Integrated study of the *Rosa* genus and evaluation of the therapeutic potential of the *Rosa canina* species on the human integument

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INTRODUCTION

In the current context, the growing interest in natural therapies and cosmetics based on natural ingredients leads horticultural research towards identifying and exploiting plants with bioactive potential. The genus *Rosa*, known for its medicinal and dermatocosmetic properties, becomes central to this scientific investigation.

Plants from the genus *Rosa*, especially *Rosa canina* and *Rosa rugosa*, are valued for their high content of vitamin C, essential fatty acids, polyphenols, and carotenoids, compounds with antioxidant, anti-inflammatory, and regenerative effects on the skin. However, studies directly linking horticultural characteristics of Rosa species to their dermatological applicability remain limited, particularly within the Romanian context.

This thesis addresses this need through an interdisciplinary approach, combining specific horticultural research methods, such as morphological characterization, molecular analysis, and plant extractions, with modern dermatological evaluation techniques through multispectral imaging within pilot studies. Thus, the thesis explores the therapeutic potential of cold-pressed oils from *Rosa canina* fruits, correlating the biochemical composition of the raw materials with observed effects on human skin.

THESIS STRUCTURE

The doctoral thesis is structured into two main parts comprising eight chapters, including 16 tables, 74 figures, and 352 bibliographic sources, totaling 153 pages, with an additional two appendices of 15 pages.

Part I presents the current state of knowledge, structured into two chapters totaling 22 pages (20%). Part II presents personal contributions based on the results obtained during the experimental period, covering 88 pages (80%), structured into six chapters, plus the bibliography.

PART I: CURRENT STATE OF KNOWLEDGE

Part I groups the first two chapters, synthesizing general and current information from specialized literature relevant to the research objectives.

Chapter 1, titled *General Considerations on Rosa Species*, covers three subchapters addressing the origin, distribution, morphological, biological characteristics, propagation, and molecular characterization methods of studied Rosa species.

Chapter 2, titled *Biochemical Characterization of Rosa Species Fruits*, contains four subchapters presenting information about bioactive compounds in fruits, therapeutic uses, dermatocosmetic effects of oils, and extraction methods of active compounds.

PART II: PERSONAL CONTRIBUTION

Part II details personal contributions structured into six chapters addressing various aspects formulated in the thesis objectives.

Chapter 3, titled *Aim and Research Objectives*, defines the thesis aim and specific objectives.

Chapter 4, titled *Morphological and Molecular Characterization of Rosa Genotypes*, includes two experiments demonstrating clear morphological differences among studied Rosa genotypes and molecular characterization to establish phylogenetic relationships.

AIM AND OBJECTIVES

The main aim of this doctoral thesis was the complex investigation of certain *Rosa* varieties, with a focus on *Rosa canina* and *Rosa rugosa*, in terms of their horticultural value and clinical applicability in dermatology. The thesis aimed to assess both the phylogenetic relationship between the studied species and the biochemical composition, antioxidant potential, and dermatocosmetic effects of oils obtained from their seeds, to support their use. The specific objectives were as follows:

1. Collection and morphological and molecular characterization of native plant material from various geographical areas in Romania.
2. Determination of the biochemical profile of oils extracted from *Rosa canina* and *Rosa rugosa* seeds, focusing on antioxidant activity, content of phenolic compounds, essential fatty acids, tocopherols, and carotenoids.
3. Evaluation through multispectral imaging analysis using OBSERV 520x and VISIA® devices of the clinical effects of rosehip oil on human skin, regarding texture, hydration, wrinkles, porphyrins, and hyperpigmentation.
4. Correlation of clinical results with skin type and individual parameters of the subjects included in the study.
5. Formulation of practical recommendations for the horticultural and dermatocosmetic use of the studied genotypes, based on the clinical and statistical results obtained. By fulfilling these objectives, the thesis provides a valuable contribution to the integration of horticultural knowledge with dermatological research, opening new opportunities for the sustainable valorization of local plant resources.

