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"Development and Profiling of a Botanically-Derived Active Extract: In Silico Prediction, In Vitro Evaluation and Microplastic-Free Inorganic Encapsulation for Cosmetic Applications"

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1. Introduction

The need for sustainable, high-performance active ingredients in cosmetics is driving innovation toward advanced botanical extraction and delivery systems. In this study, we describe the development of a multifunctional extract derived from a selected medicinal plant species, using a tailored extraction process designed to preserve and concentrate specific bioactive secondary metabolites.

Following extraction, the material was subjected to in-depth analytical profiling and *in silico* analysis to predict key biological activities, with a focus on anti-aging and skin homeostasis. To enhance the stability, efficacy, and bioavailability of the extract in formulation, we developed a microplastic-free encapsulation strategy using a 100% inorganic carrier matrix.

This integrated approach - linking phytochemistry, bioinformatics, and formulation science - aims to produce a scientifically substantiated, high-performance cosmetic ingredient.

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: % = $[(Abs_0-Abs_1)/Abs_0] * 100$, 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 min. Then, 2 mL of aqueous sodium bicarbonate (10% w/v) were added, and the mixture was incubated for 1 h 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 min at room temperature, L-DOPA was added and absorbance at 492 nm was recorded over 25 min. 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 (0.5–3%) 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-minute 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 A_2 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 *Ginkgo biloba* leaves was carried out under mild thermal conditions to preserve thermolabile metabolites. Analytical profile was obtained. The analytical profile of *Ginkgo biloba* extract allowed to identify 11 major compounds (**Figure 2**) that will further serve to predict *in silico* potential biological activities.

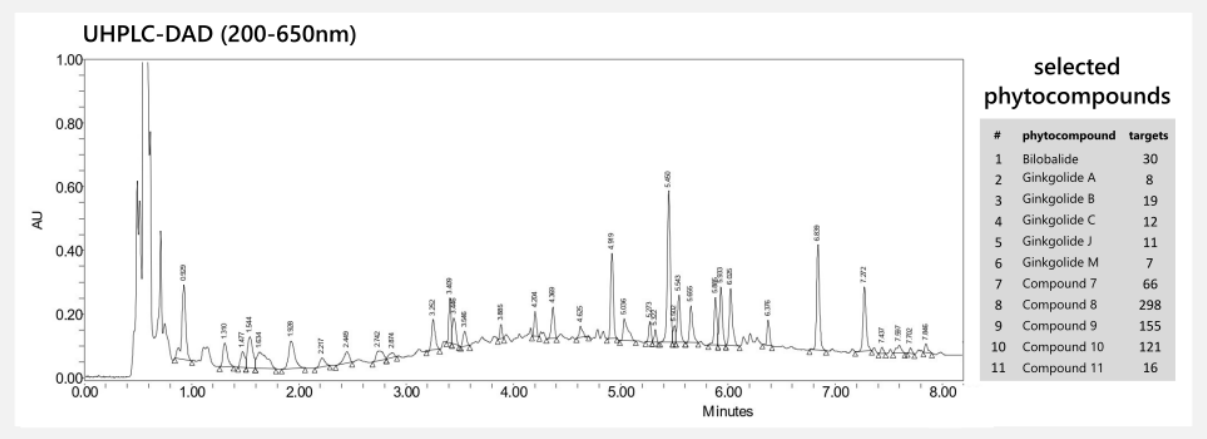

Figure 1. Chemical profiling of the extract via HRMS and compound classification.

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 G*inkgo biloba* extract,: (1) bilobalide, (2) ginkgolide A, (3) ginkgolide B, (4) ginkgolide C, (5) ginkgolide J, (6) ginkgolide M, and compounds 7 to 11. The integration of both validated and predicted targets resulted in a comprehensive list of **743 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**).

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.

