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Antioxidant properties of rose extract (Rosa villosa L.) measured using electrochemical and UV/Vis spectrophotometric methods

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The purpose of our study was to analyse the composition and antioxidant properties of phytocompounds in a rose extract (Rosa villosa L.). Spectroscopic and electrochemical methods were used to achieve this goal. UV-Vis and FTIR spectra helped to identify the polyphenols that are present in the tested extract compared with the literature data. Then, oxidation potential using cyclic and pulsed voltammetry was determined (Ep I = 0.47 V, Ep II = 0.68 V and Ep III = 1.58 V). Based on the CV and DPV parameters, which were determined from the ABTS spectroscopic methods, DPPH, FRAP and CUPRAC evaluated the antioxidant activity of the compounds that are present in the rose extract. Based on TG, the thermal stability of the rose extract was determined. A large correlation was found between the composition and the antimicrobial potential of the plant material tested, which is slightly lower than that of other plant-rich polyphenols (fruits, spices, vegetables). The extraction process itself, as well as the processing of rose fruit, has undoubtedly significantly reduced the antioxidants present in it. However, over-the-counter plant extracts can certainly compete and be an alternative for synthetic compounds, such as Trolox, BHA, and BHT, in cosmetics, food or drugs.

Keywords: Rose; UV–VIS; Electrooxidation; DPV; CPV; Antioxidant

1. INTRODUCTION

The world of plants gives us a tremendous potential for use. Most vegetables, fruits, and spices contain very valuable substances with interesting properties. Some of these substances are phytochemicals with different structures. The polyphenol molecule has strong antioxidant properties.

The position and substitution of the hydroxyl group in aromatic rings A, B and C determine their antimectant potential. Rosa (Rosa villosa L.) is a plant that belongs to wild plants and shrubs. From the literature, it appears that this plant contains enormous amounts of phenolic compounds that exhibit strong scavenging properties.

The Rosa (canina, villosa L.) plants are a valuable source for the food and drug industry. The tested rosa is a woody perennial of the Rosa genus within the family Rosaceae. Rose is the most common plant of the Rosacea family, and its accessibility is very large in different latitudes, mainly in Europe, Asia, and the Middle East and North America [1-10]. Rose heaps have been commonly used for centuries in many drinks, such as teas, jams and alcoholic beverages. Consuming various teas and functional beverages made with polyphenolic substances extracted from edible roses provides beneficial effects to human health. The literature shows that rose contains a great variety of precious substances. Its components include vitamins (B, P, PP, E, K, and C), flavonoids, carotenes, carbohydrates (mono- and oligosaccharides), organic acids, trace elements and others. Other literature reports indicate the presence of aromatics, phenolics, terpenoids, fatty acid derivatives, sugars, and other polar compounds. Most of these compounds have valuable properties, such as antioxidant, anticancer, antimutagenic and anti-inflammatory, free radical scavenging, antibacterial, antifungal, delaying or inhibiting oxidation processes [11-21].

The quality of the extract depends strictly on the process itself (temperature, pH, environment) and on plant biosynthesis, such as latitude, climate and sunlight. Vegetables containing phenolic compounds are of significant interest as alternatives because cheap alternatives are sought to replace synthetic substances. In our publication, we have proposed an interesting selection of research methodology to analyse the antioxidant properties of the compounds present in the rose fruit extract. An electrochemical, spectroscopic study was used to assess the scavenging capacity of free radicals. The composition of the extract was analysed based on the UV-Vis and FTIR spectra. The selected analytical methods are cheap, fast, and very precise, and give interesting results on the mechanism of oxidation and reduction of polyphenols [22-31].

2. EXPERIMENTAL

2.1. Chemicals

Rose samples (Rosa villosa L.) were obtained from Sadowniczy Zakład Doświadczalny Instytutu Ogrodnictwa Brzezna Sp. z o. o. The Slovak variety of this plant creates a loose shrub of a type with several stiff shoots that grow up to 2 m in height. The shoots have numerous medium-size spines. The single flowers are large and pink. The fruits are light red, large, and elongated (3-4 cm).

All chemicals used were of analytical grade and supplied by Fluka and Sigma-Aldrich. The experiments were performed in non-aqueous media. The substrate solutions were prepared by dissolving in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile. The concentration of rose was 1.995 g L^{-1} and 4.066 g L^{-1}. The solutions were thoroughly deoxygenated by purging with purified argon gas

(99.999%) for 15 min prior to the electrochemical experiments. Argon blanket was maintained over the solutions to supply an inert atmosphere during the voltammetric measurements.

