BULETINUL INSTITUTULUI POLITEHNIC DIN IAŞI
Publicat de
Universitatea Tehnică „Gheorghe Asachi” din Iaşi
Volumul 63 (67), Numărul 1, 2017
Secţia
CHIMIE şi INGINERIE CHIMICĂ

POLYPHENOLIC CONTENT EVALUATION IN BRANCHES OF *ROSA CANINA* L. AND *HIPPOPHAE RHAMNOIDES* L. SPECIES

BY

OANA TEODORA CIUPERCĂ[1,2], CARMEN ELENA ȚEBRENCU[2] and IRINA VOLF[1,*]

[1]“Gheorghe Asachi” Technical University of Iaşi,
Faculty of Chemical Engineering and Environmental Protection
[2]Research and Processing Centre for Medicinal Plants “Plantavorel” S.A., Piatra Neamț

Received: March 11, 2017
Accepted for publication: March 25, 2017

Abstract. This study evaluated the branches of *Rosa canina* L. and *Hippophae rhamnoides* L. in order to highlight new sources of polyphenols, condensed tannins and flavonoids. The phytochemical screening and ultraviolet-visible and Fourier transform infrared spectroscopy analysis on biomass showed the presence of fine chemical constituents like polyphenols, flavonoids (rutin) and condensed tannins (catechin, epicatechin). The biomass has been evaluated to quantify the total polyphenols (gallic acid) and tannins (tannic acid) by instrumental methods (UV-VIS spectrometry). The results showed that the branches of *R. canina* contain polyphenols (1.77±0.067%g gallic acid equivalent/g) and tannins (1.45±0.029%g tannic acid equivalent/g), whilst the branches of *H. Rhamnoides* are somewhat richer (1.87±0.058%g gallic acid equivalent/g and 1.94±0.038%g tannic acid equivalent/g). These results open perspectives for advanced valorization of *R. canina* and *H. rhamnoides* branches, already known only for phytotherapeutical and nutritional potential of their fruits.

Keywords: *Rosa canina*; *Hippophae rhamnoides*; branches; polyphenols; tannins; phytochemical screening.

[*]Corresponding author; *e-mail*: iwolf@ch.tuiasi.ro

1. Introduction

Polyphenols are secondary metabolites of plants and are widely distributed in all parts of them. These compounds are naturally synthesized in plants: branches, leaves, stems, roots, flowers, fruits, seeds. The quantity and quality of the bioactive compounds may differ from one part to another. The presence of a bioactive compound in a specific part of a medicinal plant may lead to its further isolation, purification and characterization. The successful determination of bioactive compounds from biomass depends on the type of solvent and on the extraction method applied (Tiwari *et al*., 2011; Charalampos *et al*., 2013; Narender *et al*., 2012; Vishnu *et al*., 2013).

Sea buckthorn (*Hippophaer hamnoides* L.) belongs to botanical family *Elaeagnaceae.* The berries are widely used as functional food supplement, wellknown for their antioxidative properties (Ignat *et al*., 2011), attributed to hydrophilic and lipophilic compounds including phenols (caffeic acid, ferulic acid, cumaric acid, gallic acid, catechin and epicatechin derivatives) (Ma *et al*., 2016; Guo *et al*., 2017), flavonoids, ascorbic cacid, condensed tannins (Tiitinen *et al*., 2005). The leaves and the branches of sea buckthorn have also antioxidant potential due to phenolic acid derivatives. Leaves were found to contain maximum value for total phenolics followed by branched (Perk *et al*., 2016).

Dog rose (*Rosa canina* L.) (*Rosaceae*) is frequently used in traditional medicine. The most studied part of these species is the hip, which is rich in many bioactive compounds, including carotenoids (Hodisan *et al*., 1997), ascorbic acid, mineral elements, phenolics and fatty acids (Hosni *et al*., 2010; Ercisli, 2007). Dog rose hips possess different kinds of pharmaceutical properties (Wenzig *et al*., 2008) and, in addition to roots and leaves, they are known as diuretic, anti-inflammatory, antioxidant agents (Orhan *et al*., 2007).

As the literature shows, from these two species, only the fruits are often used in phytotherapy. The number of studies investigating the phytotherapeutical potential of branches of *R. canina* and *H. rhamnoides* has grown in recent years, but there are still limited.

