

Quantitative analysis of some antioxidant compounds from the hydroalcoholic extracts of *Rosa canina*

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Abstract

In this study the quantitative analysis of some antioxidant compounds from the hydroalcoholic extracts of *Rosa canina* was performed by high performance liquid chromatography, using a Shimadzu Nexera X2 ultra high performance liquid chromatograph (UHPLC) equipped with a Shimadzu DAD detector (Tokyo, Japan) M30A and a Nucleosil 100-3-C18 reverse phase column. The analyzed plant material was from rose hip fruits (*Rosa canina*). Two types of hydroalcoholic extracts were analyzed (90%, 70%); the 70% ethanolic extracts had a higher content of phenolic and polyphenolic compounds, compounds with a strong antioxidant character. The analyzed antioxidant compounds were: vanillic acid, rosmarinic acid and ferulic acid. The content of ethanol extracts of *Rosa canina* in the determined compounds varies between 7.33 -18.97 mg/L. The aim is to capitalize on the *Rosa canina* plant material by obtaining dry plant extracts incorporated in active principles of therapeutic interest. As a shoulder, it was decided to carry out the extraction process with ethanol 70%, 90% as extraction solvent, and then after the quantitative analysis of the extracts, in the future we are looking at drying by the lyophilization method, with the advantage of preserving the long-term structural and functional integrity of the resulting extracts.

Key words: phenolic compound, hydroalcoholic extract, antioxidant activity, high performance liquid chromatography

1. Introduction

The antioxidant efficacy of phenols containing voluminous substituents in the ortho positions to the hydroxyl group is reduced, and they are of no practical interest. For a phenol to possess significant antioxidant activity it is necessary that the hydroxyl group be shielded by at least one big group such as t-butyl, cyclohexyl, benzyl [1]. Correlations have been established between the energy of the aromatic state of phenols and antioxidant efficacy and between the oxidation potential value and efficacy, but their practical utility is rather limited [1]. The analyzed phenolic compounds were: vanillic acid, rosmarinic acid and ferulic acid.

Ferulic acid is a powerful antioxidant, excellent in neutralizing free radicals,

protecting the skin from harmful factors in the environment, particularly useful in the composition of anti-aging cosmetic products, in treatments for mature skin and in sun protection formulas.

Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is a phenolic compound, being a derivative of dihydroxyl benzoic acid, of which a hydroxyl residue has been transformed into methyl ether.

Rosmarinic acid is a natural polyphenolic organic compound with antioxidant character, which can be widely used in oils, fat-containing foods, biomedicine, cosmetic industry.

The presence of phenolic compounds in plant tissues makes it difficult to measure each

antioxidant component separately.

Different extraction media were also tested to ensure the maximum extraction of antioxidants from the samples (in most cases, alcoholic extractions proved to be more effective than aqueous extractions) [2, 3].

It is difficult to compare the data obtained by different authors, although there are numerous publications on the effect of substituents over the antioxidant activity of phenols [4].

The rosehip (*Rosa canina*) is a thorny shrub, reaching 2-3 m, with branches arching outwards, having compound pinnate leaves with 5-7 leaflets arranged alternately and flowers of type 5 solitary or grouped to 2-3 at the top of the branches. It is a medicinal plant known since ancient times, when it was used as a remedy against rabies. Rosehip is widespread in Europe, Western Asia and North Africa [5]. Rosehip has pharmacological properties: it is a vitaminizing tonic, decreases the fragility and permeability of capillary vessels, normalizes peripheral circulation, it is cardioprotective, hepatoprotective, diuretic and vermifuge [5]. The specialized literature does not offer a very varied range of information regarding the extremely complex chemical composition of plant extracts from the species *Rosa canina* -

fam. rosaceae, although the pharmacological actions are well known. The study of these phenolic compounds led to the definition of the chemical composition of the *Rosa canina* species.

Starting from the extraction of these compounds of pharmaceutical interest, we quantitatively analyzed these compounds with antioxidant potential from *Rosa canina* fruits, in order to obtain in the future new pharmaceutical products with topical application, with the possibility of generating a potential antioxidant and antimicrobial effect.

2. Material and methods

2.1. Plant materials

The studies undertaken had as raw material the plant species: *Rosa canina* - *fam. rosaceae* – from Fares S. A. – Orăștie, Romania. Before being dried, the analyzed plant material was selected and conditioned, removing impurities and plant parts that are not useful, browned, moldy, pest-attacked plant parts, as well as organic and mineral bodies, so that, after the selection and conditioning operation, the fruit must be clean. The specifications of analyzed plant material, according the producer (Fares S.A. Orăștie) are presented in the table 1.

Table 1. Specifications of analyzed plant material

Vegetal part/plant	Aspect	Impurities [%]	Foreign bodies [%]	Moisture [%]
Rosehip fruit / <i>Rosa canina</i>	Whole, healthy fruits, picked when they are orange in color to the overripe stage	Remains of tails, leaves, max. 1	Organics, max. 0,5 Minerals, max. 0,5	Normal of fresh fruits To dried fruits, max. 0,5

2.2. Method of extraction

The hydroalcoholic extracts were obtained by static extraction [6]. The applied extraction method was unit, the operational parameters, namely the degree of plant shredding, the used solvent, the plant/solvent ratio, the extraction temperature were identical. Reagents used are: methanol and phosphoric acid for HPLC (Merck, Germany), vanillic acid ($\geq 99\%$), rosmarinic acid ($\geq 99\%$), ferulic acid ($\geq 99\%$) – Roth.

