulphuric acid and ethanol (1:1 v/v) (for mobile phase II). The plate corresponding to mobile phase II 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 hydrolised solutions: 10 ml of LAS and LES solutions were heated with 10 mL of 100 g/L hydrocholoric acid on a reflux condenser for 60 min. The mixture 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. The obtained solutions were encoded LEM and LAM.

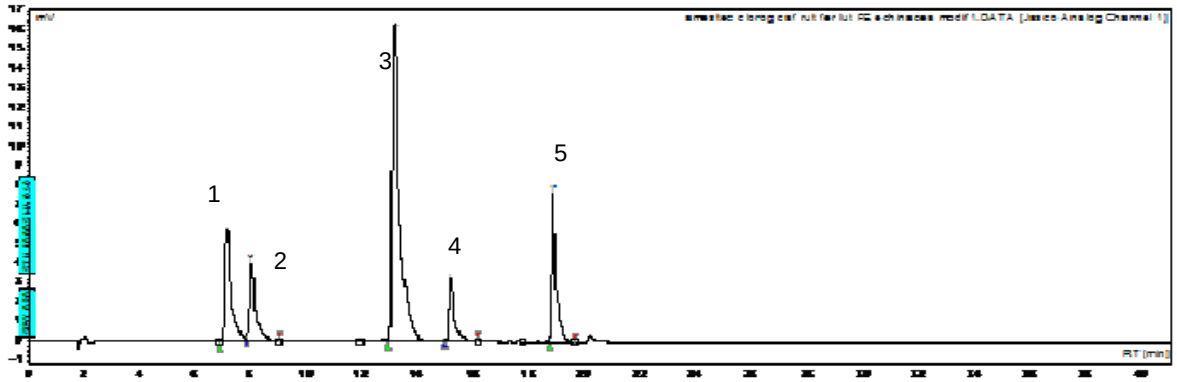
Spectrophotometric determinations

Total polyphenols (expressed as tannic acid equivalents) were determined with Folin-Ciocalteu reagent (Singleton et al., 1965, Costea et al, 2016). The flavonoid content (expressed as quercetin equivalents) was estimated based on their chelating reaction with aluminium chloride (European Pharmacopoeia 7th edition, 2011). Phenolcarboxylic acids (PCA) (expressed as chlorogenic acid equivalents) were assessed based on formation of oxymes in the presence of sodium nitrite/hydrochloric acid and sodium hydroxide (European Pharmacopoeia 7th edition, 2011). Anthocyanidins content were determined according to European Pharmacopeia 7th edition (*Myrtilli fructus* recens monography). Calibration curves of: tannic acid (2.04-9.18 µg/mL, R^2 = 0.9994, n = 8), chlorogenic acid (0.01131-0.05278 mg/mL, R^2 = 0.9998, n = 6), quercetin (2.06-14.42 µg/mL, R^2 = 0.9983,n = 8) and cyanidin chloride (1.63-9.79 µg/mL, R^2 = 0.9995) were used to determine the content of active substances. All spectrophotometric determinations were performed using a Jasco V-530 spectrophotometer (Jasco, Japan). All determinations were performed on 1% LAS and LES solutions.

HPLC-DAD analysis

HPLC analysis of phenolic compounds was performed using a Jasco HPLC MD-2015 equippped with degaser, binary gradient pump, column thermostat and UV detector according to our previously described method (Gîrd et al, 2015). Rutin, chlorogenic acid, caffeic acid, ferulic acid, apigenin-7-O-glucoside, quercetin, isoquercitrin and kampferol have been used as standard references (fig.1).

A



1 – chlorogenic acid, 2 – caffeic acid, 3 – ferulic acid, 4 – rutin, 5 - quercetin

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 27 issue 4, 2017, pp 231-238