This study was focused mainly on the bioactive compounds from branches of *R. Canina* and *H. Rhamnoides* species, in order to highlight new sources of biomass containing valuable polyphenols, condensed tannins and flavonoids.

2. Materials and Methods

2.1. Plant Material and Chemicals

Branches of *Rosa canina* L. (*Rosaceae*) and *Hippophae rhamnoides* L. (*Elaeagnaceae*) were collected from Siret Valley, Bacău region, Romania, in September 2016. The plants were identified and certified by experts from

"Stejarul" Biological Research Centre, Piatra Neamt. The biomass was dried in a well-ventilated room, in a single layer, protected from direct solar light. The dried branches were grounded using a laboratory mill Microton MB550. The samples were stored in a clean dessicator until were used for phytochemical study and FT-IR evaluation.

The loss on drying was measured with a KERN MLS Thermobalance with infrared.

All the reference standards and chemicals were of analytical grade or pure (Sigma Aldrich, Merck, Roth).

2.2. Samples Preparation

5g of powdered vegetal material of each species were dispersed separately in 100 mL of different solvents (distilled water, methanol, ethanol 30%, ethanol 50%, and ethanol 70%). It was performed a batch extraction at room temperature 20-23ºC, 24 h. Each extract was filtered and used for the UV-VIS qualitative and quantitative evaluation and for phytochemical assay.

2.3. Qualitative Analysis

Phytochemical study

In order to detect the presence of phenols, tannins and flavonoids the phytochemical tests were performed according to the methods described by Ciulei *et al*., 1993. The tests were based on the visual observation of colour change.

Tests for Phenols

Folin-Ciocalteu Test

0.5 mL Folin-Ciocalteu reagent and 2 mL 20% sodium carbonate is added at 2 mL extract; the appearance of a blue colour indicates the presence of the phenols.

Ferric chloride Test

2 mL extract was treated with aqueous 3% ferric chloride. The appearance of a olive-brown coloration indicate the presence of phenols.

Arnow Test

2.5 mL extract was treated with 2.5 mL hydrochloric acid 0.5 N, 2.5 mL Arnow reagent and 2.5 mL sodium hydroxide (shaking after each addition of reagent). The appearance of red brick coloration indicates the presence of polyphenol carboxylic acids.

Test for tannins (The ferric chloride reaction)

1 mL extract is diluted in a test tube with 2 mL distilled water and then 2-3 drops of 1% ferric chloride (R) is added. The appearance of a blue-black colour would indicate the presence of gallic tannins, whilst a dark green coloration would indicate the presence of chained tannins.

Tests for Flavonoids

Alluminium chloride test

2 mL extract is placed into two different test tubes (1 – sample, 2 – control), 5 mL 10% sodium acetate is added in each sample and stirred. In the first tube 3 mL of 2.5% aluminium chloride was added and 3 mL of methanol/ethanol in the second tube. In the presence of flavonoids a yellow colour appears.

The Shibata test (cianidol reaction)

3 mL extract is evaporated in a porcelain capsule on water bath. The residue is heat dissolved in 2 mL 50% v/v methanol. A yellow solution is obtained after dissolving the residue in methanol, which is placed in a test tube, then is added a small quantity of powder or 2-3 pieces of magnesium span and 10 drops of concentrated hydrochloric acid. (R) The presence of flavonoids aglycons is indicated by the appearance of a red or orange colour. The red colour is representative for flavonols, and the orange one is representative for the flavones.

UV-VIS and FT-IR identification

Molecular absorption spectrophotometry in ultraviolet/visible light (UV/VIS) is an analytical method based on the property of an ion or molecular species to absorb at certain wavelengths of UV/VIS radiation.

Spectroscopic analysis was performed as follows: UV-VIS absorption spectrum of extracts was recorded using a Cary 50 UV-Visible Varian spectrophotometer, in the range between 200-800 nm. The Fourier transform infrared spectrophotometer Cary 630 FT-IR Agilent was used to obtain the FT-IR spectrum for each powdered vegetal material in order to establish functional groups of the main bioactive compounds in the samples. FT-IR spectrum was recorded in 400-4000 cm^{-1} range.

2.4. Quantitative Analysis

Determination of total phenolic content

The total phenolic compound was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965), using Folin-Ciocalteu reagent. The same procedure was performed for the gallic acid standard curve. The results were expressed in mg of gallic acid equivalent (GAE) per g (% g GAE/g) taking in account the sample dilution. The analyses were performed in triplicates.

