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"Optimized Indoor Cultivation, Extraction, and Analytical Profiling of Two Plant Species for Enhanced Skin Biology and anti-aging Applications"

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1. Introduction

Innovation in the field of dermo-cosmetic ingredients relies on the identification and valorization of bioactive plant compounds. Rigorous control of cultivation conditions not only optimizes biomass production but also enables the targeted generation of key metabolites with biological activities relevant to dermo-cosmetic applications. In this context, the optimization of cultivation conditions, extraction methods, and the integration of advanced analytical and bioinformatic approaches represent a promising strategy.

This study presents an integrated approach applied to two plant species: *Calendula officinalis* and *Matricaria recutita*, grown under controlled conditions within an innovative rotating system. For *Calendula officinalis*, two cultivation conditions were explored: a warm and a cold climat, each with a control and a UV-irradiated modality. *Matricaria recutita* was cultivated under biostimulated and non-biostimulated conditions. The aim is to maximize the production of secondary metabolites with anti-aging potential by combining advanced analytical techniques and *in silico* prediction of biological activities, followed by targeted *in vitro* validations.

2. Materials and Methods

2.1 Cultivation in rotating system inside an indoor farm

A modular rotating cultivation system was used to grow *Calendula officinalis* and *Matricaria recutita* **(Figure 1)**. This rotating cultivation system operates within a vertical, circular structure that maximizes space efficiency by enabling high-density plant production within a limited footprint. Plants are grown in soil-filled trays that rotate slowly around a central axis, ensuring uniform exposure to artificial lighting and facilitating gravity-assisted, top-down drip irrigation.

The system supports a customized fertigation regime, allowing precise control over water and nutrient delivery tailored to the physiological requirements of each plant species.

