ORIGINAL PAPER

DEVELOPMENT OF DERMATO-COSMETIC HYDROGELS WITH ANTIOXIDANT ACTION USING MACERATES FROM *ROSA CANINA* L. PLANT

LUIZA MADALINA CIMA[1], GABRIELA STANCIU[2], ANA MARIA NECULAI[3], MAGDALENA MITITELU[4]

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Abstract. *In recent years, active ingredients derived from natural sources have garnered significant attention in alternative medical therapies. This study examines the therapeutic potential and pharmaceutical relevance of Rosa canina L., focusing on a comparative analysis of phytoconstituents in fresh fruits, flowers, and commercial fruit tea to inform future antioxidant-based pharmaceutical applications. Hydroalcoholic macerates (60% and 96%) were prepared from these plant materials, and spectrophotometric methods were employed to identify the compounds contributing to antioxidant properties, focusing on polyphenols and flavonoids. The total polyphenol and flavonoid content were quantified using the Folin-Ciocalteu reagent and the Romanian Pharmacopoeia method, respectively. Antioxidant activity was assessed through DPPH radical scavenging and FRAP assays. The results demonstrated that 60% of ethanolic macerates derived from fruits exhibited the highest antioxidant activity and ferric-reducing activity. Four formulations of dermato-cosmetic hydrogels were prepared with the macerates that showed the highest antioxidant potential. The physicochemical properties of the hydrogels, such as viscosity, pH, and spreadability, were analyzed to confirm their stability and appropriateness for dermato-cosmetic use. Antioxidant activity was assessed using DPPH assays, revealing a strong activity for free radical scavenging. The findings revealed a strong correlation between antioxidant activity and polyphenol content, affirming Rosa canina's potential for use in pharmaceutical formulations.*

Keywords: *natural antioxidants; R. canina macerates; phenolic compounds; flavonoids; Folin-Ciocalteu; DPPH Radical Scavenging test; FRAP method.*

1. INTRODUCTION

The significance of natural antioxidants has been widely recognized over time, leading to an intensification of scientific research into their benefits for human health. These compounds play a central role in pharmaceutical applications, contributing to the maintenance of cellular homeostasis and the prevention of pathologies associated with oxidative stress [1]. Antioxidants derived from plant sources are distinguished by their ability to protect the body

[1] Titu Maiorescu University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 040314 Bucharest, Romania. E-mail: luiza.cima@prof.utm.ro.
[2] Ovidius University of Constanta, Department of Chemistry and Chemical Engineering, 900470 Constanta, Romania. E-mail: gstanciu@univ-ovidius.ro.
[3] Ovidius University of Constanta, Department of Biochemistry, Faculty of Medicine, 900470 Constanta, Romania, E-mail: anamneculai89@gmail.com.
[4] "Carol Davila" University of Medicine and Pharmacy, Department of Clinical Laboratory and Food Safety, Faculty of Pharmacy, 020956 Bucharest, Romania. E-mail: magdalena.mititelu@umfcd.ro.

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against the harmful effects of free radicals, acting as stabilizing agents that neutralize reactive molecules and reduce oxidative damage at the cellular level. The demonstrated antioxidant activity of these compounds highlights their importance in modern strategies for the prevention and treatment of conditions caused by oxidative imbalances [2,3].

The wild rose, also known as the dog rose (*Rosa canina L.*), is a plant recognized and valued for its therapeutic properties and nutritional benefits. Widely distributed across various habitats in Europe, North Africa, and Asia Minor, *Rosa canina* belongs to the Rosaceae family and is characterized as a shrub that can reach heights of up to four meters. Its stems are protected by numerous thorny protrusions, while its compound leaves consist of five to seven leaflets. The fruit of this plant, commonly referred to as rosehip, is particularly valuable due to its complex chemical composition, with a color ranging from deep red to orange. Its structure predominantly consists of pericarp (71%) and seeds (29%), with a variable weight between 1.25 and 3.25 g [4]. Research into the chemical composition of rosehips has intensified due to the popularity of this medicinal plant and its extensive applications. Depending on species diversity and climatic influences, rosehips exhibit a significant concentration of biologically active substances, thereby contributing to numerous health benefits [5].

The use of *Rosa canina* fruits in phytotherapy has been well-documented. Studies conducted both in vivo and in vitro have consistently highlighted the multiple beneficial properties of these fruits, including antioxidant, anti-inflammatory, antimutagenic, anticancer, and anti-obesity effects [6-8]. Rosehips are a significant source of bioactive compounds such as vitamin C, carotenoids, tocopherols, polyphenols, flavonoids, tannins, pectin, organic acids, amino acids, essential oils, and unsaturated fatty acids [4]. Additionally, they serve as a valuable alternative source of lycopene, with concentrations ranging from 2.9 to 35.2 mg per 100 g [5].

The high polyphenol content of rosehips grants them strong antioxidant properties, providing numerous health benefits. Their chemical composition also includes vitamins such as C and E, flavonoids (hyperoside, astragalin, kaempferol), essential fatty acids (linoleic, linolenic, oleic), steroids (β-sitosterol, stigmasterol), and polyphenols (gallic acid, ferulic acid, ellagic acid) [9]. Among these, vitamin C stands out as one of the most important compounds found in rosehips, recognized for its potent antioxidant benefits. Its concentration varies depending on species, location, and environmental conditions, reaching peak levels during the early stages of ripening [10].