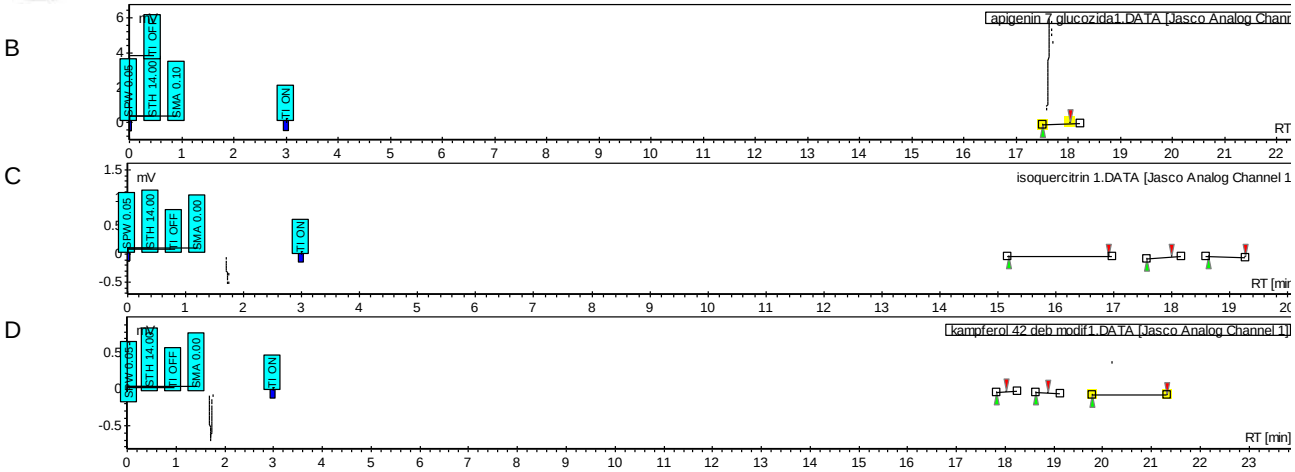


Fig. 1. Chromatogram of standards
A – mixture of chlorogenic acid, caffeic acid, ferulic acid, rutin, quercetin; B – apigenin-7-glucoside; C - isoquercitrin; D - kaempferol

Calibration curves of rutin, caffeic acid, chlorogenic acid and quercetin in the 4.06-370 µg/mL range had a good linearity (R^2 > 0.99, n =5). HPLC analysis was performed on LAS and LES solutions respectively, which were prepared as previously described.

Antioxidant activity

a. ABTS$^{\bullet}$+ radical cation scavenging capacity

The antioxidant capacity was measured using the method of Re et al, 2006. Briefly, the ABTS$^{\bullet}$+ free radical cation was generated by incubation of ABTS diammonium salt (7 mM) with potassium persulphate (2.45 mM) in the dark, at room temperature for 16 h. The absorbance of the ABTS radical solution was equilibrated to a value of 0.700 ± 0.02 at λ = 734 nm after dilution with ethanol. Briefly LAS and LES solutions were diluted to obtain a final concentration of 0.04-0.6 mg/mL. 0.5 mL of each dilution was mixed with 3 mL reagent and the absorbance of ABTS$^{\bullet}$+ radical cation was measured at 734 nm before (A_{start}) and 6 min. after adding the sample (A_{end}).

ABTS$^{\bullet}$+ radical scavenging activity (%) = $(A_{start} – A_{end}) / A_{start}$ x 100.

The concentration of ragged robin aerial parts dry extracts that inhibited 50% of the ABTS$^{\bullet}$+ free radical (EC_{50}, mg/mL) was determined graphically from the linear regression curve plotted between percent (%) of inhibition and dry extracts concentrations. The antioxidant activity was also evaluated based on ascorbic acid equivalents (mg vitamin C/g of dry extract), which were determined using a calibration curve, that was constructed in the same experimental conditions (0.02-0.12 mg/mL, R^2 = 0.991, n = 7).

b. Reducing power assay

Briefly, 2.5 mL of LAS and LES solutions (0.2-2.8 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 dry extracts solutions. A higher absorbance indicates a stronger reducing power (Oyaizu, 1986).

The dry extracts concentration providing 0.5 of absorbance (EC_{50} mg/mL) was determined graphically from the linear regression curve plotted between absorbance and concentration (mg/mL). The reducing power was also evaluated based on ascorbic acid equivalents (mg vitamin C/g of dry extract), which were determined using a calibration curve, that was constructed in the same experimental conditions (0.01 - 0.05 mg/mL, R^2 = 0.990, n = 5).

Statistical analysis

Results for spectrophotometric and antioxidant determinations represent the average ± standard deviation (SD) of three replicates, from three independent determinations and were calculated using Microsoft Office 2007 (Excel programme).

RESULTS AND DISCUSSIONS:

The macroscopic exam (fig. 2) revealed that ragged robin aerial parts are characterised by pink flowers with spoon-shaped paired lower leaves, while the middle and upper leaves are linear-lanceolate; all leaves are untoothed. Stems have barbed hairs, that makes the plant rough to touch.

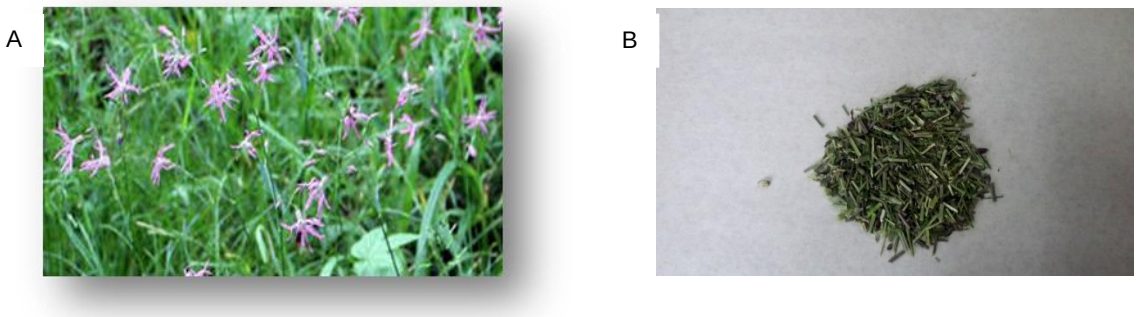


Fig.2. Macroscopic examination of ragged robin aerial parts: A - raw herbal product, B - freeze-dried herbal product

