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"Comprehensive study of a selected botanical species: extraction, analytical profiling, in silico anti-aging bioactivity prediction, and microbiome modulation for dermo-cosmetic applications"

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1. Introduction

In the quest for novel and efficacious dermo-cosmetic actives, botanical extracts rich in secondary metabolites offer vast and underexploited potential. This study focuses on a specific plant species selected for its promising composition in biologically relevant phytochemicals. A carefully optimized extraction process was developed to preserve and enrich bioactive secondary metabolites, with the objective of creating a high-value extract suitable for skin care applications. Following extraction, the material was subjected to advanced analytical profiling using high-resolution mass spectrometry (HRMS) to identify key metabolites potentially involved in skin health modulation.

To complement this analytical work, an *in silico* network pharmacology approach was applied to explore the putative biological activities of the identified compounds. This *in silico* study aimed to generate hypotheses regarding anti-aging properties, such as antioxidant potential, modulation of dermal matrix components, and regulation of inflammation-related pathways. Future experiments will leverage the predictions to design physiologically relevant assays using skin cells, reconstructed tissue models, or *ex vivo* samples.

In parallel, the study assessed the extract's potential to modulate the skin and scalp microbiome. Given the growing interest in microbiota-inclusive cosmetic science, targeted *in vitro* microbiome assays were developed to explore potential applications in skin balance, acne-prone skin, and dandruff control.

2. Materials and Methods

DPPH assay: The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as described [1]. Briefly, 0.5 mL of the test sample at various concentrations was mixed with 4 mL of a DPPH methanolic solution (45 mg/L). After incubation for 30 minutes at room temperature in the dark, the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging activity was calculated using the following formula: % = $[(Abs_0\text{-}Abs_1)/Abs_0] * 100$, where Abs_0 is the absorbance of the control (DPPH solution without extract) and Abs_1 is the absorbance in the presence of the extract. All samples were tested in triplicate.

Total polyphenol assay: Total phenolic content (TPC) of the extracts was measured by the Folin-Ciocalteu method. Briefly, 0.5 mL of extract, 2 mL of distilled water, and 0.5 mL of Folin-Ciocalteu reagent were added to a test tube and allowed to stand for 5 minutes. Then, 2 mL of aqueous sodium bicarbonate (10% w/v) were added, and the mixture was incubated for 1 hour in the dark. Absorbance was then measured at 760 nm and total phenolic content was determined using a calibration curve constructed with gallic acid standards. Results were expressed as gallic acid equivalents. Results are expressed in gallic acid equivalents, i.e. in µg of gallic acid per mL of extract [2].

Tyrosinase inhibition assay: Tyrosinase inhibitory activity was assessed by monitoring the oxidation of L-DOPA (0.25 mM) to dopachrome by mushroom tyrosinase (4 U) in 20 mM phosphate buffer (pH 6.8), as described [3] with modifications. After preincubation of the sample (0.25–5%) or kojic acid (1.25–80 µM) with the enzyme for 10 minutes at room temperature, L-DOPA was added and absorbance at 492 nm was recorded over 25 minutes. Initial reaction rates were used to calculate the percentage of inhibition: % inhibition = $[(V_0 – V_1) / V_0] \times 100$, where V_0 and V_1 are the reaction rates without and with inhibitor, respectively. IC_{50} values were determined from dose-response curves. Kojic acid was used as a positive control. All assays were performed in triplicate.

Collagenase inhibition assay: Collagenase inhibitory activity was assessed by monitoring the hydrolysis of the synthetic substrate FALGPA (0.8 mM) by *Clostridium histolyticum* collagenase (0.12 U) in 50 mM Tricine buffer (pH 7.5) containing 400 mM NaCl and 10 mM $CaCl_2$, as described [4]. After preincubation of the sample (0.1–5%) or epigallocatechin gallate (EGCG, 25 µM–1 mM) with the enzyme at 37 °C for 15 minutes, the substrate was added. The enzymatic activity was monitored by measuring the decrease in absorbance at 335 nm over time, due to substrate cleavage. The mean absorbance between 10 and 20 minutes was used to calculate the percentage of collagenase inhibition: % inhibition = $[(A_0 – A_1) / A_0] \times 100$, where A_0 is the absorbance of the control (without inhibitor) and A_1 is the absorbance in the presence of the sample or EGCG. IC_{50} values were determined from dose-response curves and expressed in % for samples and in µM for EGCG. All experiments were performed in triplicate.