STUDY 1 – MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF GENOTYPES BELONGING TO THE *ROSA* GENUS

Experiment 1 – Morphological characterization of studied *Rosa* genotypes
The aim of the study is to evaluate morphological variability of *Rosa* genotypes based on observations and measurements performed on fruits. Genotypes from different origins belonging to the species **Rosa canina**, **Rosa rugosa**, **Rosa damascena**, and *Rosa gallica* were selected for fruit morphological analysis.
Material and method
The biological material consisted of eight samples: three samples from *Rosa canina*, including two origins and the cultivar 'Can', three samples from *Rosa rugosa*, one sample from *Rosa gallica* cv. 'Tuscany', and one sample from *Rosa damascena* cv. 'Saint Nicolas'. Fruit characteristics analyzed were fruit length, fruit diameter, and number of seeds per fruit, aiming to highlight variability and identify genotypes producing the highest number of seeds, used later for oil extraction necessary for further analysis and tests.
Results and conclusions
Regarding fruit diameter, the smallest average value was recorded in *Rosa canina* from Băişoara (16.46 mm), while *Rosa rugosa* from USAMV Cluj-Napoca had the largest diameter (26.51 mm), statistically significant differences. Within *Rosa canina*, 'Can' cultivar had the largest average fruit diameter (19.22 mm). *Rosa rugosa* had larger diameter fruits with fewer

seeds, suggesting ornamental uses. Significant variations were observed among genotypes, with higher values in *Rosa rugosa*, indicating industrial and ornamental potential.

Fruit length analysis showed significant differences for *Rosa canina* 'Can', having the longest fruits. Data ranged between 33.73–27.28 mm for *Rosa canina* and 19.15–20.24 mm for *Rosa rugosa*. Statistical analysis (Duncan's test) indicated significantly shorter fruits for Rosa rugosa, *Rosa gallica*, and *Rosa damascena* compared to *Rosa canina*, which had the longest fruits due to their elongated shape.

Seed count per fruit analysis showed *Rosa canina* from Salina Turda had the highest average seed number (30.05), whereas *Rosa rugosa* from USAMV Cluj-Napoca had the lowest (17.15). Larger diameter fruits had fewer seeds compared to smaller diameter fruits with more seeds in *Rosa canina*.

The morphological results emphasize genetic diversity among eight *Rosa* genotypes, constituting valuable germplasm for breeding.

Experiment 2 – Molecular characterization of studied *Rosa* genotypes

Molecular analysis of *Rosa* genotypes is essential for understanding phylogenetic relationships, genetic diversity, and population structures.

Material and method

The biological material was identical to the morphological characterization study. Eighteen ScoT molecular markers were used for genetic diversity assessment.

Results and conclusions

UPGMA cluster analysis based on ScoT molecular markers and Euclidean genetic distances highlighted genetic relationships among four *Rosa* species. The resulting dendrogram showed high correlation with initial data (cophenetic coefficient of 0.9359), validating the analysis accuracy. Samples formed two main clusters: one with *Rosa gallica*, *Rosa rugosa*, and *Rosa damascena*, the other comprising Rosa canina genotypes. Differences within clusters reflected genetic and morphological variation, indicating valuable genetic diversity potential. ScoT molecular marker analysis provided valuable genetic characterization data of Rosa genotypes from different origins.

STUDY 2 – EXTRACTION AND BIOCHEMICAL EVALUATION OF OILS FROM ANALYZED GENOTYPES

Study objective

The purpose of this study was to characterize the biochemical composition of oils obtained through cold pressing from seeds of six *Rosa* samples (*Rosa canina* – two provenances, *Rosa rugosa* – two provenances, *Rosa gallica* and *Rosa damascena*), in order to select the sample with the highest bioactive potential. The study focused on analyzing the total content of phenolic compounds, antioxidant activity, and the carotenoid profile of the extracted oils, without including clinical evaluations on the skin at this stage. The main objectives pursued were: extraction of oils through cold pressing from the seeds of the studied genotypes; determination of the total phenolic compound content (TPC); evaluation of antioxidant activity using the DPPH method; analysis of total and individual carotenoid content using spectrophotometric and HPLC-DAD methods; comparison of the biochemical profile among the oil samples and identification of those with the most promising composition for dermatocosmetic and therapeutic uses.