The microscopic exam (fig.3,4) revealed the presence of anatomic elements characteristic to leaves and flowers – pollen grains, endotecium, papillae, epidermis with stomata, xylem vessels, calcium oxalate druses and glandular trichomes.

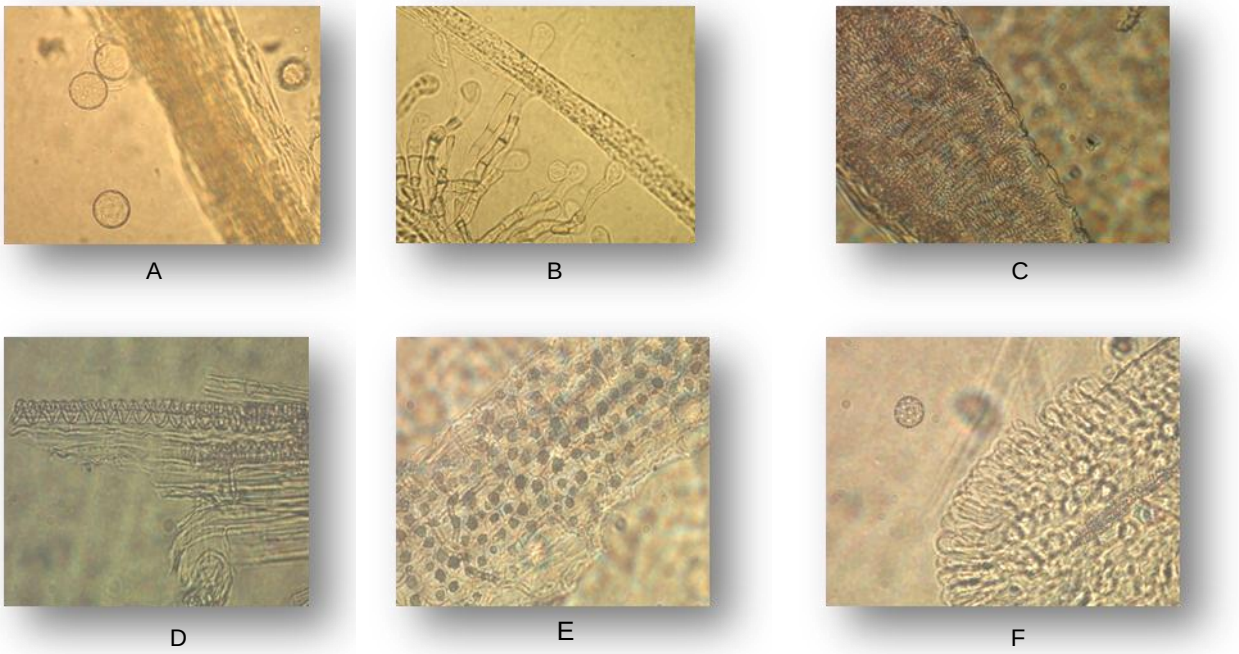


Fig.3. Microscopic exam (ob 40x).: A – pollen grains, B – glandular trichomes, C – endotecium, D - small xylem vessels, E – calcium oxalate druses, F – papillae and pollen grains