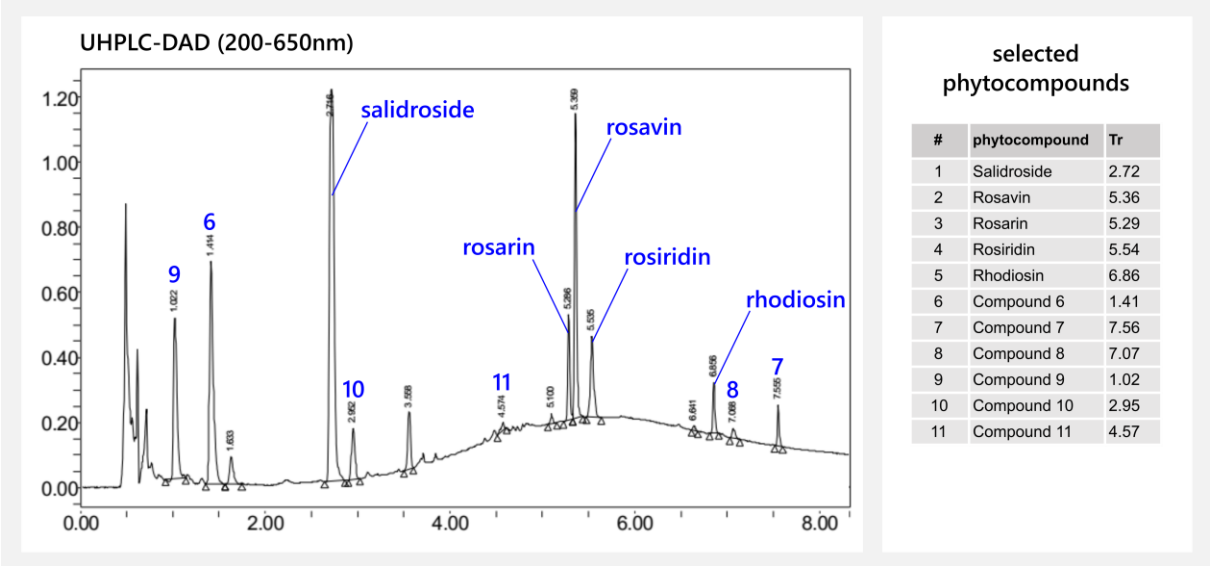
Hyaluronidase inhibition assay: Hyaluronidase inhibitory activity was evaluated by measuring the degradation of hyaluronic acid based on turbidity reduction, as described [5, 6]. The sample (0.05–1%) or tannic acid (1–200 µM) was preincubated with hyaluronidase (0.75 U) in 0.2 M acetate buffer (pH 5.35) containing BSA and NaCl, at 37 °C for 10 minutes. Then, 75 µg of hyaluronic acid in the same buffer was added as substrate. After a 45 minutes incubation at 37 °C, cetyltrimethylammonium bromide (CTAB) was added to complex the remaining (non-degraded) hyaluronic acid. Absorbance was measured at 600 nm after 10 minutes at 25 °C. The percentage of inhibition was calculated as: % inhibition = $[(A_1 - A_0) / (A_2 - A_0)] \times 100$, where

A_0 is the absorbance in the absence of inhibitor and A_1 in its presence and A2 is the absorbance of non-degraded hyaluronic acid. IC_{50} values were calculated from inhibition curves and expressed in % for samples and µM for tannic acid. All tests were performed in triplicate.

3. Results

3.1. Optimized extraction and phytochemical profiling

A solvent-controlled extraction of *Rhodiola rosea* roots was carried out under mild thermal conditions to preserve thermolabile metabolites. Analytical profile was obtained. The analytical profile of *Rhodiola rosea* extract allowed to identify 11 major compounds (**Figure 2**) that will further serve to predict *in silico* potential biological activities.



#	phytocompound	Tr
1	Salidroside	2.72
2	Rosavin	5.36
3	Rosarin	5.29
4	Rosiridin	5.54
5	Rhodiosin	6.86
6	Compound 6	1.41
7	Compound 7	7.56
8	Compound 8	7.07
9	Compound 9	1.02
10	Compound 10	2.95
11	Compound 11	4.57

Figure 1. Chemical profiling of the extract via HRMS and compound classification.