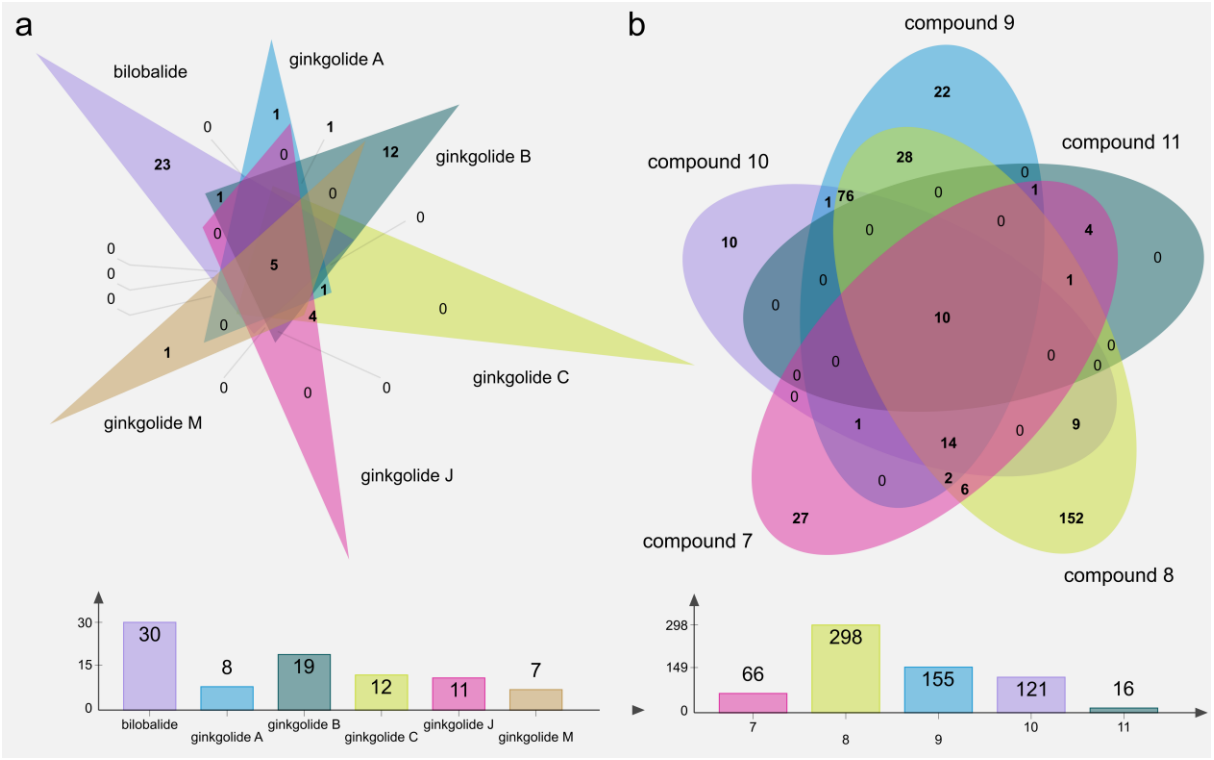

Figure 2. Number of validated and predicted targets for each of the eleven selected phytocompounds. (a) compounds 1 to 7. (b) compounds 7 to 11.