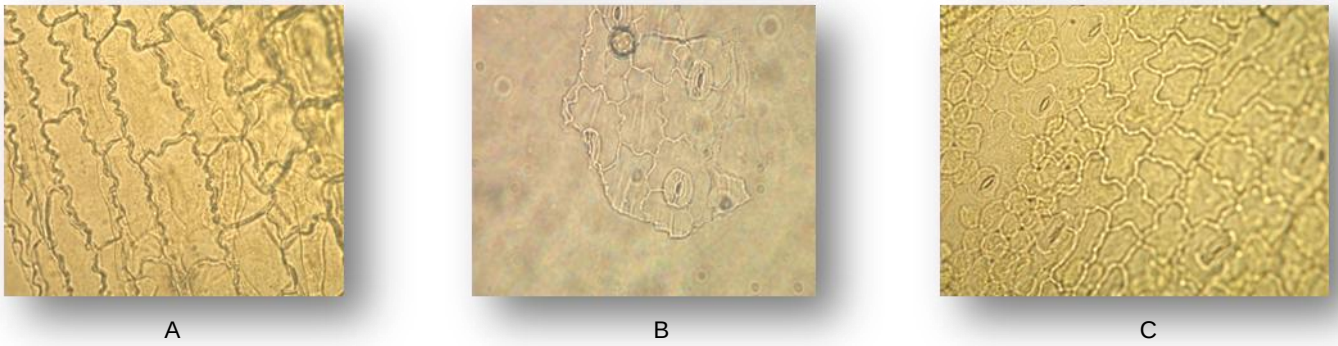


Fig.4. Microscopic exam (ob.40x): A – epidermis, B, C - stomata

The extraction yield was 20.07% for LE dry extract and 15.03% for LA dry extract. The qualitative analysis revealed the presence of flavones, phenolcarboxylic acids, coumarines, tannins, saponins and anthocyanidins in both LE and LA dry extracts. Rutin and ursolic acid were identified by means of thin layer chromatography (fig.5). The presence of triterpenic saponins and phenolic compounds in ragged

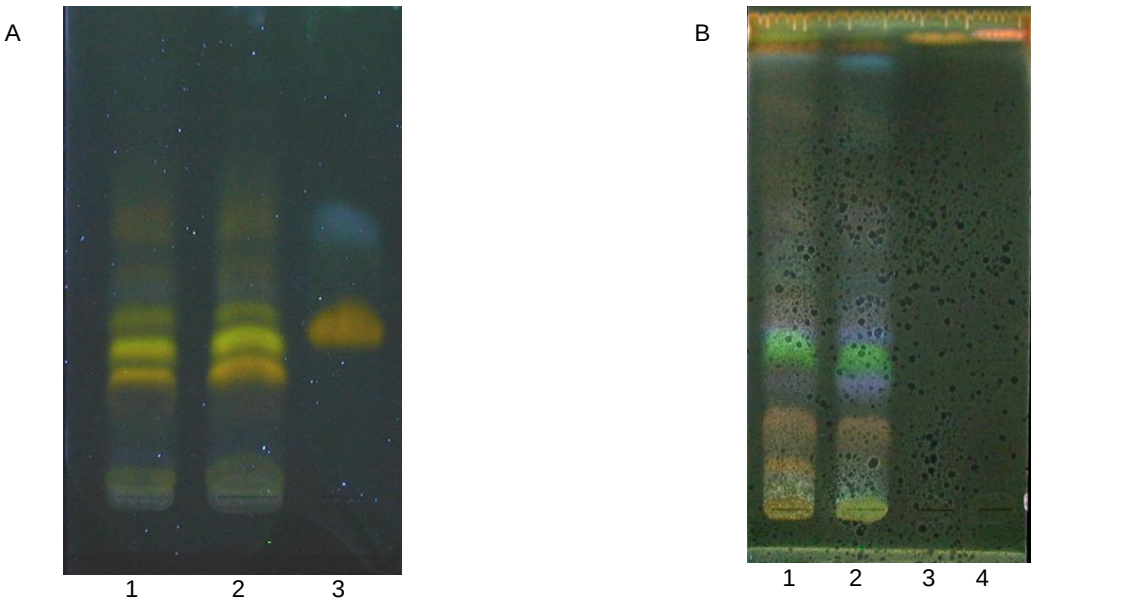
robin aerial parts is also mentioned by other authors (Jurgens et al, 2004; Tomczyk et al, 2008; Bottger et al, 2011).

Our results (table I) revealed that LE dry extract has a higher content of phenolic compounds compared to LA dry extract. According to Caunii et al (2012), the extraction yield of phenolic compounds is higher for hydroethanolic solutions compared to aqueous ones.

HPLC analysis revealed the presence of rutin in both LA and LE dry extracts (fig 6.); a higher content (table II) was observed for 50% hydroalcoholic dry extract, which is in agreement with our spectrophotometric results. Caffeic acid was determined only for LA dry extract (fig.6), while apigenin-7-O-glucoside was identified in both dry extracts (fig. 6). Rutin and apigenin-7-O-glucoside are new compounds, unmentioned by scientific literature.

The antioxidant activity was determined based on EC_{50} (mg/mL) values and ascorbic acid equivalents (mg ascorbic acid/g dry extract). Ascorbic acid equivalents were determined based on calibration curves (fig.7A,B).

Aqueous and hydroetahnolic dry extracts have a similar ferric reducing activity, similar absorbances have been determined, using the same concentration interval (fig. 8A, table III). However, LE dry extract have a slightly higher ferric reducing power compared to LA dry extract, since it has a lower EC50 value (mg/mL) (table III). However, the scavenger activity upon $ABTS^{\bullet}+$ free radical was higher for ragged robin aqueous dry extract, since we have obtained a lower EC50 value and higher ascorbic acid equivalents (table III, fig 8B). We assume that the differences among antioxidant methods are the consequence of different reaction mechanisms: $ABTS^{\bullet}+$ free radical scavenger capacity is based on both hydrogen atom and electron transfers, while reducing power assay is mainly an electron transfer based method (Tan al, 2015).