Material and method

The biological material consisted of six cold-pressed seed oil samples: two provenances of the species *Rosa canina* (from the Salina Turda and Băişoara regions); two provenances of the species *Rosa rugosa* (USAMV Cluj-Napoca and the Horticultural Research Station (SCH) USAMV Cluj-Napoca); *Rosa gallica*, cultivar 'Tuscany', and *Rosa damascena*, cultivar 'Saint-

Nicolas' (Alexandru Borza Botanical Garden, Cluj-Napoca). After oil extraction from the seeds, the carotenoid content, phenolic compounds, and antioxidant activity were determined. The total phenolic compound content (TPC) was evaluated using the Folin–Ciocalteu method. The antioxidant activity of the samples was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method in triplicate. Total carotenoid content was determined by spectrophotometric method and HPLC-DAD.

Results and conclusions

Following analyses on the phenolic compound content in the six *Rosa* genotypes from different geographical origins, the data obtained indicate varying phenolic content depending on the genotype and origin. The results highlighted the sample of *Rosa canina* from Băișoara with 2.637 mg GAE/100g, as having the highest phenolic content—more than double compared to the sample from Salina Turda with 1.161 mg GAE/100g. The other genotypes recorded values between 0.885–0.285 mg GAE/100g.

Analyzing antioxidant activity, the highest values were recorded in samples belonging to the genotypes of *R. gallica* (7.058 µM Trolox/100g) and *R. damascena* (6.017 µM Trolox/100g), while the other genotypes ranged between 5.392–5.572 µM Trolox/100g.

Through total carotenoid content analysis, the values obtained for the *R. canina* Băișoara sample (32.687 µg/mL) and *R. canina* Salina Turda (13.096 µg/mL) highlight the high therapeutic potential of rosehip oil.

Following oil sample analysis via HPLC-DAD method, a complex carotenoid profile was obtained, with high amounts of lutein, lycopene, and β-carotene. Their presence emphasizes the ability of *R. canina* oil to combat oxidative stress and protect skin cells from damage.

STUDY 3 – TESTING AND EVALUATING THE THERAPEUTIC EFFECT OF *ROSA CANINA* OIL ON HUMAN SKIN

Experiment 1 – Evaluating the therapeutic effect of *Rosa canina* oil on the skin of the hand using a multispectral and fluorescence imaging system

Study objective

The objective of this evaluation using the Observ 520x system was to investigate the effects of topical application of *Rosa canina* oil (Băișoara provenance) on the cutaneous parameters of the hand.

Material and method
Subject selection

For this case study, 10 voluntary subjects were selected, aged between 30 and 55, without active dermatological conditions, without known allergies to the products used, and without topical treatments applied to the hands in the previous 14 days. Subjects were informed in advance about the protocol and signed informed consent for participation.

Evaluated area

The analysis was carried out exclusively on the dorsal side of the hand, a region frequently used in case studies targeting hydration, evenness, and skin regeneration.

Equipment used

The Observ 520x™ system, a modern dermatological imaging device, was used, allowing skin analysis under various lighting conditions.

Application of oil and evaluation

Over a five-week period, *Rosa canina* oil (Băișoara provenance) was applied daily to the back of the hand by each subject, without combining with other cosmetic or dermatological products. Before and after the oil application, each participant underwent a scan using the Observ 520x™ under controlled lighting and temperature conditions.

Data analysis

The imaging data obtained were interpreted qualitatively and semi-quantitatively using the integrated functions of the Observ 520x™, monitoring: changes in skin texture (reduction of fine lines); increased uniformity of skin tone; improvement of visible hydration; and reduction of pigmentation spots and red areas.

Results and conclusions

The results obtained from this research showed reductions in erythematous appearance and pigmentation spots, indicating improvements in inflammation and uneven pigmentation; improvement in skin texture by achieving a smoother and more even appearance after treatment, highlighting the regenerative and hydrating effects of *R. canina* oil (Băişoara provenance), and improvement in the overall appearance of the skin, which appeared more radiant and healthier. These results suggest the beneficial potential of *Rosa canina* oil (Băişoara provenance) in ameliorating signs of skin aging and improving the general health of the skin in just five weeks of use.