2.2. Preparation of the ethanolic rose extract

The rose was cut into small pieces and extracted using a 5-fold volume of 70% ethanol under shaking conditions. The extraction was carried out in the dark at ambient temperature for 5 days. The final extract of rosa was concentrated to constant weight using a rotary evaporator under the reduced pressure conditions at 30°C.

2.3. Measurement methods

2.3.1. Cyclic and differential pulse voltammetry

The cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed using an Autolab electrochemical system (Eco Chemie, Utrecht, Netherlands) coupled to a computer and controlled using the GPES 4.9.9 software. The electrochemical cell was assembled using a conventional three-electrode system: platinum electrode as a working electrode, ferricinium/ferrocene (Fc^+/Fc) as a reference electrode, and a Pt wire as an auxiliary electrode. CV and DPV were recorded in the potential range from 0 to 2.1 V *vs* Fc^+/Fc. The DPV experiments were performed at a scan rate of 10 mV s^{-1}, pulse amplitude of 50 mV and modulation time of 1 mV. All experiments were carried out at room temperature (21 ± 1°C).

2.3.2. DPPH radical-scavenging activity

Using the DPPH procedure, the radical scavenging activity of rose samples was examined. The 2.0 mL alcohol solution of the 40 mg/mL (0.1 mM) 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was added to 0.5 mL of an ethanol solution (90%) that contained 0.02 mg/mL extract. Subsequently, 10 minutes after mixing, the absorbance of the solutions was determined using UV-Vis spectra at 516 nm. The UV-VIS spectra were recorded using a Thermo Scientific Evolution 220 spectrophotometer (2015, USA). Ethyl alcohol (70%) was used as a blank, [40-41]. The capability to scavenge the DPPH radical (AA%) was calculated using the following equation:

$$\text{Inhibition } (A\ \%) = [((A_0 - A_1)\ /A_0)\ 100] \qquad (1)$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the antioxidant sample.

2.3.3. ABTS radical-scavenging activity

Using the ABTS method, the antioxidant activity of the rose extract samples to scavenge free radicals was evaluated. Potassium peroxodisulfate (2.45 mM) and a 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) (6 mM) solution were mixed in ethyl alcohol (90%). Then, the mixture was allowed to stand for 16 h without light. The radical solution of

ABTS was diluted with ethanol to obtain an absorbance of 0.70 at 734 nm. The UV-VIS spectra were recorded using a Thermo Scientific Evolution 220 spectrophotometer, which was made in 2015 in the United States of America. After the dilution, 6.0 mL of an ABTS•+ solution was added to 50 µL of each antioxidant solution (6 mg/mL) or to (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox). The inhibition level was determined using the standard absorbance curve at 734 nm. The results are presented as the Trolox equivalent antioxidant capacity (TEAC), mmol Trolox/100 g of antioxidant [42-47].

2.4. Ferric reducing antioxidant potential - FRAP assay

The ability of rose samples to reduce the ferric ion (Fe^{3+}-TPTZ complex) under acidic conditions was determined using the FRAP test.

The analysis involves the study of the change in absorbance of the blue-coloured ferrous form (Fe^{2+}-TPTZ complex compound) at 595 nm. The FRAP reagent was obtained by mixing 25 mL of an acetate buffer (0.3 M, pH 3.6), 2.25 mL of the TPTZ solution [10 mM 2,4,6-tri(2-pyridyl)-s-triazine in 40 mM hydrochloric acid] and 2.25 mL of iron(III) chloride (20 mM) in distilled water. The reaction mixture was held at 37°C for 20 minutes. Then, the rose extract was added to the TPTZ solution. After a 4-min reaction, the absorbance of the mixture was measured.

2.5. Cupric ions (Cu^{2+}) reducing - CUPRAC assay

In total, 0.25 mL of cupric chloride (0.01 M) was mixed with 0.25 mL of 2,9-dimethyl-1,10-phenanthroline (neocuproine) (7.5×10^{-3} M in ethyl alcohol) and 0.25 mL of a buffer solution CH_3COONH_4 (1 M, pH 7.0) in the test tube, followed by the addition of the rose extract. Then, the final volume was adjusted to 2 mL using distilled water, and the solution was mixed. The test tubes were sealed and left at 24°C (room temperature). The absorbance was measured at 450 nm after a 30-minute reaction. Water was used as a reference sample. The increased absorbance of the resulting solution indicates increased reduction capacity of copper ions (Cu^{2+}).