1 – LES, 2 – LAS, 3 – mixture of rutin and chlorogenic acid

1 – LEM, 2 – LAM, 3 – ursolic acid, 4 – β - sitosterol

Fig.5. TLC chromatograms (examined in UV light λ = 365 nm) for: A – phenolic compounds (mobile phase I), B – sterols/triterpenes (mobile phase II) after spraying with detection reagents

Table I. Results for spectrophotometric determinations

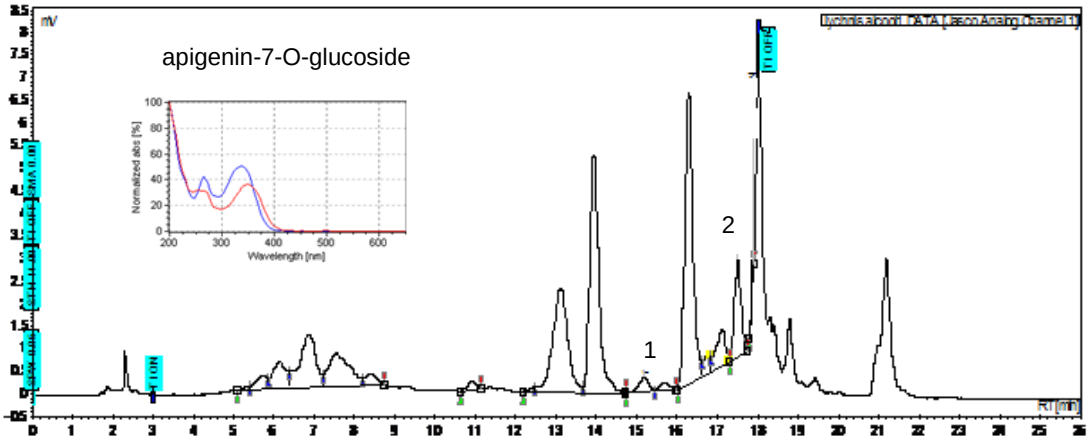
Dry extract	Total phenolic content (g tannic acid/100 dry extract)	Flavones (g quercetin/100 g dry extract)	Phenolcarboxylic acids (g chlorogenic acid/100 g dry extract)	Anthocyanidins (g cyanidin chloride/100 g dry extract)
LE	5.0962 ± 0.6158	0.3588 ± 0.0407	1.6850 ± 0.1811	0.1074 ± 0.0094
LA	3.6300 ± 0.3490	n.d	1.4816 ± 0.0239	0.0941 ± 0.0169

Legend: LE – 50% hydroethanolic dry extract, LA – aqueous dry extract, n.d. – not determined
Results are mean ± SD of three replicates, from three independent determinations

Table II. HPLC analysis of phenolic compounds

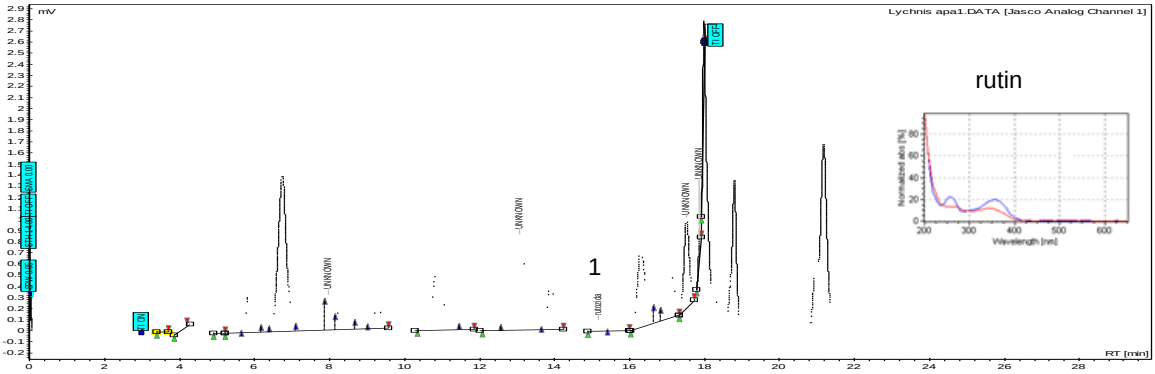
Dry extract	Rutin (mg/100 g dry extract)	Caffeic acid (mg/100 g dry extract)	Apigenin-7-O-glucoside
LE	98.67	nd	+
LA	42.66	18.82	+