Choosing the solvent and its concentration is made taking into account the substances to be extracted, as well as the inert substances that should not be entrained in the solution. Extracts were obtained 96% hydroalcoholic and 70% ethanol, with a clear appearance, having a specific color.

2.3. High performance liquid chromatography (HPLC)

The profile of polyphenolic compounds of the hydroalcoholic extracts was investigated by chromatographic analysis using a Shimadzu Nexera X2 ultra-high performance liquid chromatograph (HPLC) equipped with a Shimadzu M30A DAD detector and a Nucleosil 100-3-C18 reversed-phase column length of 125 mm x 4 mm inner diameter x 3 micrometer particle size (Macherey-Nagel GmbH, Duren, Germany). The column temperature was maintained at 30 degrees Celsius and the flow rate at 1ml/min. The solvents used for the chromatographic elution consisted of the 0.1% aqueous solution of trifluoroacetic acid, pH=3 (A) and acetonitrile (B).

The chromatographic elution schedule used in this analysis was as follows 95% A and 5% B, then the linear gradient increased to 35% B and held for 5 minutes, followed by a linear gradient of 42% B in 30 minutes. After that the eluent was changed to the initial composition, consisting of linear gradient 95% A and 5% B for 5 minutes. Measurements were performed at the wavelength of 270 nm. Once the retention time was established, calibration lines

were drawn for each analyzed standard using the series of solutions of known concentrations. The calibration curve for the identified compounds was expressed in mg/L [7-9].

3. Results and Discussion

The chromatograms of the analyzed samples are shown in figure 1.

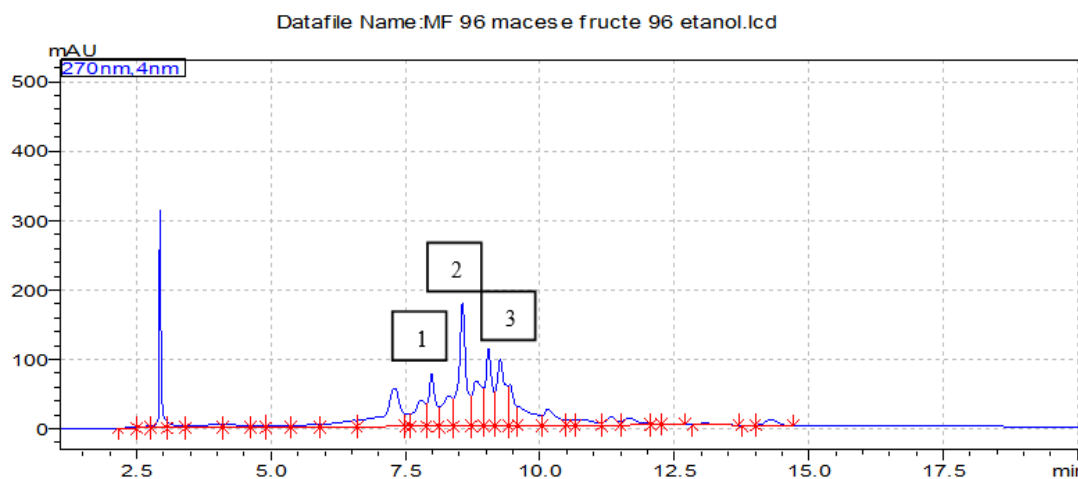


Figure 1. Chromatograms of the hydroalcoholic extracts of the analyzed *Rosa canina* species 1- vanillic acid, 2rosmarinic acid, 3-ferulic acid

Correlation coefficients of calibration lines specific to the standards analyzed by:

- vanillic acid, correlation coefficient $r^2 = 0,9946$;
- rosmarinic acid, correlation coefficient $r^2 = 0,9918$;
- ferulic acid, correlation coefficient $r^2 = 0,9935$.

The experimental results obtained from the

chromatographic analysis were in good agreement with those obtained using the external standard method for finding known amounts of pure phenolic compounds. From the data contained in table 2, the existence of a difference in the standards content of the studied extracts can be found. This difference is given only by the solvent from which the extracts were obtained.

Table 2. The content of antioxidant compounds in the hydroalcoholic extracts of *Rosa canina*.

Phenolic compounds with antioxidant character	Hydroalcoholic rosehip extracts	
	96% ethanol [mg/l]	70% ethanol [mg/l]
Vanillic acid	10.85	18.97
Rosmarinic acid	10.32	10.25
Ferulic acid	7.33	7.37

4. Conclusions

In the analyzed hydroalcoholic extracts, phenolic compounds with an antioxidant character were quantitatively determined by high performance liquid chromatography. Knowing the chemical composition of the rosehip is necessary, because the phenolic compounds determined together with other antioxidants are responsible for the antioxidant and antiradical properties of the rosehip.

As a result of the analysis carried out, it can be stated that the 70% ethanol hydroalcoholic extracts present higher amounts of phenolic compounds, compared to the 96% ethanol hydroalcoholic extracts obtained in the same way.

The work is based on pure research, namely exploratory and theoretical work, carried out with the aim of acquiring new knowledge, without seeking long-term benefits other than

the advancement of knowledge. In the future, we aim to obtain medicinal gels with antioxidant potential from rosehip fruits.

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