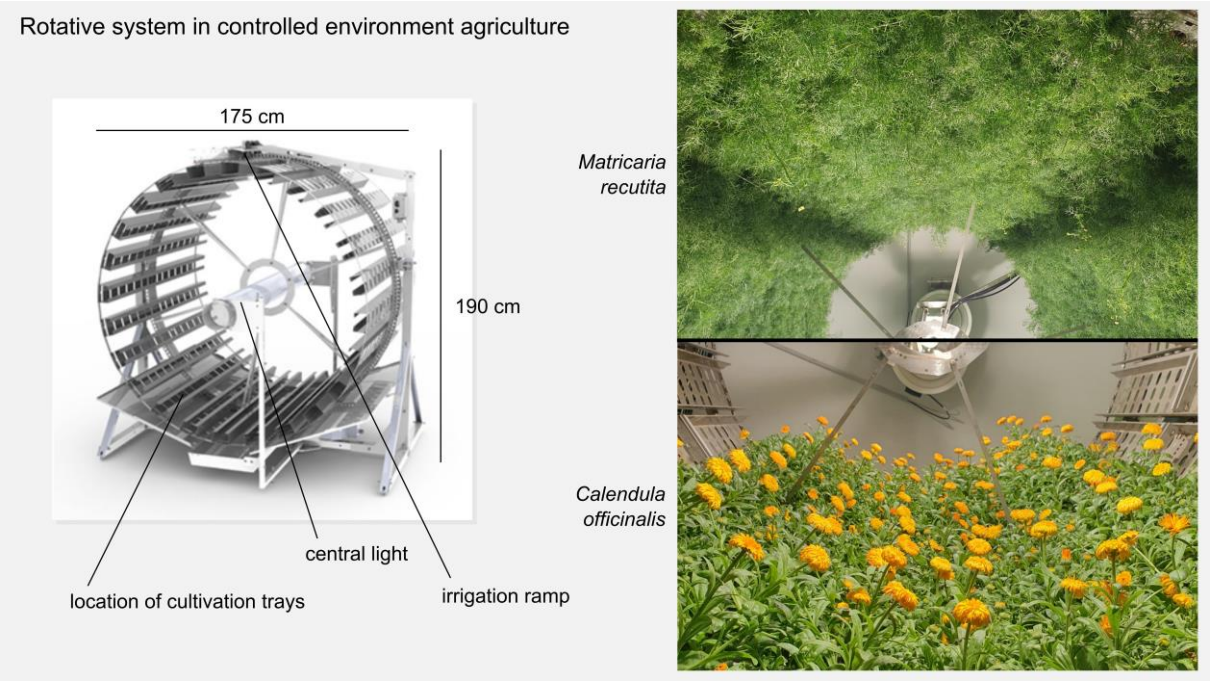


Figure 1. Schematic representation of the controlled rotating plant cultivation system, including lighting modules, elicitation zones, and nutrient flow.

2.2 Plant cultivation and elicitations

Table 1. Description of all modalities and their cultivation parameters

Modality	Plant species	Variety	Elicitation	Cultivation time (days)	Climat
CT Hot	*C. officinalis*	Bonbon	Control	37	Hot : 25/21°C 87%RH
UV Hot	*C. officinalis*	Bonbon	UV-C		
TOR Hot	*C. officinalis*	Touch of red	Variety		
CT Cold	*C. officinalis*	Bonbon	Control	48	Cold : 20/16°C 83%RH
UV Cold	*C. officinalis*	Bonbon	UV-C		
TOR Cold	*C. officinalis*	Touch of red	Variety		
CT Goral	*M. recutita*	Goral	Control	48	22/17°C 73%RH
CT Lutea	*M. recutita*	Lutéa	Control		
BS Goral	*M. recutita*	Goral	Biostimulation		
BS Lutea	*M. recutita*	Lutéa	Biostimulation		

Genetics is one of the major factors influencing the phytochemical profile of plants and the production of secondary metabolites. In the case of *Calendula officinalis*, two cultivars were compared: “Bonbon Orange Indoor” (Voltz) as the control, and “Touch of Red” (Promesse de Fleur) as the test variety [1].

To evaluate the effect of UV-C irradiation on secondary metabolite production, a UV-C lamp (BIO-SCAN Light 255, BIO-UV, France) was used as an elicitor [2]. The UV-C treatment was

applied three times prior to harvest—on day -7, day -4, and day -1—resulting in a cumulative dose of approximately 5.25 kJ·cm^{-2}.

The cultivation of these three treatment modalities was conducted under two distinct climate conditions: a warm environment as the control and a cold environment as an elicitation condition, as cold stress is known to modulate the plant's antioxidant system [3]. Details of each modality are given in **Table 1**.

For *Matricaria recutita*, a species recognized for its resilience to abiotic stress [4, 5], a biotic stress was introduced through soil biostimulation. This involved a dual application of *Pseudomonas fluorescens* (Marknature) during both the germination and transplantation stages at a dose of 4.10^8 and 2.10^8 UFC/kg respectively. As with *Calendula*, two different genotypes were compared: "Goral" and "Lutea," both selected and sourced from Pharmasaat [6].

Seeds were sown in 144-cell germination trays filled with seedlings substrat (080, Klasmann-Deilmann, France). During the germination phase, environmental conditions were maintained at 24 °C with 70% relative humidity. Plantlets were grown under LED lighting (T10 LED Grow Tube Light, HW-GL-T10-1200-36W-3Y, China) providing a light intensity of 130 µmol·m^{-2}·s^{-1}, with a 16/8 photoperiod (16h light /8h dark). Seedlings were transplanted at the two-leaf stage, which occurred after 15 days for *C. officinalis* and 22 days for *M. recutita*.

Throughout the cultivation of both species, plants were grown under a light intensity of 270 ± 15 µmol·m^{-2}·s^{-1} provided by CMH lamps (630 W double-ended, 4200 K spectrum, Lumatek Ltd, Malta). A 16/8 photoperiod was also maintained and trays were filled with potting soil (UAB 1NP, Klasmann-Deilmann, France). Plants were harvested at full bloom and cultivation durations inside the rotating system are detailed in **Table 1**. The aerial parts were then dried in a dehydrator at 30 °C for 72 hours.