The evaluation of the therapeutic effects of *Rosa canina* oil (Băişoara provenance), applied topically to the skin of the hand and analyzed using the multispectral imaging system OBSERV 520X™, highlighted positive changes in skin parameters relevant to the dermatocosmetic field and skin regeneration therapies, ensuring standardized, non-invasive, and reproducible evaluation of skin effects, thus providing a high level of observation validity.

The case studies allowed non-invasive monitoring of skin evolution following treatment using standardized and reproducible analysis technologies. Following daily application of *R. canina* oil (Băişoara provenance), a reduction in pigmentation spots and a decrease in local chromatic variations were observed, suggesting a regulatory action on melanin distribution, leading to a more even complexion. Another notable result was the reduction of erythema, supporting the conclusion that the applied oil may have an anti-inflammatory and soothing effect on skin exposed to environmental factors.

Experiment 2 – Evaluating the therapeutic effect of *Rosa canina* oil on human skin using an advanced multispectral imaging system

Study objective

The aim of the study was to analyze in detail, using the VISIA® system, the effects of topical application of *Rosa canina* oil on various skin characteristics. The evaluation targeted improvement of skin appearance regarding erythematous areas, pigmented spots, brown spots, and UV-induced spots, as well as parameters related to texture, wrinkles, pores, and presence of porphyrins.

Material and method

Oil used in the study – preliminary characterization Preliminary biochemical analysis of oil obtained from *Rosa canina* seeds (Băişoara provenance) revealed high antioxidant capacity due to significant content of polyphenols and carotenoids, particularly β-carotene, zeaxanthin, and lycopene. Therefore, the oil was selected for evaluation of its therapeutic effect on the skin in the pilot clinical study.

Subjects and selection criteria

Randomized tests were performed on a group of 86 volunteers, of whom 27 were selected based on strict eligibility criteria. Subjects included in the study were aged between 25 and 65, covering a wide adult age range. Each subject received a numeric code, from RC01 to RC27, and was assigned to one of four age groups: Group 1 (25–35 years, n=8) – AG1; Group 2 (36–45 years, n=8) – AG2; Group 3 (46–55 years, n=5) – AG3; Group 4 (56–65 years, n=6) – AG4.

Study design

To evaluate the therapeutic effects of cold-pressed *Rosa canina* seed oil on the skin, each volunteer applied three drops of oil daily using a dropper across the entire face, avoiding the eye area. This protocol was followed consistently throughout the study to ensure treatment uniformity.

Evaluation of results with Visia® analysis system

Facial scanning with the VISIA® system uses 3D images to identify and accurately quantify all aesthetic aspects of the skin before visible signs of damage or aging occur. The system captures multiple angles of the skin and represents an innovative tool in dermatological and anti-aging research, providing comprehensive and accurate evaluations of the skin.

Evaluated parameters

The parameters evaluated in the study were: erythematous areas, macules, pigmented spots, texture, wrinkles, pores, presence of cutaneous porphyrins, and assessment of the biological skin age.

Results and conclusions

Macule

The macule score indicated the treatment's effectiveness in reducing their visibility, depending on the age group and face side evaluated. Differences among age groups proved significant, especially between AG1 and the other groups ($p < 0.01$).

Erythematous area

Improved results were reported in age group AG2, especially in cases RC04, RC08, RC13, and RC20, where unpleasant symptoms diminished, and erythema was visibly reduced, as shown by the score decreasing from 12.603 ± 3.94 to 12.264 ± 2.291. In AG3, erythema scores remained constant after five weeks, with no significant inflammatory responses reported. In contrast, most individuals in AG4 showed a mild inflammatory response and increased erythema visibility, with scores varying from 16.568 ± 2.895 to 19.582 ± 7.308.