3.2. *In silico* prediction of potential anti-aging activities and skin health related properties

The major compounds identified in the extract were subjected to computational modeling to predict bioactivities relevant to skin health and aging. Initially, a literature mining step was conducted to collect experimentally validated molecular targets. In parallel, a bioinformatic workflow was designed to predict proteins potentially modulated by each of the selected phytocompounds. By combining a chemical structure similarity approach (ligand-based target prediction) with database mining, a list of predicted molecular targets was established [7].

Eleven major phytocompounds were selected in the *Rhodiola rosea* extract: (1) salidroside, (2) rosavin, (3) rosarin, (4) rosiridin, (5) rhodiosin, and compounds 6 to 11. The integration of both validated and predicted targets resulted in a comprehensive list of **542 targets**. Details regarding the number of targets associated with each compound are shown in **Figure 2.**

The prediction of biological activities based on selected phytocompounds was achieved through a network pharmacology approach, integrating analytical chemistry with bioinformatics and artificial intelligence. After identifying eleven major phytocompounds, computational tools were used to predict their potential target genes and associated pathways. This involved virtual screening based on structural similarity, followed by data mining from scientific literature and

public databases. The resulting phytocompound-target associations (**Figure 3a**) were subjected to gene enrichment analysis, uncovering biological processes and signaling pathways potentially modulated by the extract (**Figure 3b**).

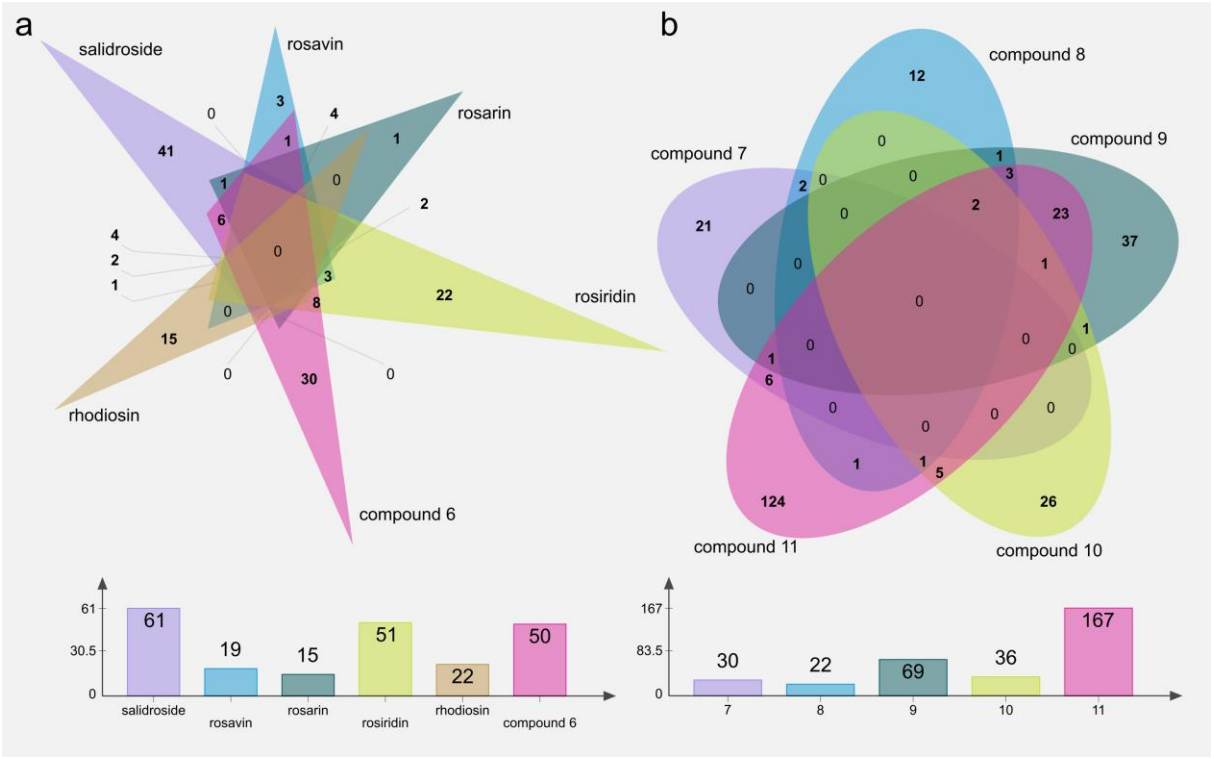


Figure 2. Number of validated and predicted targets for each of the eleven selected phytocompounds. (a) compounds 1 to 6. (b) compounds 7 to 11.