Determination of total tannins content

The total tannins compound was determined according Folin-Denis method (Katoch, 2011), using Folin-Denis reagent. The absorbance was measured at 726 nm on spectrophotometer (UV-VIS CARY50). A standard

curve was obtained for the tannic acid following the same procedure. The results were expressed in g of tannic acid equivalent per g (%g/g), taking in account the sample dilution. The analyses were performed in triplicates.

3. Results and Discussion

The chemical composition of branches of *R. canina* and *H. rhamnoides*is less studied and this is the reason why the following chemical and instrumental methods of identification were used in order to confirm the presence of interest polyphenolic compounds.

3.1. Qualitative Analysis

Phytochemical study

The results of phytochemical study are presented in Table 1. The results revealed that the various alcoholic and aqueous extracts of branches of *R. canina* and *H. rhamnoides* contain polyphenols, condensed tannins and flavonoids. Phenols were detected in all type of extracts obtained from the two selected species, with high and moderate intensity.

Table 1
Qualitative Phytochemical Analysis of Various Alcohol and Aqueous Extracts of Rosa Canina and Hippophae Rhamnoides Branches

Plant constituents		Methanol		Water		Ethanol 70%		Ethanol 50%		Ethanol 30%	
		RC	HR	RC	HR	RC	HR	RC	HR	RC	HR
Phenols	The Folin-Ciocalteu test	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	The ferric chloride test	++	+++	++	++	+++	+++	+++	+++	+++	+++
	Arnow test	++	++	+++	++	+++	+++	+++	+++	+++	+++
Tannins	The ferric chloride test	++	++	++	+	+++	+++	+++	+++	++	++
Flavonoids	The alluminium chloride test	+	++	+	+	+++	++	+++	++	++	++
	The Shibata test	–	+	+	+	–	++	–	++	–	++

+++: highly present, ++: moderately present, +: low, –: absent, RC: *Rosa canina* branches extract, HR: *Hippophae rhamnoides* branches extract.

The ferric chloride test showed a dark-green coloration, which has indicated the condensed tannins presence in all extracts, with high intensity in those obtained with 70% and 50% ethanol. The lowest intensity of dark-green colour, specific for condensed tannins, was obtained for aqueous extract of *H. Rhamnoides* branches. Flavonoids were detected in all type of extracts, with high content in 70% and 50% ethanol extracts of *R. canina.* The Shibata test revealed a low or absent presence of flavonoids in *R. canina* extracts whilst in *H. rhamnoides* extracts are moderately present.

UV-VIS and FT-IR identification

UV-VIS analysis of phenolic compounds was performed on methanol extracts and the spectra are presented in Figs. 1 and 2. Depending on the specific absorption, were identified flavonoid compounds like rutin between 200-220 nm and condensed tannins like catechin, epicatechin between 200-280 nm. The results proved that methanolic extracts showed maximum absorption peaks attributed to flavonoids such as rutin which has a maximum peak absorption at 205 nm, present in *R. canina* extract and condensed tannins such as catechin and epicatechin, with a maximum peak absorption at 210 nm, 275 nm present in both species extract.

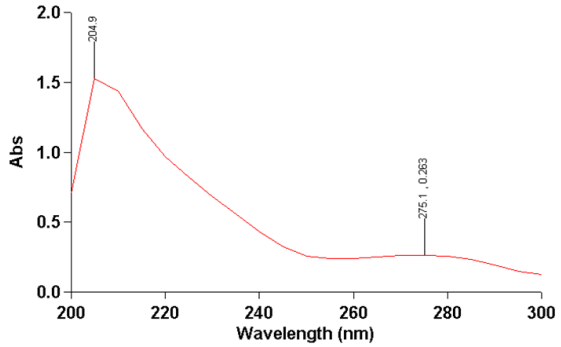

Fig. 1 – UV-VIS absorption spectra of methanolic *Rosa canina* extract.