Wrinkle

Wrinkle assessment is essential in dermatological and anti-aging studies to determine treatment effectiveness. Overall, scans revealed a visible reduction in the number and depth of wrinkles, confirming the efficacy of the applied treatment. Wrinkle reduction or increase was confirmed by the relative score fluctuations depending on the age group, especially between AG1 and AG4 ($p < 0.01$). Comparing differences between the right and left side of the face, no significant variations in wrinkle visibility were observed, as confirmed by the small effect size on both sides: left (CI95% = [-6.4869; 8.1555]; Cohen's d = 0.06224) and right (CI95% = [-7.4878; 8.4385]; Cohen's d = 0.0326).

UV-Induced spot

Although individual variations were observed in UV spot scores, the general trend was a slight increase in their visibility after treatment. This is confirmed by the small effect size recorded in different facial areas: frontal area: CI95% = [-1.6947; 6.3331]; Cohen's d = 0.3156; right side: CI95% = [-2.8422; 4.6293]; Cohen's d = 0.1306; left side: CI95% = [-6.4869; 8.1555]; Cohen's d = 0.06224. These results suggest that rosehip oil was not equally effective in reducing UV spots for all participants. This variability may be influenced by factors such as skin type, individual sensitivity to oil components (e.g., vitamin C content), and adherence to post-treatment care, especially the use of SPF products. The data indicated a moderate reduction in UV pigment spots, suggesting a photoprotective potential of the bioactive compounds in *R. canina* oil (Băişoara provenance).

Pigmented spot

No significant differences were recorded in hyperpigmentation on the frontal part of the face at the beginning and end of the treatment period; however, an improvement in skin tone uniformity was observed on the right side of the face, with significant differences between AG1 and AG2. The results demonstrated a moderate reduction in pigmented spots in AG3 and

AG4, with individual variations partially attributed to the antioxidant and anti-inflammatory effects of carotenoids (lutein, lycopene, β-carotene, zeaxanthin) and phenolic compounds.

Skin texture

Skin texture analyses showed significant improvements across all age groups, with a more pronounced effect in subjects from AG1 (25–35 years) and AG2 (36–45 years), indicating an early positive impact of antioxidant treatment.

Pore and porphyrin

In AG1 (25–35 years), a significant decrease in porphyrin levels was observed, reflecting possible inhibition of cutaneous bacterial activity and overall improvement in skin health. Pore analysis did not show statistically significant differences in any age group, suggesting this parameter is not directly influenced by the tested oil application.

Biological skin age evaluation To assess changes and improvements in skin appearance, the parameter True Skin Age was used. The results showed significant differences before and after topical treatment with *R. canina* oil (Băişoara provenance), especially in AG1, on the right side of the face, where average values were 33.5 ± 4.87 years before treatment and 29.625 ± 6.07 years after treatment.

This study demonstrated the significant potential of *Rosa canina* oil (Băişoara provenance) in improving skin characteristics, especially in reducing wrinkles, UV-induced pigmented spots, and porphyrin levels.

RECOMMENDATIONS

Based on the obtained results, the following practical application directions and valorization of the research findings presented in this thesis are recommended:

1. *Rosa canina*, being a species that grows spontaneously in Romania, is recommended for morphological and molecular analysis to correctly identify genotypes belonging to this species, thus avoiding the use of other *Rosa* species in the production of dermatocosmetic products.
2. To obtain a sufficient amount of oil, it is recommended to harvest elongated fruits that contain a higher number of seeds rather than flattened ones with fewer seeds.
3. To valorize local genetic resources, it is recommended to select mountain areas for rosehip harvesting for the production of *Rosa canina* oil used in dermatocosmetic products, due to the increased content of bioactive compounds.
4. To increase income in mountainous and sub-mountainous regions, it is recommended to establish commercial plantations of *Rosa canina* (Băişoara provenance) for oil production.
5. For the conservation and valorization of *Rosa* genetic resources, it is recommended to combine molecular analyses with morphological ones in order to develop breeding programs.
6. Expand clinical studies to include diverse samples (skin types, phototypes) to validate the long-term efficacy of *Rosa canina* oil.
7. Create interdisciplinary partnerships between horticultural research, processors, and the dermatocosmetic industry to ensure full traceability from harvesting to the final product.
8. Integrate rosehip seed oil—particularly from the *Rosa canina* genotype of Băişoara provenance—as an active ingredient in dermatocosmetic products aimed at preventing and alleviating signs of skin aging, considering its proven effects in wrinkle reduction, UV pigmentation fading, and bacterial activity regulation.