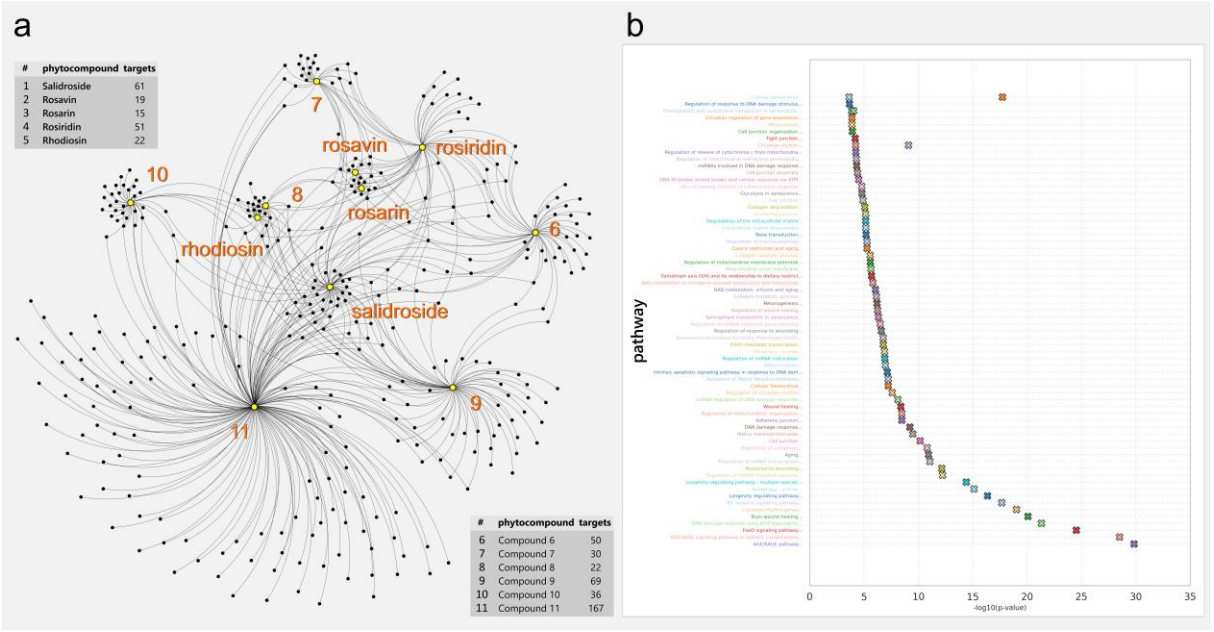


Figure 3. (a) Phytocompounds-Targets Network. The number of validated and predicted targets for each of the eleven selected phytocompounds is mentionned. (b) Selection of computed pathways with enrichment score.

This systems pharmacology strategy enables the *in silico* prediction of key skin-related effects—such as anti-aging, anti-inflammatory, antioxidant, and senescence-modulating activities—prior to experimental validation.

3.3. Preliminary *in vitro* evaluation and exploratory biological assays

Experimental results provided preliminary evidence of the extract's biological potential. *Rhodiola rosea* extract demonstrated significant free radical scavenging activity in the DPPH assay, and an elevated level of total polyphenols as measured by the Folin–Ciocalteu method (**Figure 4a**). In addition, enzyme inhibition assays showed a dose-dependent inhibitory effect on tyrosinase, hyaluronidase, and collagenase (**Figure 4b, c, d**).