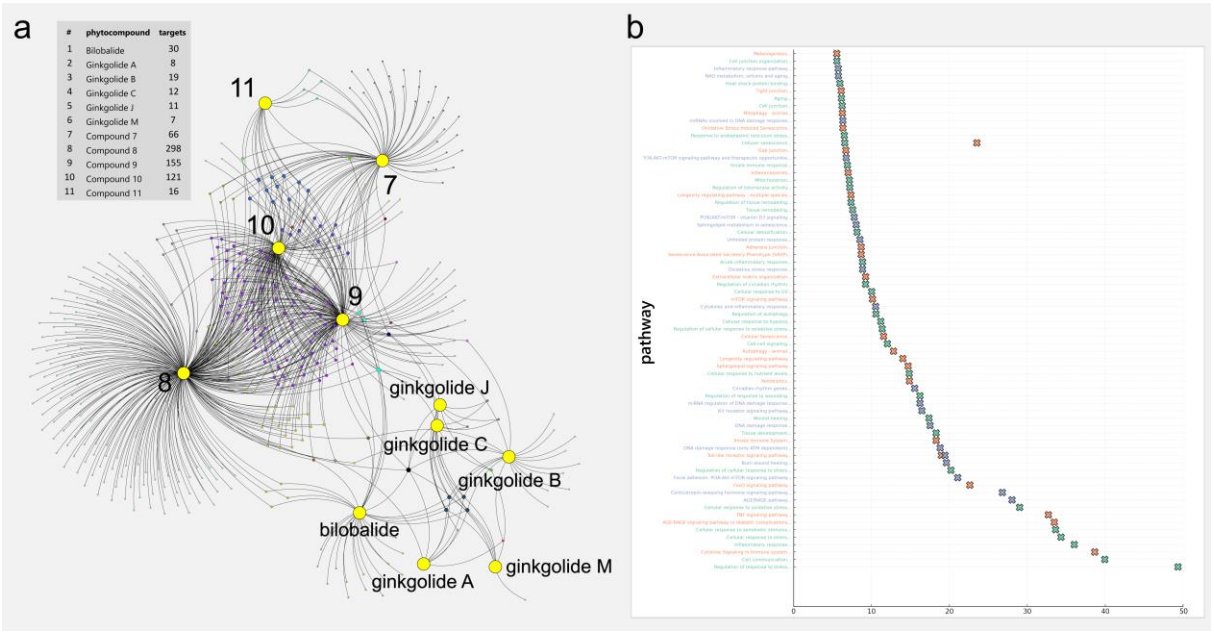

Figure 3. (a) Phytocompounds-Targets Network. The number of validated and predicted targets for each of the eleven selected phytocompounds is mentionned. (b) Selection of computed pathways with enrichment score.

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

Experimental results provided preliminary evidence of the extract's biological potential. The *Ginkgo biloba* 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**), suggesting potential benefits in skin brightening, anti-wrinkle, and anti-aging applications.

The antioxidant capacity of the aqueous extract of *G. biloba* 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). Three concentrations of the *G. biloba* extract were tested across all assays: 1%, 2%, and 3%. At a concentration of 1%, the *G. biloba* extract exhibited a mean antioxidant activity of 38.77%, whereas at 2%, this activity increased significantly, reaching 68,50% (**Figure 4a**, left).

The TEAC results followed a similar trend: at 1% extract concentration, a value of 39.87 µg Trolox equivalents/mL was recorded, while the 2% extract showed a 1.7-fold increase **(Figure 4a**, right). The total phenolic content, expressed as gallic acid equivalents, was determined to be 22.52 µg/mL, and 46.99 µg/mL, for the 1%, and 2% *G. biloba* extract, respectively (**Figure 4a**, right). In each measurement, the 3% extract demonstrated the highest capacity, with an average antioxidant activity of 88.97% (**Figure 4a**, left), a TEAC of 88.37 µg Trolox equivalents/mL, as well as a total phenolic content of 67.90 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 *G. biloba* extract rises. This highlights the natural antioxidant potential of the extract.

The inhibitory effect of *G. biloba* extract on tyrosinase activity was evaluated. Kojic acid was used as a reference inhibitor and exhibited an IC_{50} value of 13.54 µM. The extract showed dose-dependent inhibition, reaching a maximum of 72.36% at a concentration of 5%. The calculated IC_{50} of the extract was 1.15% (**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. *G. biloba* extract demonstrated hyaluronidase inhibition, reaching 100% inhibition at 5% concentration. The calculated IC_{50} of the extract was 1.10% (**Figure 4c**).

Finally, the inhibitory effect on collagenase was investigated. EGCG was used as the reference inhibitor and exhibited an IC_{50} of 854 µM. *G. biloba* extract showed weak, dose-dependent collagenase inhibition, reaching 25.37% at a concentration of 5%. The estimated IC_{50} of the extract was 9.98% (**Figure 4d**).