Legend: LE – 50% hydroalcoholic dry extract, LA –aqueous dry extract, nd – not determined, + - ,,identified



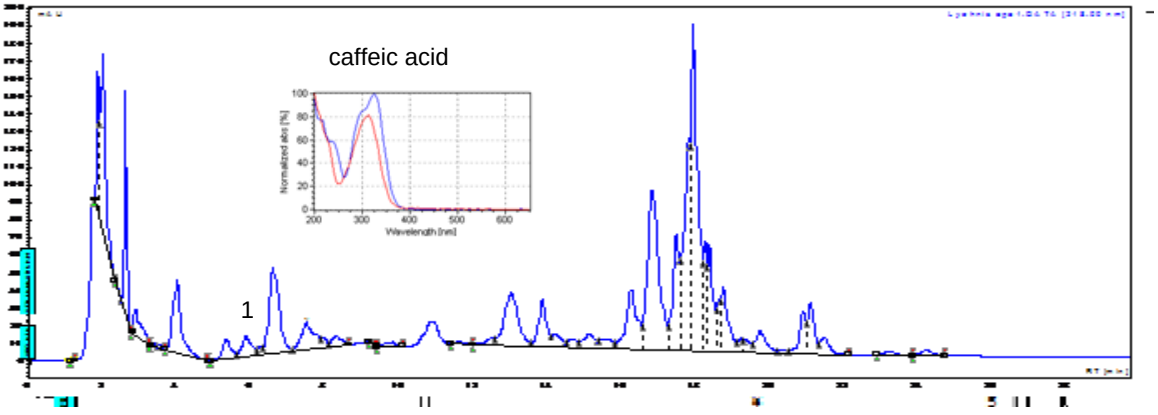
Legend: 1 – rutin, 2 – apigenin-7-O-glucoside

A



Legend: 1 - rutin

B



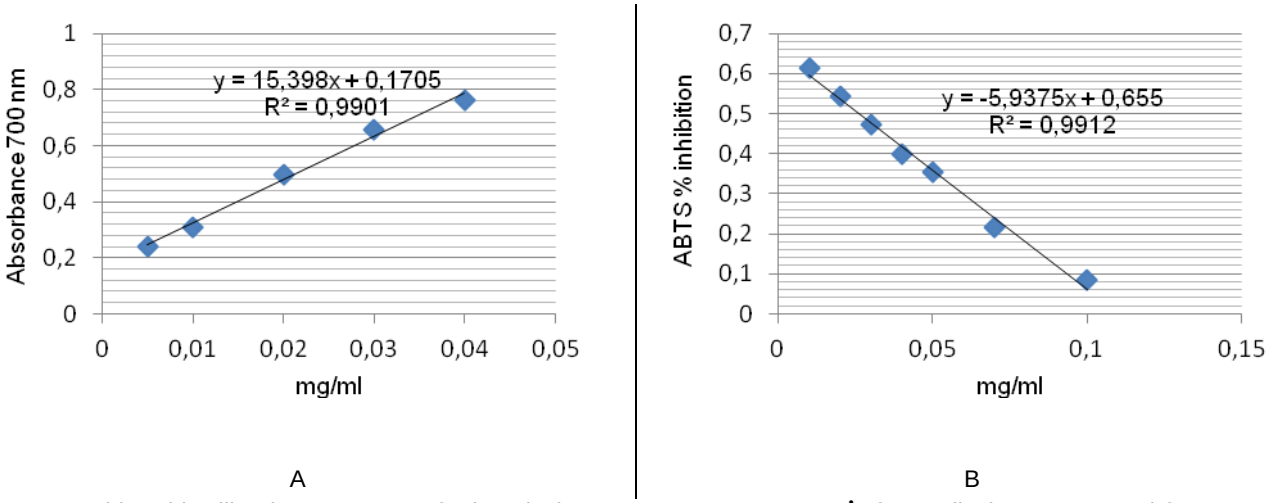
Legend: 1 – caffeic acid

C

Fig.6. HPLC analysis: A – LES solution (330 nm); B – LAS solution (330 nm); C – LAS solution (218 nm)