The antioxidant capacity of the aqueous extract of *Rhodiola rosea* was evaluated using the DPPH assay, through two indicators: the percentage of antioxidant activity (**Figure 4a**, left) and the Trolox equivalent antioxidant capacity (TEAC), expressed in µg of Trolox equivalents per mL of extract (**Figure 4a**, right). To complement these data regarding antioxidant potential, the total phenolic content (TPC) was also assessed using the Folin–Ciocalteu assay, expressed in µg of gallic acid equivalents per mL of extract (**Figure 4a**, right). Five concentrations of the *Rhodiola rosea* extract were tested across all assays: 0.1%, 0.5% 1%, 2%, and 3%. At a concentration of 0.1% *Rhodiola rosea* extract exhibited a relatively low antioxidant activity of 7.42% (**Figure 4a**, left), corresponding to 6.99 µg Trolox equivalent and 2.97 µg gallic acid equivalent per mL of extract (**Figure 4a**, right). When the concentration increased to 0.5%, a marked increase in the antioxidant activity was observed, reaching 41.91% (**Figure 4a**, left), with values of 41.40 µg Trolox equivalent and 18.96 µg gallic acid equivalent per mL of extract (**Figure 4a**, right). At a concentration of 1%, the *Rhodiola rosea* extract exhibited a mean antioxidant activity of 77.47%, whereas at 2%, this activity increased significantly, reaching 95.14% (**Figure 4a**, left). The TEAC results followed a similar trend: at 1% extract concentration, a value of 86.88 µg Trolox equivalents/mL was recorded, while the 2% extract showed a 1.15 fold increase (**Figure 4a**, right). The total phenolic content, expressed as gallic acid equivalents, was determined to be 29.49 µg/mL, and 74.79 µg/mL, for the 1%, and 2% *Rhodiola rosea* extract, respectively (**Figure 4a**, right). In each measurement, the 3% extract demonstrated the highest capacity, with an average antioxidant activity of 95.32% (**Figure 4a**, left), a TEAC of 100.25 µg Trolox equivalents/mL, as well as a total phenolic content of 100 µg/mL gallic acid equivalents (**Figure 4a**, right).

These results suggest a dose-dependent enhancement of antioxidant efficacy, which parallels the increase in total phenolic content as the concentration of the aqueous *Rhodiola rosea* extract rises. This highlights the natural antioxidant potential of the extract.

The inhibitory effect of *Rhodiola rosea* extract on tyrosinase activity was evaluated. Kojic acid was used as a reference inhibitor and exhibited an IC_{50} value of 15.92 µM. The extract showed dose-dependent inhibition, reaching a maximum of 82.18% at a concentration of 5%. The calculated IC_{50} of the extract was 1.34% (**Figure 4b**).

The inhibitory effect on hyaluronidase was also assessed. Tannic acid served as the reference inhibitor, with an IC_{50} of 4.80 µM. *Rhodiola rosea* extract demonstrated potent hyaluronidase inhibition, with an IC_{50} of 0.11% (**Figure 4c**).

Finally, the inhibitory effect on collagenase was investigated. EGCG was used as the reference inhibitor and exhibited an IC_{50} of 779 µM. *Rhodiola rosea* extract showed weak, dose-dependent collagenase inhibition, reaching 36.44% at a concentration of 5%. The estimated IC_{50} of the extract was 9.74% (**Figure 4d**).

While these effects are in partial agreement with the predicted *in silico* activities, they do not yet capture the full extent of the mechanistic hypotheses generated computationally.