The antibacterial activity of various rosehip species has been attributed to polyphenolic compounds, which play a crucial role in combating pathogenic microorganisms. Additionally, chemical analyses have demonstrated that *Rosa canina* extracts possess strong antioxidant properties, contributing to the anti-inflammatory effects observed in in vivo studies. These findings suggest that *Rosa canina* may serve as an adjunctive therapeutic tool for managing inflammatory conditions [11].

Ethanolic macerates of *Rosa canina*, rich in phenols and flavonoids, have demonstrated the ability to inhibit digestive enzymes such as α-amylase, suggesting significant potential in glycemic regulation and diabetes management. By slowing starch digestion, the regular consumption of rosehip infusion may help maintain better glycemic control, particularly after carbohydrate-rich meals [12, 13].

Given the substantial volume of existing data on this plant species, which is also found in Romania, this research aims to conduct a comparative analysis of the phytoconstituents present in fruits, flowers, and commercial teas of *Rosa canina*. The phytoconstituents were extracted through maceration using ethanol at concentrations of 96% and 60%. Specific spectrophotometric techniques were employed to identify compounds responsible for antioxidant properties, such as polyphenols and flavonoids. Furthermore, the total antioxidant

activity of the obtained ethanolic macerates was assessed using specific methods, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays. The primary goal of these investigations is to identify the extract with the highest antioxidant potential for application in the development of pharmaceutical products with therapeutic effects.

The development of dermato-cosmetic formulations with enhanced antioxidant properties has gained significant attention due to the increasing demand for products that combat oxidative stress and promote skin health. This study focuses on the formulation and evaluation of hydrogels incorporating macerates from *Rosa canina* L., a plant known for its rich content of bioactive compounds, particularly flavonoids, phenolic acids, and vitamin C. The macerates were prepared using optimized extraction protocols to maximize antioxidant yield, followed by their incorporation into hydrogel bases. In this regard, physicochemical properties (including viscosity, pH, and spreadability) were assessed to ensure product stability and suitability for dermato-cosmetic applications. The antioxidant activity of the hydrogels was evaluated through DPPH assays, demonstrating significant free radical scavenging activity.

2. MATERIALS AND METHODS

2.1. MATERIALS AND ANALYTICAL EQUIPMENT

All reagents used in the analyses were of analytical purity and purchased from Sigma-Aldrich, Germany. The absorbance measurements, following the applied methods, were performed using a Jasco-550 UV-VIS double-beam spectrophotometer (Jasco International Co, LTD., Tokyo, Japan). For the preparation of dermato-cosmetic hydrogels, a turbine stirrer in a Heidolph RZR 2020 (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) was used, and rheological analysis was performed with a Rotational Viscometer ST-2020 R, manufactured by Laboquimia, Spain.

2.1.1. Determination of total polyphenol content

The concentration of total polyphenolic compounds (TPC) was determined using the Folin-Ciocâlteu method, according to the ISO 14502-1:2005 standard [14]. Spectrophotometric analyses were performed at a wavelength of 765 nm, employing substances such as gallic acid, methanol, and anhydrous sodium carbonate using a Jasco-550 UV-VIS double-beam spectrophotometer (Jasco International Co, LTD., Tokyo, Japan).

2.1.2. Determination of total flavonoid content

The total flavonoid content was determined according to the method described in the Romanian Pharmacopoeia, 10^{th} Edition. In this analysis, the following reagents were used: rutin solution, sodium acetate, aluminum chloride, methanol, and distilled water.

2.1.3. Antioxidant activity analysis

The antioxidant activity of hydroalcoholic macerates obtained from the fruits, flowers, and fruit tea of the *R. canina* plant was evaluated using two complementary methods: DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power). For the

DPPH method, the reagents used included methanol and the DPPH reagent, which are essential for assessing free radical scavenging activity [15].

The FRAP method involved the use of an acetate buffer solution (pH 3.6) to stabilize the reaction, a TPTZ solution (10 mmol/L) as a complexing agent, ferric chloride ($FeCl_3$, 20 mmol/L) as an oxidizing agent, and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) as a standard for constructing the calibration curve. The FRAP working reagent was prepared by combining the acetate buffer solution with the TPTZ and $FeCl_3$ solutions, according to the specifications of the established method [16-17].

2.1.4. Plant material

The aerial parts (fresh fruits and flowers) of the *Rosa canina* L. plant were harvested during the flowering period: flowers in June 2023 and fruits in September 2023. *Rosa canina* L. is a perennial plant that grows in Romania's wild flora and was collected from Giurgiu County, Romania (Figs. 1-2). To conduct a comparative study of bioactive compounds with antioxidant properties, an additional analysis was performed on a rosehip fruit tea purchased from a tea shop in the city of Giurgiu (Figure 3). The plant materials were thoroughly washed with distilled water and naturally dried on mesh trays placed in a well-ventilated room. Subsequently, the dried plant material was finely ground using a laboratory mill [18].