2.6. UV-VIS Spectra

The UV-VIS spectra of the propolis extract solution were recorded from the mixture of 25 mL of each extract plus 30 mL of 96% ethanol. The mixture was scanned at 190-1100 nm using a UV-spectrophotometer (UVMini 1240, Shimadzu Co.).

2.7. Thermal decomposition

The thermogravimetric (TG) analysis of the rose fruit was performed using a Mettler Toledo Thermobalance. The samples of approximately 5 mg were placed in aluminium pans and heated from

20°C to 700°C under a dynamic flow of nitrogen (50 mL /min). Five heating rates (5°C/min) were used.

3. RESULTS AND DISCUSSION

3.1. UV-Vis and FTIR analysis

First, the composition of the studied extracts was determined. Thus, FTIR and UV-Vis spectrophotometry were used. It is clear from the literature that such spectrum is a "fingerprint" that reflects the composition and structure of the substance in the extract. The spectra of the dry plant material and of the extract were used to determine the composition and substances that were extracted. The FT-IR spectra of the investigated Rosa villosa L. ethanolic extract of the fruit are shown in Figure 1.

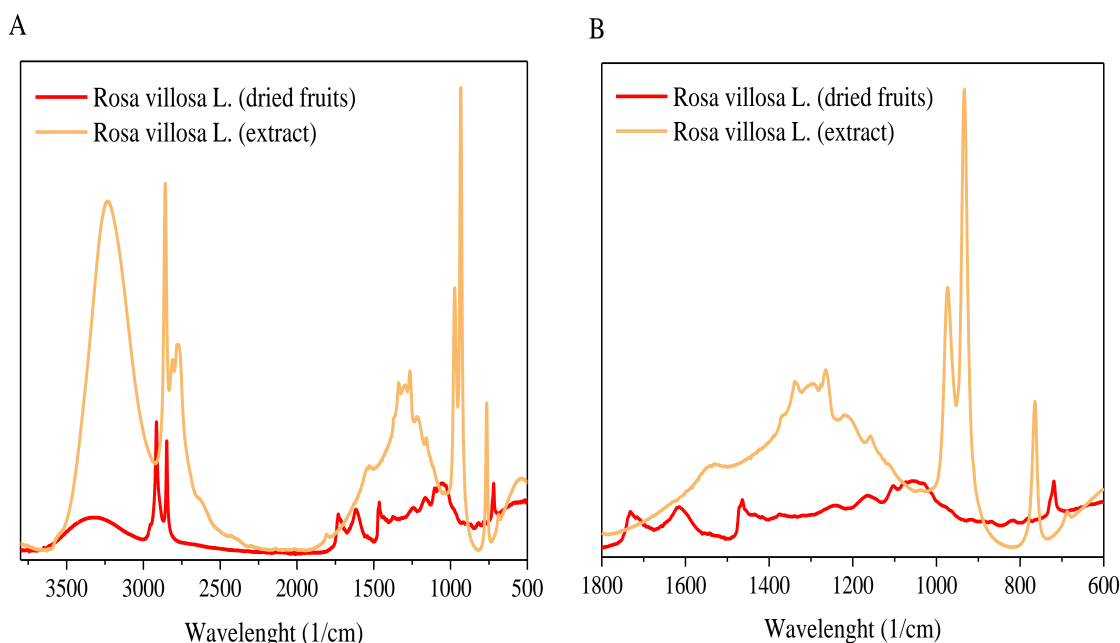

Figure 1. FT-IR spectra of the rose and rose extract (Rosa villosa L.) in the range of 3500-500 cm-1 (1800-600 cm-1).

Wenzig et al. [1] has already tested the total phenolic, ascorbic acid, and antiradical activity of Rosa canina by applying the Soxhlet method with dichloromethane, n-hexane and methanol. Lattanzio et al. [2] studied the rose extract in aqueous ethanol solutions.