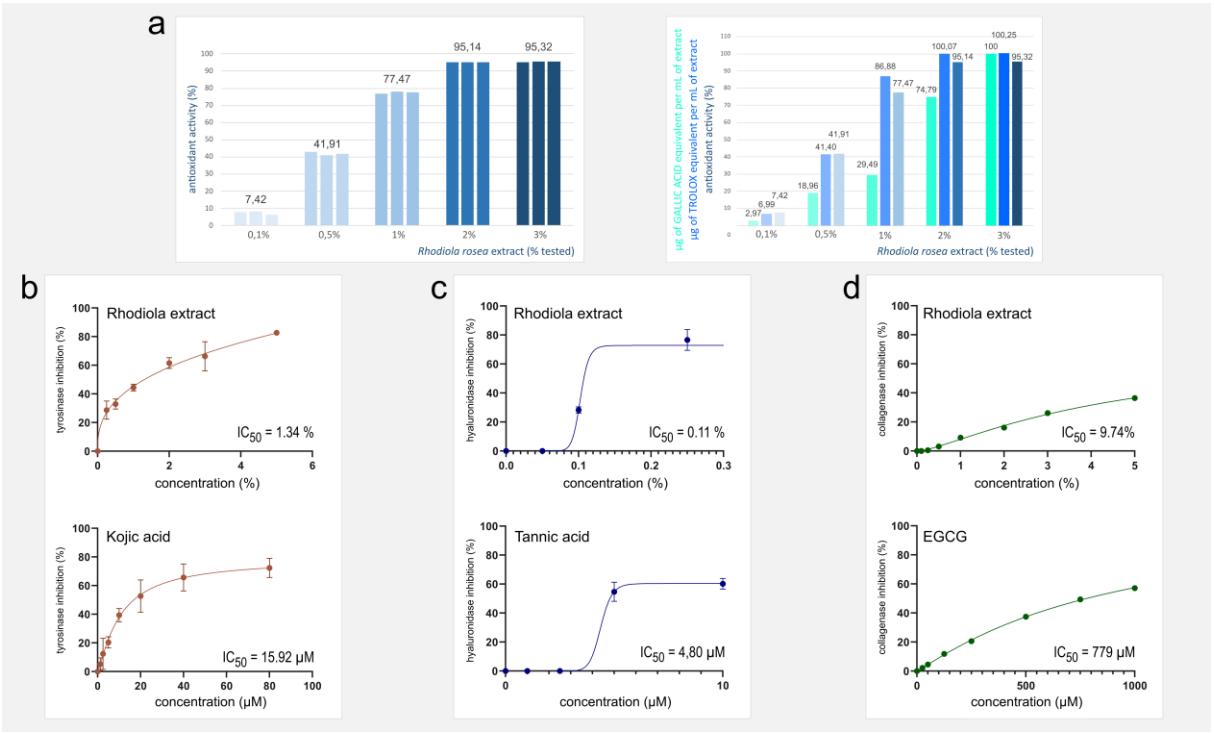


Figure 4. *In vitro* assessment of antioxidant and enzyme inhibitory activities of *Rhodiola rosea* extract. **(a)** Evaluation of the antioxidant capacity of the *Rhodiola rosea* extract expressed as the percentage of antioxidant activity at five different concentrations (0,1%, 0,5%, 1%, 2%, and 3%). Correlation between antioxidant capacity and total phenolic content of *Rhodiola rosea* extract at five different concentrations (0,1%, 0,5%, 1%, 2%, and 3%). Antioxidant capacity was expressed both in percent of antioxidant activity and in µg Trolox equivalents/mL, whereas total phenolic content was expressed in µg gallic acid equivalents/mL. **(b)** Inhibitory activity of *Rhodiola rosea* extract and the reference inhibitor kojic acid on tyrosinase. **(c)** Inhibitory activity of *Rhodiola rosea* extract and the reference inhibitor tannic acid on hyaluronidase. **(d)** Inhibitory activity of *Rhodiola rosea* extract and the reference inhibitor EGCG on collagenase.

To assess the extract's potential for microbiome-conscious dermo-cosmetic applications, its effect on the growth *Staphylococcus aureus* (an opportunistic pathogen) was evaluated. This assay aimed to determine whether the extract could help maintain microbiome homeostasis. The results demonstrated dose-dependant *S. aureus*-modulating activity of the extract**.** The assay, carried out in duplicate, consisted in exposing *S. aureus* cultured in a liquid medium to increasing concentrations of the extract and measuring the evolution of the absorbance versus time **(Figure 5)**.