Figure 1. *Rosa canina* flower

Figure 2. *Rosa canina* fresh fruit

Figure 3. Rosehip fruit tea (*Rosa canina*)

2.2. METHODS

2.2.1. Preparation of *Rosa canina* ethanolic macerates

The preparation process of ethanolic macerates (MFL96, MFL60, MFR96, MFR60, MC96, and MC60) involved finely grinding 2.5 g of plant material obtained from the flowers, fruits, and fruit tea of the *Rosa canina* plant, to which ethanol at concentrations of 96% and 60% was added, up to 25 mL, respectively (1:10 ratio). The macerates were stored for 10 days under optimal conditions, protected from light and humidity, in a cool place. During the 10 days, the extracts were carefully observed and shaken 2-3 times a day. In the end, they were

filtered through cotton filters to separate the plant material and brought to volume with solvent. The liquid collected during filtration was stored in sterile, dark containers [18].

2.2.2. Determination of total polyphenol content

To determine the total polyphenol content, the calibration curve was constructed using gallic acid as the standard. Three control tests were performed for validation. The resulting equation was y = 0.0118x + 0.0973, with a correlation coefficient R^2 = 0.9952, confirming a linear relationship. The 6 ethanol macerates (MFL96, MFL60, MFR96, MFR60, MC96, and MC60) were initially diluted in a 1:10 ratio with the corresponding solvent (96% or 60% ethanol). From each diluted extract, 10 mL were measured and transferred into 25 mL volumetric flasks, which were then filled with water to the final volume. Then, 5 mL of diluted Folin-Ciocâlteu reagent was added to each flask, and after a 5-minute interval, 4 mL of 7.5% Na_2CO_3 solution was added. After one hour of reaction at room temperature, the absorbance of the samples was measured at a wavelength of 765 nm [19,20].

Equation (1) was used to determine the TPC concentration, measured in milligrams of gallic acid per 100 grams of plant material.

$$\text{TPC (mg GAE/100g dry weight)} = \frac{C \times 10^{-3} \times 100 \times V_m \times d}{m_{p \times V_p}} \quad (1)$$

where: *C* represents the concentration of gallic acid, expressed in µg/mL; V_m is the volume of the macerate used, measured in mL; mp refers to the mass of the plant material, expressed in grams; V_p indicates the volume of the solution used in the experiment, in mL; *d* is the dilution factor applied to the macerate.

2.2.3. Determination of total flavonoid content

For the determination of total flavonoid content, according to the method described in the Romanian Pharmacopoeia, 10th Edition, a calibration curve was achieved using varying concentrations of rutin. The obtained linear regression equation was y = 0.0361x + 0.028, with a correlation coefficient R^2 = 0.9991. Based on this curve, TFC values were calculated for each analyzed sample.

In the analysis, the six macerates were diluted in a 1:10 ratio with the corresponding solvent (96% or 60% ethanol). From each diluted extract, 10 mL were transferred into 25 mL volumetric flasks, to which 5 mL of 100 g/L sodium acetate solution and 3 mL of 25 g/L aluminum chloride solution were added. The mixture was shaken and completed to the mark with methanol. After a 15-minute reaction time, the absorbance of the solutions was measured spectrophotometrically at a wavelength of 430 nm. The samples were analyzed three times, and the average value was reported.

The flavonoid content in the sample, expressed in milligrams of rutin per 100 g of plant material, was calculated using equation (2):

$$\text{TFC (mg rutin/100 g dry weight)} = \frac{C \times 10^{-3} \times V_m \times 100 \times d}{m_{p \times V_p}} \quad (2)$$

where *C* represents the concentration of rutin (µg/mL) read from the calibration curve, *Vm* represents the volume of the extract (mL), *mp* is the mass of the analyzed sample (g), and *d* is the dilution factor of the extract.

2.2.4. Antioxidant activity analysis

The antioxidant activity of the *Rosa canina* macerates using the DPPH test was evaluated by applying the method of the initial color loss percentage, according to the procedure described by Murokore, with minor adjustments to the original protocol [21]. To quantify the antioxidant activity, 0.4 mL of each sample were transferred into 10 mL volumetric flasks, to which 2 mL of DPPH solution were added. The flasks were then filled to the mark and kept in a dark place for 30 minutes to allow stabilization at room temperature. The absorbance of the solutions was measured spectrophotometrically at a wavelength of 517 nm, using methanol as a control [18]. The DPPH radical scavenging activity was calculated according to equation (3).

$$\%\ \text{DPPH radical scavenging activity} = \frac{(\Delta A517\ \text{blank} - \Delta A517\ \text{sample})}{\Delta A517\ \text{blank}} \times 100 \quad (3)$$

The FRAP method is based on the ability of antioxidants to reduce the yellow-colored ferric tripyridyltriazine complex (Fe(III)-TPTZ) to the blue-colored ferrous tripyridyltriazine complex (Fe(II)-TPTZ) through electron donation by antioxidants. The calibration curve, constructed using standard Fe(II) solutions with known concentrations, showed a positive linear relationship ($y = 0.158x + 0.135$, with a correlation coefficient $R^2 = 0.9882$) between the average FRAP values and the concentrations of the $FeSO_4 \cdot 7H_2O$ standards. This was used to accurately assess the antioxidant activity of the analyzed samples. The procedure involved diluting the *Rosa canina* L. macerates (MC60, MFR60, MFL96) with purified water in a 1:10 ratio, except for the MFL60 macerate, which was diluted 1:100, and the MC96 and MFR96 macerates, which did not require further dilution. After dilution, the samples were placed in a water bath at 37°C for 5 minutes. Then, 100 µL of sample or standard solution were added to each test tube, and 100 µL of water were added to the control test tube. Each test tube was completed with 3 mL of pre-heated FRAP reagent, followed by incubation of the samples in the water bath. After 4 minutes, the contents of each test tube were transferred to a cuvette, and the absorbance was measured at 593 nm [22].