From the presented spectra, one can infer the content of polyphenol compounds as well as other information. Namely, there are strong broad bands and strong bands in the range of 3393 - 3418 cm^{-1}, which can be attributed to the overlapping -OH and -NH stretching. The peak with a value of 1644 cm^{-1} represents the C = C relation; the 1384 cm^{-1} peak corresponds to the phenolic OH group. The band in the range of 2926 - 2968 cm^{-1} is attributed to the C-H stretching vibrations. The band at 1629 - 1638 cm^{-1} is due to the C = O stretching (aldehydes, ketones, and esters). The peaks at 1618 and 1407 cm^{-1} correspond to the symmetrical and asymmetrical stretching vibration of carboxylic acid (COO-), which

indicates the existence of carboxylic acid, ester, or carbonyl groups, based on the presence of FTIR spectra in the region. It is also easy to see the presence of free fatty acids (1710 cm^{-1}) and glycerides (1740 cm^{-1}). In the range of 800-1750 cm^{-1}, it is suggested that the band contains C = C-C aromatic ring stretches.

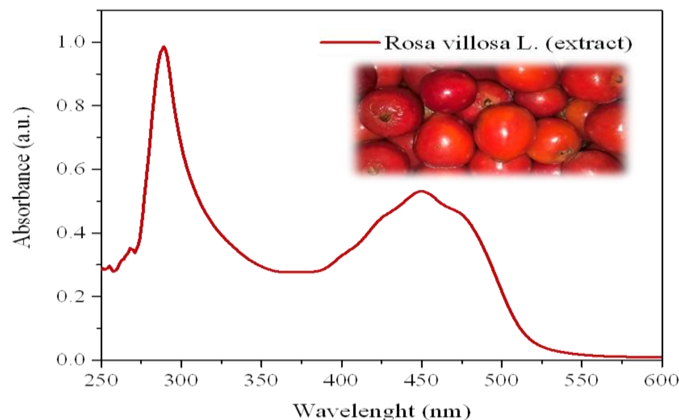

Figure 2. UV/visible absorption spectra of the rose extract (Rosa villosa L.).

The literature shows that most flavonoids have absorption bands in the range of 240 to 280 nm and 300 to 400 nm [32]. This range of wavelengths of the absorption bands strictly follows the structure of the polyphenol molecule, including the conjugation degree, the number and position of substituents, and the OH groups. The UV-VIS spectrum of the rose extract was evaluated in the wavelength range of 190-600 nm.

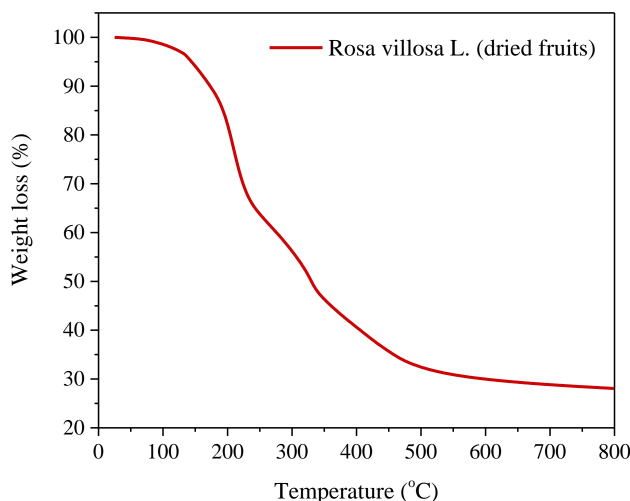

Figure 3. TG curve of the rose extract (Rosa villosa L.).

Figure 2 shows the UV-VIS spectrum of the rose extract. In the 300-450 nm range we noticed two maxima of absorption as a shoulder, which corresponds to different phytocompounds. The spectral characteristics (UV) of flavanols, which are present in the extract studied, give the absorption bands in

the range of 270-290 nm. Chrysin, hesperetin, eriodictyol, taxifolin give an absorption band at 288 nm, (+) catechin at 280 nm, and (-) epicatechin at 278 nm.

There are various compositions of rose honeys, including those determined by Ognyanov and Mihaylova [5], indicating high levels of organic acids (gallic, cinnamic, and ellagic), polyphenolic compounds, and flavonoids (rutin, kaempferol, quercetin, and catechin) [33-44].

As a result of the investigation of the rose extract properties, thermogravimetry at air conditions was measured to determine the thermal stability of the material under investigation. Oxidation and reduction temperatures are very important given the thermal processing of plant materials, such as the extraction process itself. Figure 3 shows the fruit TG curve of the rose. On the curve, there are two endothermic changes, which are associated with mass loss. The first change we attributed to dehydration, i.e., the evaporation of physically bound water (2%). In addition, 50% (T_{50}) was recorded at 328°C. The analysis shows that the rose fruit can be processed at 180°C. Above that temperature, polyphenols undergo oxidation and reduction processes.