2.3 *In vitro* testing assays

DPPH assay: The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as described by [7]. 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 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 [8].

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 [9]. After preincubation of the sample (0.1–5%) or epigallocatechin gallate (EGCG, 25 µM–1 mM) with the enzyme at 37 °C for 15 min, 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 min 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.

3. Results

3.1 Yields data of each modality and statistical analysis

Controlled Environment Agriculture (CEA) offers significant advantages for the cultivation of plants intended for cosmetic applications. The precise regulation of environmental parameters—such as temperature, humidity, light spectrum, and nutrient availability—enables local, year-round production under pesticide-free conditions. Moreover, the proximity of cultivation to processing facilities enhances traceability and reduces the environmental footprint associated with transportation and land use.

Indoor cultivation also facilitates the implementation of elicitation strategies (e.g., light stress, nutrient modulation, climate variation, mild drought stress) during plant development. These controlled stressors can stimulate and standardize the biosynthesis of target secondary metabolites, leading to high-quality, reproducible biomass enriched in active compounds suitable for cosmetic formulations.

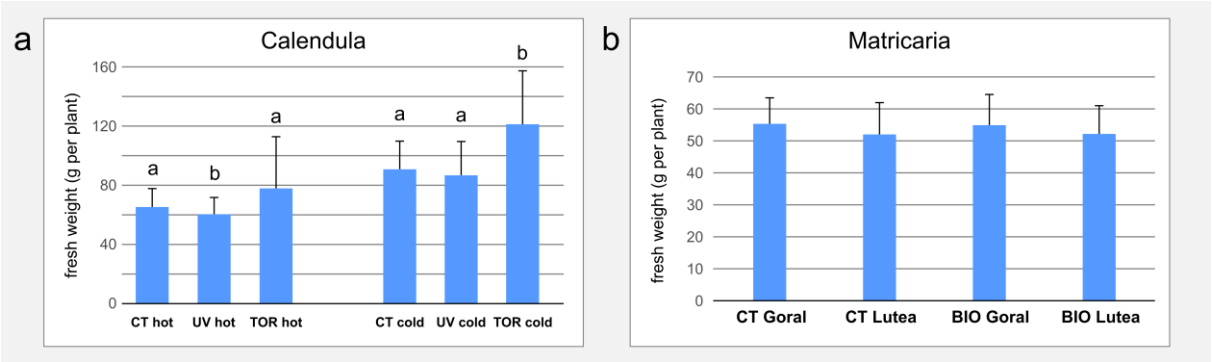


Figure 2. *Graphical representation and statistical analysis of yield data by species and modality.* **(a)** *C. officinalis* hot : significant difference of the UV modality compared to the CT (Kruskal-Wallis, p-value = 0,000063). *C. officinalis* cold : significant difference of the TOR

variety compared to the CT (Kruskal-Wallis, p-value = 0,006528). **(b)** *M. recutita* : no significant difference between the 4 modalities (Kruskal-Wallis, p-value = 0,132).

3.2 Analytical profiling of extracts

The various extracts were analyzed by UHPLC–QToF–MS in ESI+ mode (dual AJS) to obtain a Base Peak Chromatogram (BPC) after blank subtraction, in order to generate chromatograms free from background noise. The aim here is to qualitatively and semi-quantitatively compare the most comprehensive chemical profiles possible, with the objective of highlighting significant differences that will then be the focus of further investigation **(Figure 3)**.

In this context, compounds from different chemical families could be monitored (amino acids, coumarins, flavonoids, phenolic acids, polyynes, sesquiterpenes, etc.), and notable differences were observed between species and according to the cultivation/elicitation conditions. Among these differences, one profile (biostimulated *Matricaria* Lutea) appeared to stand out based on part of its composition, particularly its content in volatile substances **(Figure 3)**.