The FRAP values of the samples, expressed in µmol/g, were calculated based on the absorption of the standard using equation (4).

$$\frac{\text{Absorbance at 593 nm of the reaction mixture of the sample}}{\text{Absorbance at 593 nm of the } Fe^{2+} \text{ standard reaction mixture}} \times Fe^{2+}\ \mu\text{moli/L} = \mu\text{moli FRAP/g} \quad (4)$$

2.2.5. Formulation of hydrogels with *Rosa canina* L. macerates

Four hydrogels were made with macerates from rosehip flowers (MFL60 and MFL96) and those obtained from fruits (MFR60 and MFR96), those that presented the highest content in compounds with antioxidant action. To develop four hydrogels with Carbopol 940 (2%) and the specified macerates, a structured approach involving preformulation studies is essential. These hydrogels are intended to combine the functional benefits of rose hips with a stable, user-friendly formulation.

Carbopol 940 was weighed to achieve a 2% concentration and slowly dispersed in distilled water under constant stirring to avoid clumping. The dispersion was left undisturbed for 24 hours to allow complete hydration and swelling of the polymer. Each macerate was gradually added to separate batches of hydrated Carbopol, maintaining continuous stirring for uniform mixing. The volume of macerate added ranged between 5% and 20% of the total hydrogel formulation to balance the active content with the structural stability of the gel. After

macerate incorporation, the pH of each formulation was measured. Since Carbopol forms a gel only at specific pH levels, triethanolamine (TEA) was added dropwise while stirring to neutralize the formulation to a pH range of 5.5–6.5, ideal for skin application. This step was critical to ensure the gel achieved the desired consistency and stability. The mixtures were homogenized using a mechanical homogenizer to achieve a smooth, uniform gel free from air bubbles or particulate clumps. This step enhanced the aesthetic quality and usability of the hydrogels.

Once prepared, the hydrogels were assessed for the following characteristics:

- Each hydrogel was visually inspected for clarity, uniformity, and the intensity of the natural color imparted by the macerates. The odor was noted to ensure the characteristic aroma of rose hips was preserved without overpowering.
- The gels were evaluated for their ease of application by measuring the spreadability on a glass plate under standardized conditions.
- Viscosity measurements determined the gel's flow behavior, ensuring it was neither too thick for application nor too thin to lose structure.
- The formulations were tested for pH consistency over time at different storage conditions, including 4°C, room temperature, and 40°C.
- The antioxidant activity of the hydrogels was measured using DPPH assays to confirm the active phenolic compounds were retained post-formulation.
- The development of these hydrogels resulted in four distinct formulations, each incorporating the unique characteristics of its respective macerate. The preformulation studies provided insights into their compatibility, stability, and potential functional benefits. These hydrogels hold promise for applications in cosmetics or dermatology, offering antioxidant-rich formulations derived from natural sources.

Based on the evaluations, the following optimal compositions were established (Table 1).

Table 1. Composition of hydrogels with *Rosa canina* macerates

Components	Mass [g]			
	Formula P1	**Formula P2**	**Formula P3**	**Formula P4**
Carbopol 940	2 g	2 g	2 g	2 g
Glycerine	5 g	5 g	5 g	5 g
Triethanolamine	q.s	q.s	q.s	q.s
MFL60	10 g	-	-	-
MFL96	-	-	10 g	-
MFR60	-	10 g		-
MFR96	-	-	-	10 g
Purified water	until 100 g	until 100 g	until 100 g	until 100 g

Carbopol 940, was hydrated using purified water for at least 24 hours in the presence of glycerin as a dispersing agent (purity over 99%; Glycerin from Merck, Darmstadt, Germany). Triethanolamine (purity over 99%; Triethanolamine from Carl Roth GmbH + Co. KG., Karlsruhe, Germany) was used for neutralization at the end of 24 hours in the cold and added to the semisolid matrix generated by hydration of the hydrophilic polymer under intense stirring (2000 rpm for 10 minutes, using a turbine stirrer in a Heidolph RZR 2020).

Triethanolamine (TEA) was added dropwise to neutralize the pH to 5.8–6.2, ensuring the gel's stability and compatibility with skin application. Finally, *Rosa canina* macerates were added to the semisolid matrix under stirring until complete homogenization.

Physicochemical properties such as pH, appearance, rheological behavior, and spreadability assessment were evaluated for formulas P1-P4 according to the methodology used in similar studies carried out [23]. The rheological properties were assessed by varying rotational speeds (ω) between 4 and 200 rpm. The measurements were conducted with a Rotational Viscometer ST-2020 R, using 10-second intervals for each determination.