3.2. Cyclic and differential pulse voltammetry

The volumetric (CV) and pulse differential (DPV) voltammetry were used to determine the antioxidant properties of the rose extracts. The voltammetric as potentiodynamic assay methods are based on the recording of current intensity at controlled potential variation and exploit the reducing ability of antioxidants or the reversibility of redox active substances [45-48]. Therefore, voltammetry has distinguished itself among the methods applied for both qualitative analysis and quantitation of molecules. The method allows to study the antioxidant molecules' electrochemical behaviour, mutual influence and interaction with oxygenated species. The examples of CV and DPV voltamperograms are shown in Fig. 4,

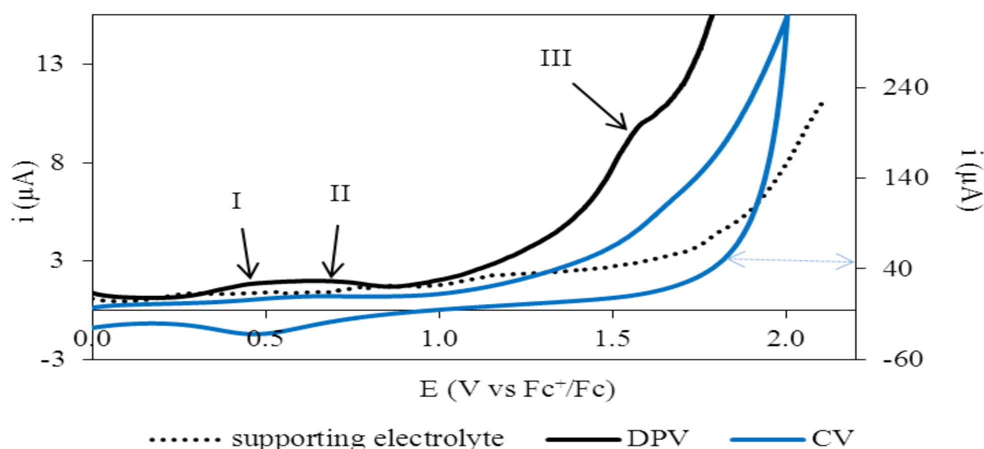

Figure 4. CV i DPV electrooxidation of the rose extract in 0.1 mol L-1 (C4H9)4NClO4 in acetonitrile recorded at the Pt electrode; v = 0.1 Vs-1.

At DPV, there are three electrolysis peaks of the compounds contained in the rose extract at the potentials. However, one oxidation peak of the compounds contained in the extract is identified as peak III at a potential of 1.65 V (Figure 4). In thermodynamic terms, the extract is characterized by good antioxidant properties, indicating a low oxidation potential of the compounds contained in this extract, that is, the extract is a good electron donor. Thus, in most cases, the peak power of the compounds expresses the antioxidant power of each compound [4-6]. The strength of the antioxidant properties of the compounds also determines the peak current, which depends on the concentration of the compounds contained in the test sample and on the nature of the electrochemical reaction. [49-50]. The higher the peak current (kinetic and concentration parameter) is, the higher is the electron transfer rate and/or the amount of electroactive species. Compounds that are more difficult to thermodynamically oxidize, with a potential of 1.58 volts, are more powerful than the oxidizing compounds at a potential of 0.47 V and 0.68 V, because the peak current is approximately 6 times higher. Fig. 5. Shows voltamperograms of the CV recorded for different scan rates, which are presented to observe the electrolyte peak of the extracted sample I and II.