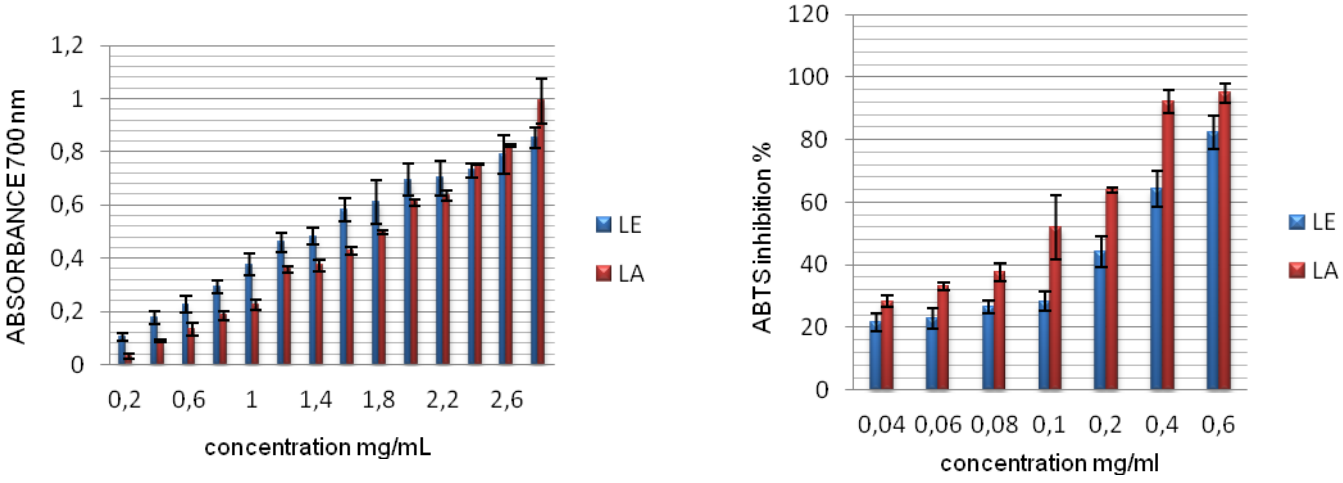
We assume that the antioxidant capacity of both dry extracts is the consequence of their chemical composition, mainly phenols (phenolcarbocylic acids, anthocyanidins, flavones), which are known as metal chelators, reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers. They are also involved in activation of humans antioxidant defense system; they enhance superoxide dismutase, catalase, glutathione peroxidase activities (Gülcin et al,2012). Among identified compounds (through qualitative and quantitative assays), rutin was found to have antioxidant activity, anti-dyslipidaemic properties (Nishimura et al, 2016). Rutin antioxidant activity was also determined on different cell lines (HUVEC- human umbilical endothelial cell line) through up-regulation of glutathione synthesis (Sthijns et al, 2017). Caffeic acid was shown to have antioxidant activity using different in vitro methods (Chiou et al, 2017). Apigenin-7-O- glucoside showed antioxidant activity using several *in vitro* methods

(scavenger activity towards ABTS/DPPH free radicals) (Vitalini et al, 2011) and prevents acute lung injury via down-regulation of oxidative enzymes expression and anti-inflammatory properties (Li et al, 2015). Ursolic acid, which we have identified by means of thin layer chromatography, has antioxidant activity in a MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) Parkinson model (Rai et al, 2016).



A B

Fig.7. Ascorbic acid calibration curves: A – ferric reducing power assay, B – ABTS$^{\bullet}$+ free radical scavenger activity



A B

Fig. 8. Antioxidant activity of analysed dry extracts: A – ferric reducing power, B – ABTS$^{\bullet}$+ free radical scavenger activity

Table 3.
Evalution of dry extracts antioxidant activity

Dry extract	METHOD			
	ABTS$^{\bullet}$+		Reducing power	
	EC_{50} (mg/mL)	mg vitamin C/g extract	EC_{50} (mg/mL)	mg vitamin C/g extract
LE	0.2899 ± 0.0184	286.85 ± 1.2092	1.4832 ±0.1461	14.8488 ± 2.1964
LA	0.1513 ± 0.0123	522.89 ± 1.2684	1.6614 ±0.0246	12.1942 ± 2.3964

Legend: LE – 50% hydroalcoholic dry extract, LA –aqueous dry extract
Results are mean ± SD of three replicates, from three independent determinations

CONCLUSIONS:

Ragged robin aerial parts (*Lychinis flos cuculi* L.) are an important source of bioactive compounds. The solvent is a key factor that influences both chemical composition and antioxidant activity. New compounds (rutin and apigenin-7-O-glucoside), unmentioned by scientific literature, were identified by us using HPLC analysis. Both dry extracts might be used in therapeutics for their antioxidant activity. Future reserch (pharmacological studies) is needed in order to

determine te exact mechanism of action and also the pharmaco-toxicological profile.

REFERENCES:

Bottger S, Melzig MF, Triterpenoid saponins of the *Caryophyllaceae* and *Ilecebracea*e family. Phytochemistry Letters, 4, 59-68, 2011.

Caunii A, Pribac G, Grozea I, GaitinD, Samfira I, Design of optimal solvent for extraction of bioactive ingredients from six varieties of *Medicago sativa*. Chemistry Central Journal, 6, 123, 2012.

Chandra S, Ravat DS, Medicinal plants of the family *Caryophyllaceae*: a review of ethnomedicinal uses and pharmacological properties. Integratie Medicine Research, 4, 123-131, 2015.