2.2.6. Determination of antioxidant activity of hydrogels with *Rosa canina* macerate

The antioxidant activity of the four rose hip hydrogels (MFL60, MFL96, MFR60, and MFR96) was evaluated using the DPPH radical scavenging assay. This method measures the hydrogels' ability to neutralize free radicals, an indicator of their potential to reduce oxidative stress when applied to the skin. A solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared by dissolving 0.1 mM DPPH in methanol. The solution appeared deep violet due to the presence of free radicals. Approximately 1 g of each hydrogel (P1, P2, P3, P4) was mixed with 10 mL of methanol to extract the antioxidant compounds. The mixture was vortexed for 5 minutes and filtered to obtain a clear extract. In a 96-well microplate, 200 µL of DPPH solution was added to 50 µL of the hydrogel extract (in triplicates for accuracy). A blank (methanol instead of the extract) and a control (DPPH solution alone) were also prepared. The reaction mixtures were incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A decrease in absorbance indicated radical scavenging activity. The DPPH radical scavenging activity was calculated according to equation (3).

2.2.7. Statistical analysis

All determinations were performed in triplicate, and the results were expressed as mean ± SD (standard deviation). Statistical evaluation of clinical results was performed by Student's t-test and analysis of variance (ANOVA) [24].

3. RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1. Determination of total polyphenol content

The results obtained for the analysis of the total polyphenol content (TPC) of the *Rosa canina* L. macerates, expressed in mg GAE/100 g plant material, are presented in Table 2. These indicate values ranging from 225.21 mg GAE/100g dry sample to 396.13 mg GAE/100g and are in accordance with data reported in the scientific literature [25-28].

Table 2. Total polyphenol content in *Rosa canina* macerates

Sample	Total polyphenol content [mg GAE/100 g dry weight]
MFL96	359.32 ± 2.55
MFL60	273.43 ± 1.33

Sample	Total polyphenol content [mg GAE/100 g dry weight]
MFR96	272.85 ± 1.25
MFR60	396.13 ± 2.66
MC96	225.21 ± 1.36
MC60	252.62 ± 1.16

3.1.2. Determination of total flavonoid content

Table 3 presents the results of the TFC analysis, expressed as mg rutin/100 g dry weight, for the ethanolic macerates prepared from the flowers (MFL96, MFL60), fruits (MFR96, MFR60), and fruit tea (MC96, MC60) of *Rosa canina*. The values obtained ranged from 82.32 mg rutin/100 g dry weight to 174.71 mg rutin/100 g dry weight, and are in accordance with data from the scientific literature [25,26].

Table 3. Total flavonoid content in *Rosa canina* macerates

Sample	Total flavonoid content [mg rutin/100 g dry weight]
MFL96	174.71± 2.72
MFL60	152.65 ± 1.42
MFR96	110.93 ± 1.18
MFR60	157.45 ± 1.65
MC96	99.76 ± 0.27
MC60	82.32 ± 0.36

3.1.3. Antioxidant activity analysis

The antioxidant activity of the hydroalcoholic macerates of *Rosa Canina*, evaluated through the DPPH radical scavenging test, is presented in Table 4.

Table 4. DPPH % results for *R. canina* macerates

Sample	Absorbance [517 nm]	DPPH [%]
Blank	2.6575	-
MFL96	0.1160	92.38
MFL60	0.1490	94.39
MFR96	0.9790	63.16
MFR60	0.2025	95.63
MC60	0.1769	93.34

The antioxidant activity of the hydroalcoholic macerates of *Rosa canina* in ethanol was quantified according to the FRAP procedure. The quantitative results, expressed as μmoles FRAP/g, are presented in Table 5 and are in accordance with the scientific literature data [26].

Table 5. FRAP method results for *R. canina* macerates

Sample	FRAP [μmoli/ g]
MFL96	40.30 ± 1.66
MFL60	39.69 ± 1.65
MFR96	11.47 ± 0.25
MFR60	38.24 ± 0.26
MC96	9.20 ± 0.33
MC60	37.69 ± 0.42

3.1.4. Characteristics of hydrogels with macerates of *Rosa canina*

Characteristics of hydrogels with macerates of *Rosa canina* in ethanol are presented in Table 6.

Table 6. Main characteristics of hydrogels with macerates of *Rosa canina*

Characteristics	Formula P1	Formula P2	Formula P3	Formula P4
Organoleptic evaluation – initial time	*appearance*: homogeneous, translucent; *colour*: pinkish; *smell*: specific	*appearance*: homogeneous, translucent; *colour*: pinkish; *smell*: specific	*appearance*: homogeneous, translucent; *colour*: pinkish; *smell*: specific	*appearance*: homogeneous, translucent; *colour*: pinkish; *smell*: specific
Organoleptic evaluation – after 30 days	constant initial characteristics	constant initial characteristics	constant initial characteristics	constant initial characteristics
Organoleptic evaluation – after 60 days	constant initial characteristics	constant initial characteristics	constant initial characteristics	constant initial characteristics
pH – initial time	5.5 – 5.6	5.5 – 5.7	5.5 – 5.6	5.5 – 5.7
pH – after 30 days	5.5 – 5.6	5.5 – 5.7	5.5 – 5.6	5.5 – 5.7
pH – after 60 days	5.7	5.8	5.7	5.8
Viscosity – initial time	670 ± 0.65 mPa·s	645 ± 0.27 mPa·s	674 ± 0.55 mPa·s	684 ± 0.22 mPa·s
Viscosity – after 30 days	652 ± 0.73 mPa·s	623 ± 0.33 mPa·s	664 ± 0.25 mPa·s	672 ± 0.46 mPa·s
Viscosity – after 60 days	624 ± 0.36 mPa·s	610 ± 0.52 mPa·s	632 ± 0.33 mPa·s	634 ± 0.18 mPa·s