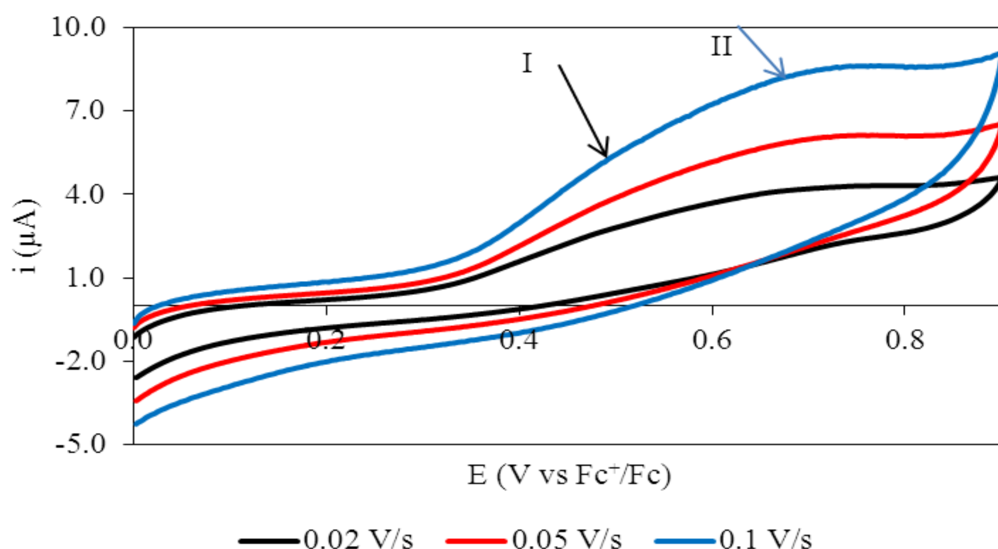

Figure 5. CV of the extract oxidation in 0.1 mol L-1 (C4H9)4NClO4 in acetonitrile recorded at the Pt electrode for various scan rates.

The CVs shown in Fig. 5 show two oxidation peaks at 0.47 and 0.68 V potentials that were not shown in the CV in Fig. 5 As the scan rate increases, the peak currents increase. It can be concluded that the compounds contained in the extracted rose extract already oxidize at the potential of 0.47 V. The low values of oxidation potentials of the compounds indicate good antioxidant properties of rose extracts.

Based on the CV and DPV voltammograms and the peak potential (E_p) and peak current (i_p) parameters, the antioxidant capacity (AC) is calculated according to equation [51-53]:

$$AC = \frac{i_{pI}}{E_{pI}} + \frac{i_{pII}}{E_{pII}} + \frac{i_{pIII}}{E_{pII}} \quad (1)$$

where i_p is the anodic current of the peak, E_p is the anodic potential of the same peak.

The results are shown in Table 1.

Table 1. Values of E_p, i_p, AC for the electro-oxidation of the compounds contained in the rose extract

Method	Peak I		Peak II		Peak III		AC_{total}
	E_p (V)	i_p (μA)	E_p (V)	i_p	E_p (V)	i_p	
CV for v=0.1 Vs^{-1}	0.51	5.29	0.71	8.49	1.67	118.35	93.19
DPV for v=0.01 Vs^{-1}	0.47	1.31	0.68	1.49	1.58	9.05	10.71
AC for CV	10.37		11.96		70.87		
AC for DPV	2.79		2.19		5.73		

The determined antioxidant capacity (AC) for I and II of the electro-oxidation peak is comparable, while for peak III, it is higher. The AC values determined for CV and DPV are different because CVs were registered at the 0.1 Vs^{-1} scan rate, while DPVs were recorded at 0.01 Vs^{-1}. In addition, CV and DPV differ in the current measurement technique.

3.3. Antioxidant capacity (ABTS, DPPH) and the ferric reducing antioxidant power (FRAP, CUPRAC) of the rose extract (Rosa villosa L.) measurement of the UV/Vis spectrophotometric assays

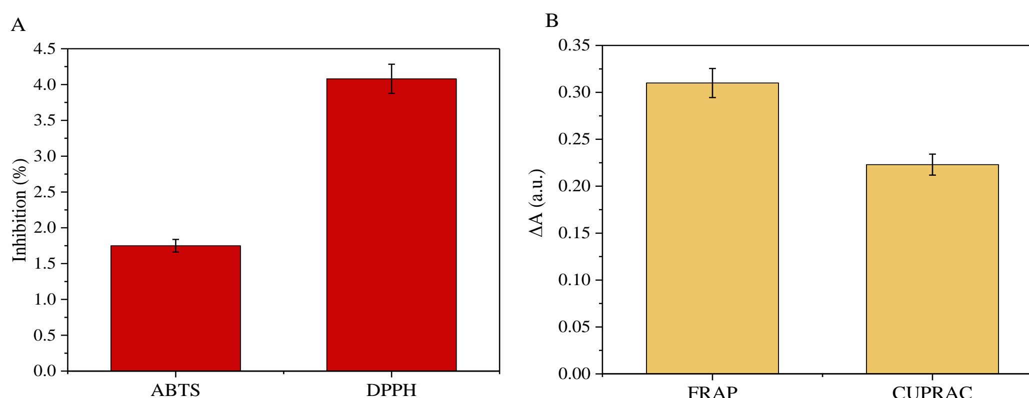

Figure 6. Radical cation scavenging activity (%) of the methanolic rose (Rosa villosa L.) extracts were evaluated against radical ABTS, DPPH.