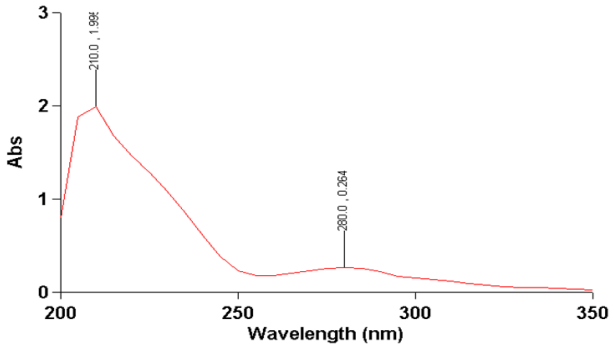

Fig. 2 – UV-VIS absorption spectra of methanolic *Hippophae rhamnoides* extract.

The FT-IR analysis showed intense absorption bands that correspond to aliphatic and aromatic functional groups from the interest compounds.

The vibrational spectrum for the powdered vegetal material (*R. canina* - branches) is shown in Fig. 3. Several absorption peaks are centred around 3737, 3280, 2894, 2329, 2104, 1414, 1232 cm^{-1} which are assigned to the stretching vibrations of the O–H group at 3280 cm^{-1}. Other characteristic stretching modes are found at 2894 cm^{-1} for C–H aromatic, 1414 cm^{-1} and 1512 cm^{-1} for C–C stretch (in the aromatic ring), C–O stretch mode at 1232 cm^{-1}.

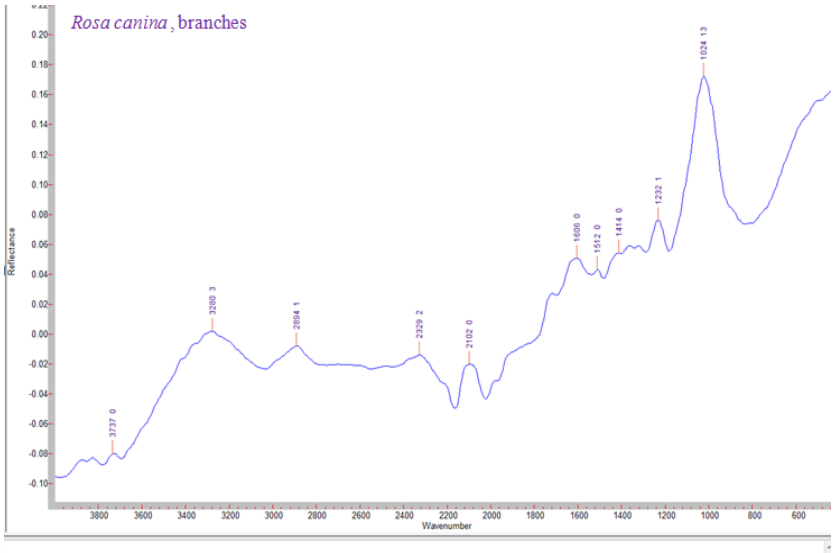

Fig. 3 – FT-IR spectra of powdered material plant (branches) of *Rosa canina*.

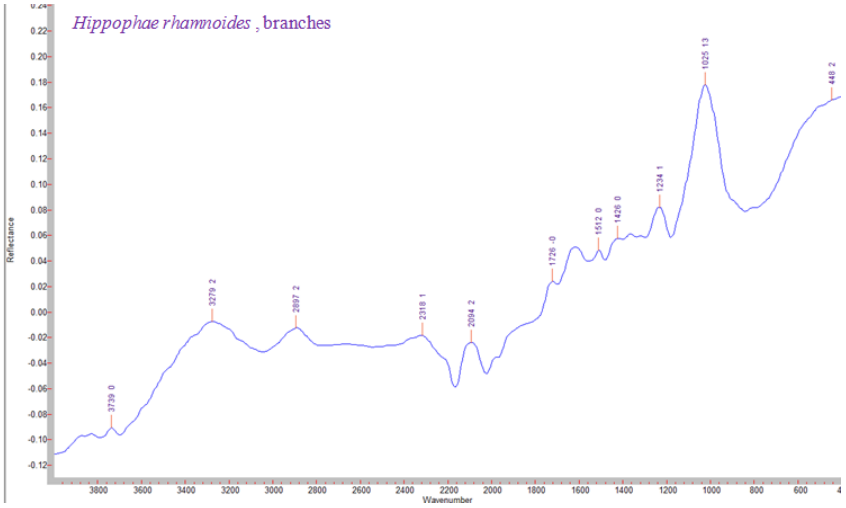

Fig. 4 – FT-IR spectra of powdered material plant (branches) of *Hippophae rhamnoides.*

FTIR spectral data for the powdered vegetal material (*H. rhamnoides* - branches) (Fig. 4) are attributable to the O–H group at 3279 cm^{-1}. A C–H stretch existsin the region of 2850–3000 cm^{-1} and a carbonyl bond stretching vibrations from aldehydes, ketones, carboxylic acids at 1726 cm^{-1}. Peaks at 1512 cm^{-1} and 1426 cm^{-1} can be assigned to C–C stretching (in the aromatic ring) and at 1234 cm^{-1} is attributable to the C–O group.