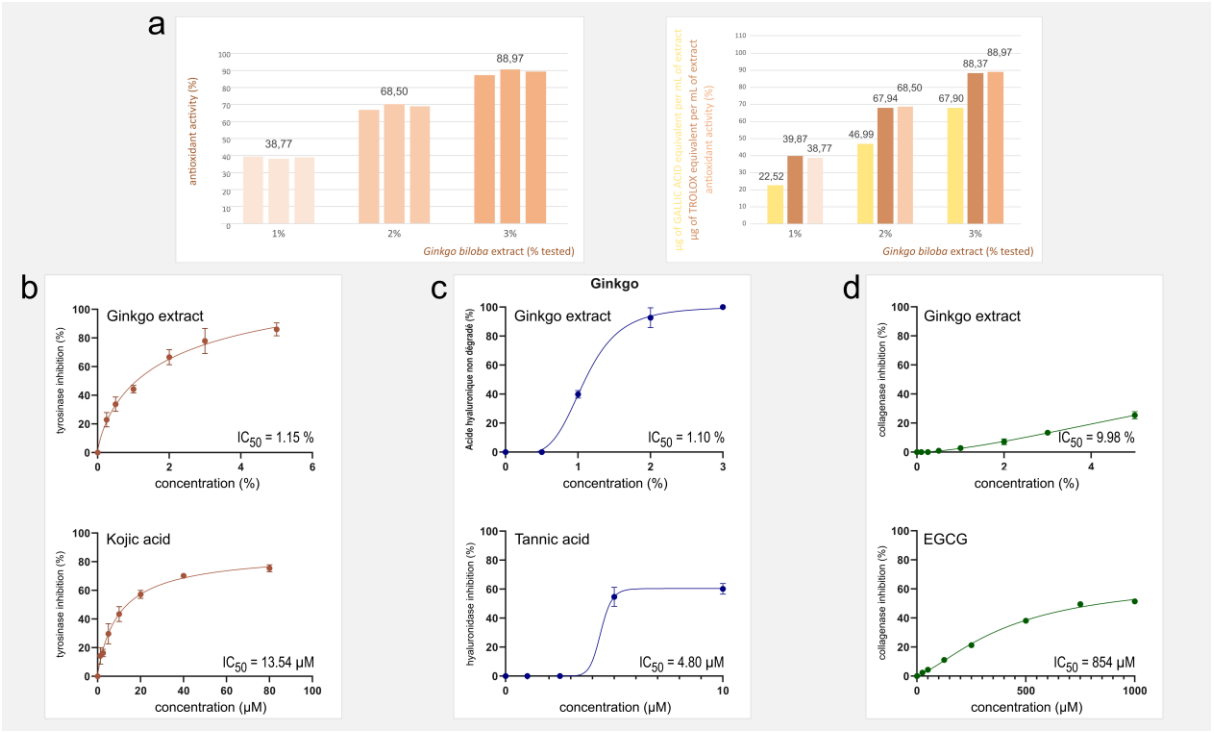

Figure 4. *In vitro* assessment of antioxidant and enzyme inhibitory activities of *Ginkgo biloba* extract. Evaluation of the antioxidant capacity of the *G. biloba* extract expressed as the percentage of antioxidant activity at three different concentrations (1%, 2%, and 3%). (right): Correlation between antioxidant capacity and total phenolic content of *G. biloba* extract at three different concentrations (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 *G. biloba* extract and the reference inhibitor kojic acid on tyrosinase. **(c)** Inhibitory activity of *G. biloba* extract and the reference inhibitor tannic acid on hyaluronidase. **(d)** Inhibitory activity of *G. biloba* extract and the reference inhibitor EGCG on collagenase.

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.