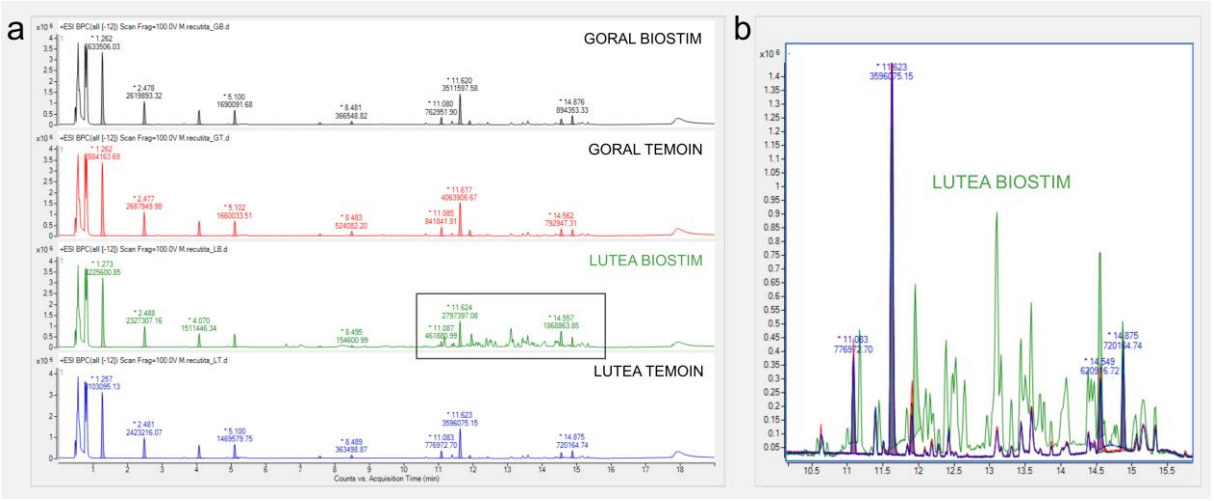
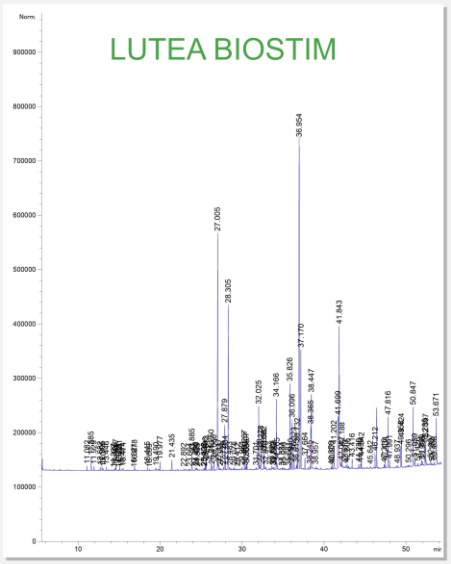


Figure 3. Comparative analysis of metabolite profiles via HPLC-PAD and HRMS (ESI+ mode). **(a)** Comparison of control and biostimulated *Matricaria* Goral and Lutea. **(b)** Superposition of the four chromatograms.

Biostimulated *Matricaria recutita* Lutea was selected for further exploration, based on its differential analytical profile, when compared to control Lutea or to Goral variety. Therefore, a specific analysis by GC-FID and GC-MS was carried out to focus on the highlighted compounds **(Figure 4)**.