All formulations exhibited smooth, non-greasy textures with excellent spreadability, making them easy to apply. Stability studies at 4°C, room temperature, and 40°C showed no significant changes in viscosity, pH, or appearance after three months.

Spreadability is a key parameter in evaluating the performance of topical hydrogels. It measures how easily the gel spreads under an applied force, reflecting its usability and user experience. The spreadability of all hydrogels showcases a fine balance between natural enrichment and functional design, driven by the synergistic effects of *Rosa canina* macerates and the tailored hydrogel matrix (Figs. 4-7).

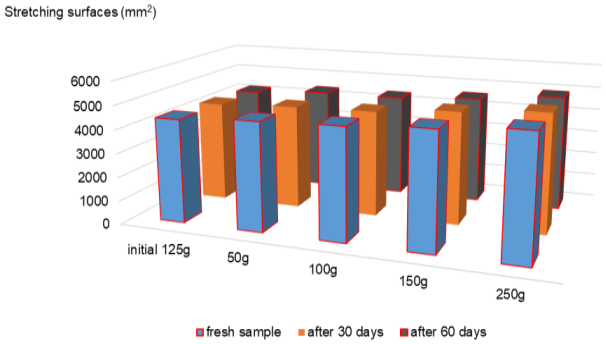

Figure 4. Spreadability of Formula P1.

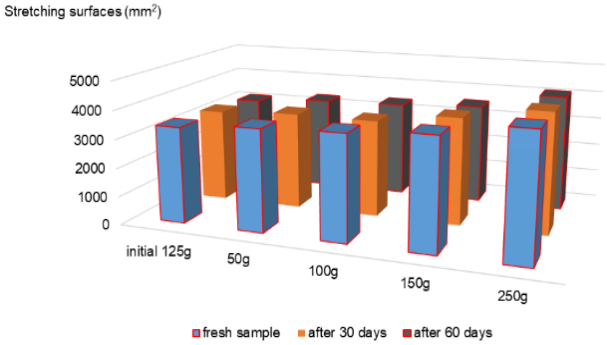

Figure 5. Spreadability of Formula P2.

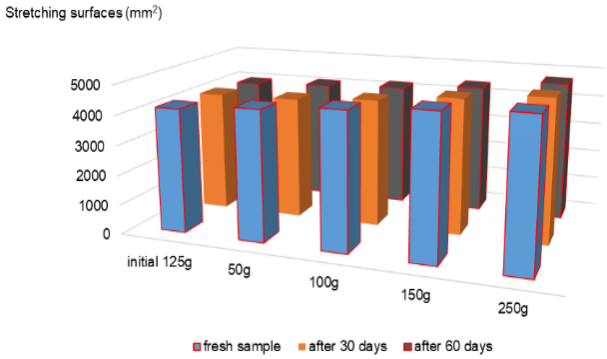

Figure 6. Spreadability of Formula P3.

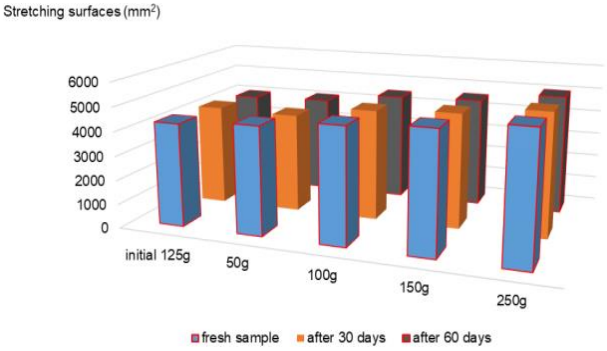

Figure 7. Spreadability of Formula P4.

Rheological properties describe how a material flows and deforms under applied forces, and these are critical for assessing the usability, spreadability, and stability of topical hydrogels. The gels exhibited pseudo-plastic flow behavior, ideal for topical application. The rheological profiles of the four rose hip hydrogel formulations were analyzed to determine their viscosity, flow behavior, and structural integrity under stress (Fig. 8). Excellent structural recovery after deformation, ensuring the gel retains its shape and stability over time. Flow behavior exhibits pseudo-plastic (shear-thinning) behavior, where the viscosity decreases as the shear rate increases. This makes the gels easy to spread during application while maintaining structure when at rest.