Flavonoid substances are ubiquitous secondary metabolites in different plants. Most of them are characterized by excellent reducing properties. The purpose of the presented manuscript was to analyse the composition of rose fruit components and their ability to sweep free radicals.

The antioxidant activity of the rose extract was evaluated based on the ABTS and DPPH scavenging capacity. The ability to reduce iron (FRAP) and copper (CUPRAC) iron has also been studied. These methods are distinguished by their mechanism of action and are complementary to other methods for determining the oxidation potentials of phytocompounds. The study was performed to compare the antioxidant activity in the (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

diammonium) radical scavenging method used, which is applicable for lipophilic and hydrophilic antioxidants. DPPH, on the other hand, is most often used to evaluate activity in a hydrophobic environment. In addition, DPPH radical scavenging can be divided into two free stages, fast stage up to 90 seconds, and next longer stage up to 30 minutes. Thus, this method is helpful for determining the mechanism of antioxidative action. During the first rapid stage of the "DPPH assay", *o*-hydroxyls of the *B*-ring most likely react.

The antioxidant activity of the tested rose does not seem to be very high compared with other plant materials. The extract of rose showed good antioxidant ability because it is reach in phenolic and flavonoid compounds. The degree of inhibition of DPPH is 4.08 ± 0.20, and for ABTS, it is 1.75 ± 0.09. On the other hand, the reduction potential (ΔA) of Fe is 0.310 ± 0.02, and for copper, it is 0.223 ± 0.01. However, these studies may not accurately reflect the antioxidative properties of the extract tested because the solution itself is coloured and the described spectrometric methods are based on measuring the change in the maximum absorption of the 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic; 2,2-Diphenyl-1-picrylhydrazyl complex, which is accompanied by a significant change in colour. Thus, the intense colour of the extract itself often does not provide reliable test results. From the literature, the results show that the rose fruit contains large amounts of vitamin C, which is characterized by high antioxidant activity [53].

The high activity for sweeping free radicals is also due to the high content of catechins and tannins. The study in the literature of the total phenolics and ascorbic acid contents clearly indicates their great wealth in rose extracts. Nadpal et al. [54] presented 44 phenolics and quinic acid in rose heap extracts, which were identified using the LC-MS/MS technique. This also indicates, based on De Paepe et al. [55] that the heat treatment of rose heaps causes irreversible changes in the degradation of polyphenols contained therein.

Moreover, some of the literature points to the constituent content of minerals (N, P, K, Ca, Mg, Fe, and Zn), carbohydrates, and ascorbic acid. Various rose plants were analysed to assess the plant type, origin, growth conditions, latitude, composition and quality of the extract. Thus, a comparison of R. arvensis and R. canin was performed, confirming a much higher wealth of antioxidants in the first variant [53-55].

However, from the obtained results, it is clear that the process of obtaining and processing the rose results in significant losses in the composition and stability of the compounds contained therein. Reactive oxygen species play a very important role in inflammatory diseases, cardiovascular diseases, neurodegenerative disorders, cancer and ageing. Therefore, recent publications on the properties of natural polyphenolic compounds have a significant interest in many areas.

4. CONCLUSION

The studies in this publication concerned the analysis of the composition and properties of the substances present in rose fruit (Rosa villosa L.). Spectroscopic, electrochemical and calorimetric methods were used. The electrochemical studies (CV, DPV) of oxidation of the rose extract provided essential information about their potential of antioxidant capacity. A large correlation was found

between the composition and the antimicrobial potential of the plant material tested. Activity to sweep away free radicals and to reduce metal ions is small compared with other polyphenol-rich plants.

The extract is characterized by good antioxidant properties, indicating low oxidation potential of the compounds contained in this extract, that is, the extract is a good electron donor. Certainly, the extract tested can compete with synthetic antioxidants, which are used in human food, medications and cosmetics. This study provides constructive information about the antioxidant properties of rose and its application possibilities.

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