The extract was encapsulated using a proprietary inorganic microcarrier system, free from carbon-based materials and non-microplastics (compliant with EU Regulation 2023/2055). It was formulated with silica-based microparticles **(Figure 5a)**, with a median particle size below 10 µm (determined by laser diffraction), ideal for cosmetic formulas and maintaining a smooth texture. Particle size distribution indicated a D50 of 8 µm and a D90 of less than 70 µm **(Figure 5b)**.

Despite mild particle aggregation during extract loading, efficient dispersion was achieved in aqueous media. The slightly off-white aqueous suspension significantly reduced the color intensity of the original dark brown extract. The microcarrier system naturally provided a barrier to external degradation of actives ingredients (oxidation, photodegradation). The extract-loaded suspension was easily incorporated into an oil-in-water day cream without noticeable changes in color, spreadability, or organoleptic properties **(Figure 5c)**.

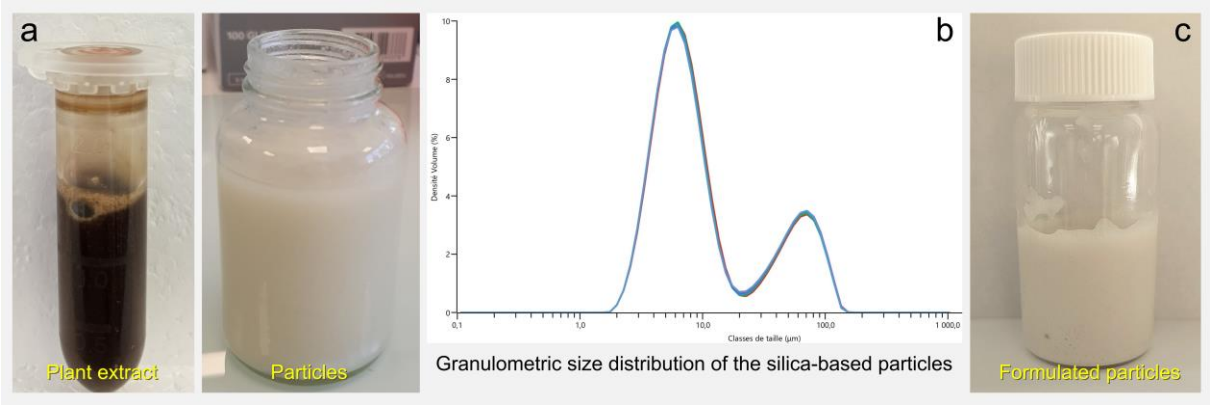

Figure 5. (a) Visual aspect of the extract before (left) and after incorporation in silica-based particles (righrt). **(b)** Granulometric size distribution (by volume) of silica-based particles determined by laser diffraction. **(c)** Skin care cream formulation.

4. Discussion and conclusions

The development of this *Ginkgo biloba*-derived extract exemplifies the potential of an integrative strategy combining optimized green extraction, advanced phytochemical profiling, *in silico* activity prediction, and innovative formulation via microplastic-free encapsulation. The *in silico* workflow successfully identified molecular targets and biological pathways relevant to skin health, notably those involved in oxidative stress, pigmentation regulation, extracellular matrix remodeling, and cellular senescence.

Experimental assays validated several predicted activities, demonstrating potent dose-dependent antioxidant capacity and significant inhibitory effects on tyrosinase and hyaluronidase, with moderate activity on collagenase. These effects align with anti-aging, skin-brightening, and anti-inflammatory claims, suggesting the extract's potential as a multifunctional cosmetic ingredient.

The encapsulation approach using silica-based microparticles improved the physical appearance and dispersibility of the extract while complying with regulatory requirements for microplastic-free formulations. Although the system is expected to provide enhanced protection against photodegradation and oxidation, as well as facilitate incorporation into complex cosmetic matrices, these functional advantages remain to be fully demonstrated through extended stability studies and formulation testing.

Altogether, these results underscore the relevance of combining phytochemistry, bioinformatics, and formulation science to design high-performance, sustainable active ingredients. Future investigations will aim to deepen the mechanistic understanding of the observed effects and assess clinical efficacy in human studies.

5. Bibliographic references

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