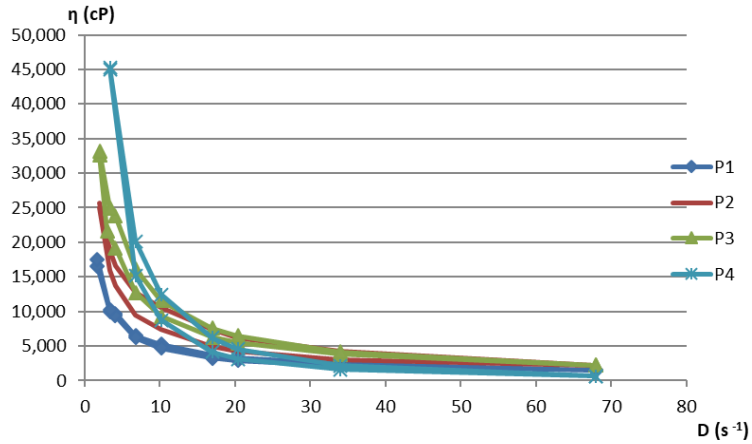

Figure 8. Flow curve for hydrogels with macerates of *Rosa canina*.

3.1.5. Determination of the antioxidant activity of hydrogels with *Rosa canina* macerates

All four hydrogels exhibited significant antioxidant activity, with variations depending on the type of macerate and alcohol concentration (Table 7).

Table 7. DPPH % results for hidrogels with *R. canina* macerates

Sample	Absorbance [517 nm]	DPPH [%]
Blank	2.6575	-
P1	0.7324	72.52
P2	0.1690	85.74
P3	0.1840	67.35
P4	0.1924	58.94

The DPPH assay confirmed that the hydrogels have strong antioxidant properties, with P2 (60% ethanolic macerates derived from fruits of *Rosa canina L.*) hydrogel emerging as the most effective formula. These findings suggest that the choice of maceration method and alcohol concentration significantly impacts the antioxidant activity of the final product. This makes the P2 hydrogel ideal for applications targeting oxidative stress-related skin conditions. By targeting oxidative stress pathways, the P2 hydrogel represents a versatile and promising solution in pharmaceutical and cosmeceutical applications for enhancing skin health and resilience [29]. The hydrogel's antioxidant properties make it an excellent component in after-sun gels or formulations designed to repair UV-induced damage, mitigating erythema and photoaging effects.

3.2. DISCUSSION

The results of the TPC analysis showed significant variations in the levels of total polyphenolic compounds depending on the type of solvent used, 96% and 60% ethanol. The determined TPC values ranged from 225.21 mg GAE/100 g dry weight to 396.13 mg GAE/100 g dry weight. The highest polyphenol content was obtained for the rosehip fruits macerate with 60% ethanol, which recorded a value of 396.13 mg GAE/100 g dry weight. For rosehip flowers, 96% ethanol was found to be more effective, yielding a content of 359.32 mg GAE/100 g dry weight. These differences highlight the importance of selecting the solvent based on the plant part being analyzed. 60% ethanol was identified as the most effective formula for extracting polyphenolic compounds from rosehip fruits. For the commercial fruit tea, the ethanolic solvent (60%) provided the highest values, with a content of 252.62 mg GAE/100 g dry weight, followed by the concentrated solvent (96%), which recorded 225.21 mg GAE/100 g dry weight. Based on these results, freshly harvested rosehip fruits prove to be a superior option, having higher concentrations of phenolic compounds compared to commercial fruit tea. Moreover, macerates obtained from rosehip flowers represent a significant source of bioactive compounds, demonstrating remarkable antioxidant activity due to the exceptional stability of the polyphenols identified in the analyzed samples.

The analysis of the total flavonoid content (TFC) in the macerates obtained from *Rosa canina* allowed for the quantitative assessment of these bioactive compounds, recognized for their antioxidant properties and health benefits. The results highlighted that macerates from rosehip flowers (MFL60 and MFL96) and those obtained from fruits (MFR60 and MFR96) exhibited the highest amounts of flavonoids, with values ranging from 152.65 mg rutin/100 g dry weight (MFL60) to 174.71 mg rutin/100 g dry weight (MFL96) and 110.93 mg rutin/100 g dry weight (MFR96) to 157.45 mg rutin/100 g dry weight (MFR60). Ethanol, in both concentrations used (96% and 60%), proved to be significantly more effective in extracting flavonoids from flowers and fruits, compared to commercial fruit tea. The macerates obtained

from rosehip tea showed a lower total flavonoid content, ranging from 82.32 mg rutin/100 g dry weight to 99.76 mg rutin/100 g dry weight (MC60, MC96). This variation can be explained by differences in the concentration of flavonoids in the various parts of the plant, as well as the differing solubility of these compounds, which is influenced by the plant matrix and the type of solvent used.

To evaluate the antioxidant properties, two different spectrophotometric methods were used: the DPPH method, which measures the free radical scavenging activity, and the FRAP test, which measures ferric-reducing antioxidant power.

The antioxidant activity of the macerates obtained from *Rosa canina*, measured by the DPPH method, ranged from 63.16% to 95.63%, showing significant differences depending on the type of plant material and the solvent used. The results revealed that the ethanolic macerates derived from the flowers of *Rosa canina* (MFL60 and MFL96) exhibited the highest antioxidant activity, with values ranging from 94.39% to 92.38%, compared to the macerates obtained from fruits (63.16%-95.63%) and commercial fruit tea (66.73%-93.34%). The selection of solvent proved to be a critical factor in the extraction of antioxidant compounds, with the ethanolic solvent (60%) being significantly more effective for extracting bioactive compounds from fruits, while concentrated ethanol (96%) was more effective for extracting from rosehip flowers.