9. Given the reduction in porphyrins observed in the study, it is recommended to use rosehip oil as an adjuvant in dermatocosmetic products for oily, sensitive, or acne-prone skin, due to its potential in regulating bacterial activity.

10. It is recommended to associate topical application of rosehip oil with consistent use of sunscreen, as the slight increase in UV spot scores observed in some subjects suggests that ultraviolet exposure can diminish treatment efficacy and promote hyperpigmentation.

11. Based on the results regarding wrinkle reduction and skin texture improvement, *Rosa canina* oil is recommended as a natural alternative to topical vitamin C, due to its antioxidant and regenerative properties.

12. Cold-pressed rosehip oil should be stored under controlled conditions of temperature and light (preferably in opaque containers and at low temperatures) to ensure the stability of bioactive compounds, especially polyphenols and essential fatty acids, whose degradation can compromise product quality and therapeutic efficacy.

ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

This doctoral thesis brings a series of original and innovative contributions in the field of horticulture applied to dermatology, by investigating the clinical effects of oil from genotypes of the *Rosa* genus on human skin. The originality elements of the work are highlighted as follows:

1. Establishing phylogenetic relationships among *Rosa* genotypes. The use of ScoT primers allowed the evaluation of genetic relationships among the studied *Rosa* genotypes and their positioning into two distinct groups, results also confirmed by morphological analysis.

2. Identification of the *Rosa canina* genotype from Băişoara with therapeutic potential. The thesis contributes originally by identifying this specific genotype as exceptionally suitable for dermatocosmetic and clinical use, based on its superior composition profile and geographical origin.

3. Biochemical characterization and therapeutic potential of oils obtained from *Rosa* seeds with different native origins. A detailed analysis of the bioactive compounds present in oils extracted from six *Rosa* genotypes collected from different regions was carried out, correlating antioxidant content with therapeutic potential.

4. Recommendation of the *Rosa canina* genotype from Băişoara as raw material for dermatocosmetic applications. The thesis highlights the potential of this mountain genotype through its use in seed oil production for dermatocosmetic purposes.

5. Application of advanced multispectral and fluorescence imaging systems for evaluating the dermatological effects of *Rosa canina* oil. This study represents a national premiere in using multispectral and fluorescence imaging to monitor skin changes following topical application of *Rosa canina* oil.

6. Validation of the topical effect of *Rosa canina* oil on skin parameters. To our knowledge, this is the first comprehensive study in Romania to investigate the effects of cold-pressed *Rosa canina* seed oil on wrinkle reduction, texture improvement, porphyrin level decrease, and facial hyperpigmentation. The pilot study results offer a new perspective on the benefits of rosehip oil for skin health, highlighting its potential as a natural and effective skincare solution.

7. Correlation of skin changes with age and skin type. The thesis introduces a complex analysis of differential responses to rosehip oil treatment based on age group, facial side (left/right/frontal), and skin typology, contributing to personalized dermatocosmetic recommendations.

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8. Proposal to use rosehip oil as a natural alternative to topical vitamin C. Based on results regarding wrinkle reduction and skin texture improvement, the thesis proposes the use of *Rosa canina* oil as an adjunct therapeutic option in anti-aging products.
9. Interdisciplinary contribution. The thesis proposes an integrative vision connecting horticultural research with dermatocosmetic applications. This comprehensive approach represents an original contribution by identifying viable solutions for developing dermatocosmetic products with full traceability from plant to final product.

Through these contributions, the thesis adds a valuable set of practical and theoretical knowledge both in the field of horticulture applied to human health and in dermatological research on natural active ingredients, opening new perspectives for the use of *Rosa* species in dermatocosmetic therapies. By integrating horticultural, biochemical, and clinical elements, the thesis opens new directions of research and applicability for *Rosa* species