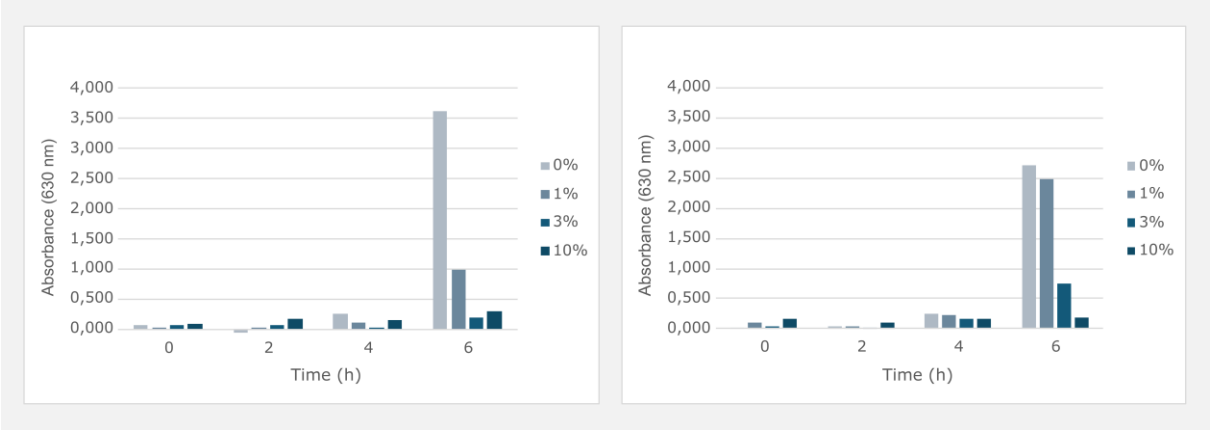


Figure 5. Evolution of the absorbance (630 nm) versus time of an *Staphylococcus aureus* culture exposed to increasing concentrations of *Rhodiola rosea* extract (assay in duplicate).

In the conditions of the assays, it was observed that concentrations of 1, 3 and 10 % of the *R. rosea* extract had a significant inhibitory effect on the growth of *S. aureus* at 6 hours of culture.

4. Discussion

This study provides a comprehensive evaluation of a botanical extract derived from *Rhodiola rosea*, combining optimized extraction, high-resolution analytical profiling, *in silico* prediction of bioactivities, and *in vitro* validation assays. The integrated approach offers valuable insights into the dermo-cosmetic potential of the extract, particularly in the areas of antioxidant protection, enzymatic regulation, and microbiome balance.

The *in silico* prediction based on eleven major phytocompounds identified in the extract indicated potential modulation of key biological pathways associated with aging, inflammation, and oxidative stress. These computational findings provided a strong rationale for subsequent experimental validations.

The antioxidant capacity of the aqueous extract was confirmed by the DPPH and Folin-Ciocalteu assays. A clear dose-dependent relationship was observed across the five tested concentrations (0.1% to 3%). Notably, a substantial increase in antioxidant activity and total phenolic content occurred between 0.1% and 1%, with a plateau effect seen from 2% to 3%. These findings suggest an early saturation of radical scavenging potential.

Enzymatic inhibition assays further demonstrated the extract's functional relevance. Tyrosinase and hyaluronidase activities were potently inhibited, with IC_{50} values of 1.34% and 0.11%, respectively, supporting the extract's application in pigmentation control and skin hydration preservation. However, the collagenase inhibition was more modest (36.44% at 5%; IC_{50} = 9.74%), indicating that the extract may be less effective in preventing collagen degradation. Complementary in vitro assays will be necessary to better characterize the potential bioactivity observed, based on the computational predictions.

Microbiome-oriented assays provided initial insights into the extract's dermo-cosmetic relevance. The observed inhibition of *Staphylococcus aureus* suggest a potential role in promoting microbiome balance. However, these findings remain preliminary and further targeted *in vitro* studies will be needed to confirm these effects and better understand the mechanisms involved in microbiome modulation.

Altogether, the results validate the utility of a multidisciplinary approach in developing next-generation botanical ingredients. While the antioxidant and tyrosinase/hyaluronidase inhibitory effects are particularly promising, further research is required to confirm the long-term benefits of microbiome modulation and to improve the anti-collagenase performance, possibly through formulation synergies or extract combination strategies.

5. Conclusion and perspectives

This work illustrates the potential of combining optimized extraction, deep analytical profiling, predictive bioinformatics, and microbiome-targeted assays in the development of advanced dermo-cosmetic ingredients. The selected extract showed preliminary promising predicted anti-aging potential, and microbiome-balancing effects.

Future directions include expanding validation on complex skin models, integrating the extract into prototype formulations, and conducting clinical assessments. Complementary in vitro studies will also help refine the understanding of observed biological effects and elucidate mechanistic pathways.

Additional perspectives include investigating the effect of the extract on *Cutibacterium acnes* growth (a bacterium related to acne) and evaluating its impact *in vitro* on *Malassezia sp.* to explore modulation of scalp microbiota potentially linked to dandruff control. This integrated approach opens new avenues for the development of next-generation cosmetic products that holistically support skin health and microbiome equilibrium.

6. Bibliographic references

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