#	Tr	N° CAS	Composés	% Fid
1	36.95	4575-53-5	(Z)-Spiroether	18,829
2	27.01	18794-84-8	(E)-Béta-Farnésène	10,057
3	41.84	463-40-1	Acide Linolénique	8,982
4	28.31	502-61-4	(E,E)-Alpha-Farnésène	6,497
5	41.70	60-33-3	Acide Linoléique	4,697
6	37.17	50257-98-2	(E)-Spiroéther	4,567
7	38.45	57-10-3	Acide Palmitique	3,695
8	53.67	630-03-5	n-Nonacosane	2,954
9	35.83	-	Néophytadiène*	2,823
10	50.85	593-49-7	n-Heptacosane	2,633
11	34.17	22567-36-8	Oxyde A d'Alpha-Bisabolol	2,617
12	32.02	26184-88-3	Oxyde B d'Alpha-Bisabolol	2,170
13	47.82	629-99-2	n-Pentacosane	1,892
14	27.88	23986-74-5	Germacrène-D	1,748
15	36.10	-	Inconnu masse molaire 220	1,664
16	38.37	206062-17-1	(E)-Tibétin-Spiro-Ether	1,489
17	41.20	150-86-7	(E)-Phytol	1,158
18	36.73	-	Néophytadiène*	0,993
19	49.36	630-01-3	n-Hexacosane	0,755

* unidentified isomer

Figure 4. Gas chromatography analysis of an hexanic extract of biostimulated *Matricaria recutita* Lutea.

3.3 *In silico* prediction of biological activities

The major compounds identified in the Biostimulated *M. recutita* Lutea extract **(Figure 4)** 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 [10].

Twelve major phytocompounds were selected in the biostimulated *Matricaria recutita* Lutea extract. The integration of both validated and predicted targets resulted in a comprehensive list of 362 putative targets. Details regarding the number of targets associated with each compound are shown in **Figure 5**.