The reducing activity (FRAP) was evaluated for the macerates obtained from *Rosa canina*, with values ranging between 9.20 μmol FRAP/g and 40.30 μmol FRAP/g. Among them, the macerate from the flowers, for both solvent variants used (60% and 96% ethanol), recorded the highest values, ranging from 39.69 μmol FRAP/g to 40.30 μmol FRAP/g, indicating high antioxidant activity, while the MC96 macerate showed the lowest reducing activity, with 9.20 μmol FRAP/g. These results are consistent with the data obtained from the DPPH analysis for *Rosa canina* macerates, as well as with reports from the literature. These findings highlight the significant variations in antioxidant activity between different types of macerates, emphasizing the influence of the plant material type and solvent used on the efficiency of bioactive compound extraction.

The results demonstrate that hydrogels formulated with *Rosa canina* macerate exhibit favorable physicochemical properties, stability, and antioxidant activity, making them suitable for dermato-cosmetic applications. All hydrogels were characterized by smooth textures, excellent spreadability, and pseudo-plastic flow behavior, ensuring ease of application and structural integrity. Among the formulations, the P2 hydrogel, prepared using 60% ethanolic macerates from *Rosa canina* fruits, exhibited the highest antioxidant activity, underscoring the influence of maceration methods and solvent concentration on bioactive compound extraction.

These findings highlight the potential of the P2 hydrogel for addressing oxidative stress-related skin conditions, such as UV-induced damage, erythema, and photoaging. Its combination of stability, usability, and therapeutic efficacy positions it as a promising candidate for cosmeceutical and pharmaceutical products aimed at enhancing skin health and resilience. Future research should focus on clinical evaluations and expanding applications in skincare innovations.

4. CONCLUSIONS

Rosa canina represents an important source of valuable compounds used for therapeutic purposes to maintain health. In this study, the phytochemical composition (TPC, TFC) of *Rosa canina* was correlated with antioxidant activity, and assessed using two methods such as DPPH and FRAP.

According to the results obtained for TPC, the macerate from rosehip fruit with 60% ethanol recorded the highest concentration of polyphenols (396.13 mg GAE/100 g dry weight material). In contrast, the macerate from flowers with 96% ethanol also showed a considerable polyphenol content (359.32 mg GAE/100 g dry weight), highlighting the importance of selecting the appropriate solvent based on the plant material used. These results reflect the significance of extraction strategies in maximizing the phytochemical potential of *Rosa canina*.

The results of the total flavonoid content (TFC) analysis highlight rosehip flowers and fruits as the richest source of flavonoids among all the parts of *Rosa canina* analyzed. The macerates from flowers (MFL96 and MFL60) and fruits (MFR96 and MFR60) showed values ranging from 152.65 mg rutin/100 g dry weight to 174.71 mg rutin/100 g dry weight for flowers and values between 110.93 mg rutin/100 g dry weight and 157.45 mg rutin/100 g dry weight for fruits. Thus, rosehip flowers represent a valuable option for formulations in the pharmaceutical and cosmetic industries, where flavonoids play a crucial role in cellular protection and the prevention of negative effects caused by oxidative stress.

The antioxidant activity of macerates from *Rosa canina* was evaluated using the DPPH radical scavenging test and the FRAP method. The results indicated high values, ranging from 63.16% to 95.63% (DPPH test) and from 9.20 μmol FRAP/g to 40.30 μmol FRAP/g (FRAP method). The differences recorded between the hydroalcoholic macerates are based on the complex and variable chemical composition of the studied plant material, as well as on the concentration of the solvent used for extraction. The most effective extraction solvent was 60% ethanol. These results confirm the existence of a strong correlation between the polyphenol content (TPC), flavonoids (TFC), and antioxidant activity, highlighting their essential role in the development of products with enhanced antioxidant potential.

The phytochemical analysis (TPC and TFC) and evaluation of the antioxidant potential of the *Rosa canina* plant highlight that both the type of solvent and the plant part used have a significant impact on the efficiency of bioactive compound extraction. The appropriate selection of solvent, depending on the plant material, is essential for optimizing the antioxidant potential of the extracts derived from rosehips. Following the phytochemical analysis (TPC, TFC) and evaluation of the antioxidant potential, *Rosa canina* is classified as a valuable natural source with significant antioxidant properties, having considerable potential in the pharmaceutical industry. The strong antioxidant properties of the P2 hydrogel, as confirmed by the DPPH assay, highlight its potential as a therapeutic agent for managing oxidative stress-related skin conditions.

This study highlights the significant antioxidant potential of *Rosa canina L.*, particularly in 60% ethanolic macerates derived from fruits, which demonstrated the highest polyphenol content and antioxidant activity. The successful incorporation of these macerates into stable and effective dermato-cosmetic hydrogel formulations underscores their pharmaceutical relevance. The strong correlation between polyphenol content and antioxidant activity affirms *Rosa canina* as a valuable natural source for developing antioxidant-based therapeutic and cosmetic products. Future work could focus on clinical evaluations and scaling up production to fully realize its potential in commercial applications.

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