Chiou S-Y, Sung J-M, Huang P-W, Lin S-D, Antioxidant, antidiabetic and antihypertensive properties of *Echinacea purpurea* flower extract and caffeic acid derivatives using in vitro models. Journal of Medicinal Food, 20(2), 171-179, 2017.

Costea T, Lupu AR, Vlase L, Nencu I, Gîrd CE, Phenolic content and antioxidant activity of a raspberry leaf dry extract. Romanian Biotechnological Letters, 21(2), 11345-11356, 2016.

Gîrd CE, Duţu LE, Popescu ML, Iordache AT, Tudor I, Costea T, Bazele teoretice şi practice ale analizei farmacognostice, vol I, ediţia a II – a .Bucureşti, Ed. Curtea Veche, 41-47, 2010.

Gîrd CE, Costea T, Nencu I, Popescu M L, Duţu LE, Phytotochemical and phytobiological research upon aerial parts and seeds from *Peucedan*um officinale L. Farmacia, 63(2), 247-253, 2015.

Guerrero JLG, Lopez-Martinez JCL, Navaro-Juarez R, Maroto-Garcia F, Campra-Madrid P, γ-linolenic acid from Caryophylaceae seed oil. JAOCS, 81(7), 659-66, 2004.

Gülcin I, Antioxidant activity of food constituents: an overview. Arch Toxicol, 86, 345-349, 2012.

Jűrgens A, Flower scent composition in diurnal Silene species (*Caryophyllaceae*): phylogenetic constraints or adaption to flower visitors ?. Biochemical systematic and Ecology, 32(10), 841-859, 2004.

Li KC, Ho YL, Hsieh WT, Huang SS, Chang YS, Huang GJ, Apigenin-7-glycoside prevents LPS-induced acute lung injury via downregulation of oxidative enzyme expression and protein activation through inhibition of MAPK phosphorylation. International Journal of Molecular Sciences, 16(1), 1736-1754, 2015.

Malinski MP, Mihalska AD, Tomczykowa M, Tomczyk M, Thiem B, Ragged robin (*Lychnis flos cuculi*) a plant with potential medicinal value. Rev Brav Pharmacogn, 24, 722-730, 2014.

Nishimura M, Ohkawara T, Sato Y, Satoh H, Suzuki T, Ishiguro K, Nishihira J, Effectiveness of rutin-rich Tartary buckwheat (Fagopyrum tataricum Gaertn.)'Manten-Kirari'in body weight reduction related to its antioxidant properties: A randomised, double-blind, placebo-controlled study. Journal of Functional Foods, 26, 460-469, 2016.

Oyaizu M, Studies on products of browning reactions: antioxidative activities of browning reaction prepared from glucosamine. *Jpn. J.Nutr*, 44, 307-315, 1986.

Popescu ML, Aramă C, Dinu M, Costea T, Contributions to the pharmacognostical and phytobiological study of Sojae semen. Farmacia,57(5), 562-569, 2009.

Rai SN, Yadav SK, Singh D, Singh SP, Ursolic acid attenuates oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in MPTP-induced Parkinsonian mouse model. Journal of Chemical Neuroanatomy,71, 41-49, 2016.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C, Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26(9-10), 1231-1237, 1999.

Singleton VL, Rossi JA, Colorimetry of total polyphenols with phosphomolybdic - phosphotungstic acid reagents. Am. J. Enol. Vitic, 37, 144-158, 1965.

Sthijns MM, Schiffers PM, Janssen GM, Lemmens KJ, Ides B, Vangrieken P, Johansson K. Rutin protects against H_2O_2-triggered impaired relaxation of placental arterioles and induces Nrf2-mediated adaptation in Human Umbilical Vein Endothelial Cells exposed to oxidative stress. Biochimica et Biophysica Acta (BBA)-General Subjects, 1861(5), 1177-1189, 2017.

Tan JBL, Lim YY, Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts. Food Chemistry, 172, 814-822, 2015.

Tomczyk M, Preliminary phytochemical investigation of *Lychnis flos cuculi* herbs. J. Nat. Med, 62, 473-475, 2008.

Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, Dall'Acqua SM, Fico G, Phenolic compounds from *Achillea millefolium* L. and their bioactivity. Acta Biochim. Pol, 58(2), 203-209, 2011.

Wichtl M, Anton R, Plantés therapeutiques tradition, practique officinale, science et thérapeutiques. Editura Tec & Doc, Paris, 529-530, 2002.

xxx - European Pharmacopoeia, 7th edition, Ed. EDQM Council of Europe, Strassbourg, 1059, 1147-1148, 1071-1072, 2011.

Yang CH, Li RX, Chuang LY, Antioxidant activity of various parts of *Cinnamomum cassia* extracted with different extraction methods. Molecules, 17, 7294-7304, 2012.