3.2. Quantitative Analysis

Determination of total phenolic content and total tannins content

The *R. canina* vegetal material (branches) has 7.95% loss of drying and *H. Rhamnoides* 7.50%. The obtained results showed that the branches of *R. canina* contain polyphenols (1.77±0.067%g gallic acid equivalent/g) and tannins (1.45±0.029%g tannic acid equivalent/g), whilst the branches of *H. rhamnoides* contain polyphenols (1.87±0.058%g gallic acid equivalent/g) and tannins (1.94±0.038%g tannic acid equivalent/g) (Table 2).

Table 2
Determinations of the Main Constituents

Parameters	*R. canina*	*H. rhamnoides*
	branches	
Loss of drying (%)	7.95	7.50
Total phenolic content (as gallic acid) (%g GAE/g)	1.77 ± 0.067	1.87± 0.058
Total tannins content (as tannic acid) (%g/g)	1.45 ± 0.029	1.94 ± 0.038

4. Conclusions

The present study provides information about the polyphenols that can be found in branches of *R. canina* and *H. rhamnoides*.

The phytochemical screening and UV-VIS spectroscopy analysis showed the presence of valuable chemical constituents like polyphenols, flavonoids (rutin, with a maximum of absorption at 205 nm) and condensed tannins (catechin and epicatechin, with a maximum of absorption at 210 nm and 275 nm). The FT-IR analysis reveals intense absorption bands (1750-600 cm^{-1}) corresponding to aliphatic and aromatic functional groups from gallic acid, catechin, epicatechin, tannic acid, rutin.

The content in polyphenols and tannins (1.77±0.067%g gallic acid equivalent/g and 1.44±0.029%g tannic acid equivalent/g for *R. canina* branches; 1.87±0.058%g gallic acid equivalent/g and 1.94±0.038%g tannic acid

equivalent/g for *H. rhamnoides* branches) suggest an advanced potential of the two species.

These results open perspectives for advanced valorisation of *R. canina* and *H. rhamnoides* branches, two species already known only for phytotherapeutical and nutritional potential of fruits.

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EVALUAREA CONŢINUTULUI POLIFENOLIC AL RAMURILOR DE MĂCEŞ (*ROSA CANINA* L.) ŞI CĂTINĂ (*HIPPOPHAE RHAMNOIDES* L.)

(Rezumat)

Acest studiu a avut ca obiectiv evaluarea compoziției chimice a ramurilor de *Rosa canina* L. şi *Hippophae rhamnoides* L. precum şi posibilitatea utilizării acestora ca o nouă sursă de polifenoli, respectiv taninuri condensate şi flavonoide. Screeningul fitochimic, analiza UV-VIS şi FT-IR a biomasei a indicat prezența compuşilor chimici de interes cum ar fi polifenoli, flavonoide (rutin) şi taninuri condensate (catehina şi epicatehina). De asemenea biomasa a fost evaluată cantitativ prin dozarea polifenolilor (acid galic) şi taninurilor (acid tanic) prin metode intrumentale (spectrometrie UV-VIS). Rezultatele obținute au indicat că ramurile de *R. canina* conțin polifenoli (1.77±0.067%g echivalent acid galic/g) şi taninuri (1.44±0.029%g echivalent acid tanic/g), iar ramurile de *H. rhamnoide* conțin de asemenea polifenoli (1.87±0.058%g echivalent acid galic/g) şi taninuri (1.94±0.038%g echivalent acid tanic/g), dar în cantități ceva mai mari. Rezultatele obținute reprezintă premizele de valorificare avansată a acestor resurse şi de obținere a unor compuşi polifenolici din ramurile speciilor medicinale de *R. canina* şi *H. rhamnoides*. Cele două specii sunt cunoscute în prezent doar pentru potențialul fitoterapeutic şi nutrițional al fructelor.