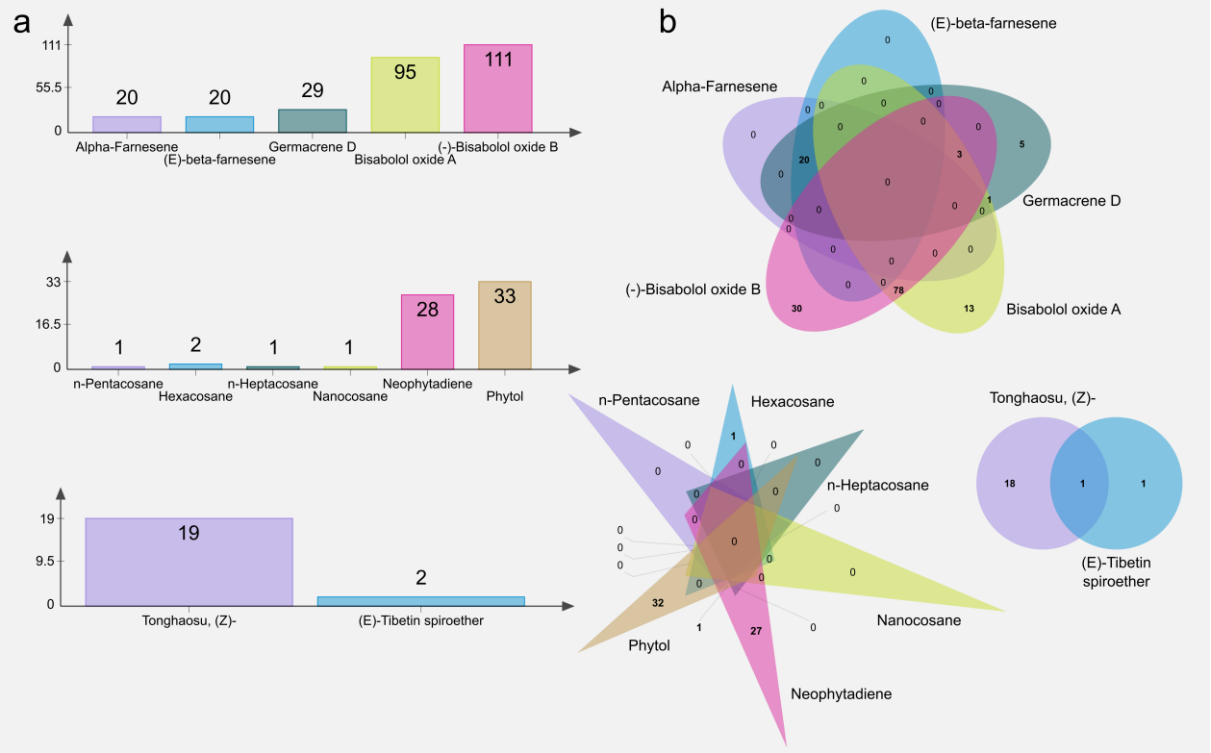
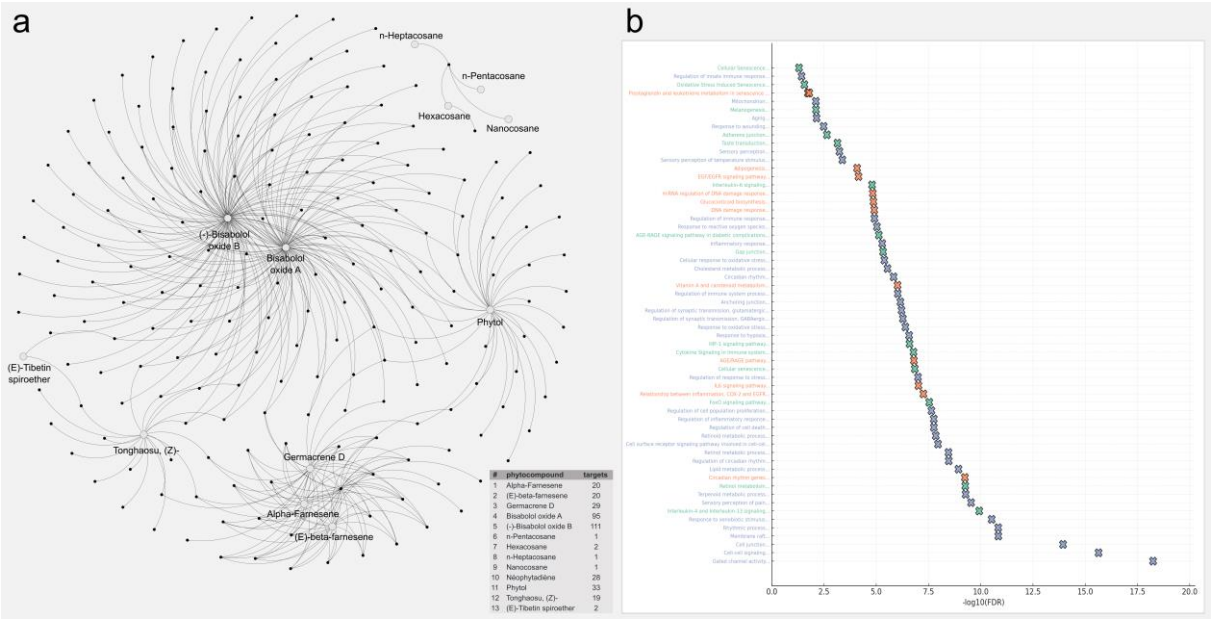


Figure 5. Number of validated and predicted targets for each of the selected phytocompounds.

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 twelve 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 6a)** were subjected to gene enrichment analysis, uncovering biological processes and signaling pathways potentially modulated by the extract **(Figure 6b)**.

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.



#	phytocompound	targets
1	Alpha-Farnesene	20
2	(E)-beta-farnesene	20
3	Germacrene D	29
4	Bisabolol oxide A	95
5	(-)-Bisabolol oxide B	111
6	n-Pentacosane	1
7	Hexacosane	2
8	n-Heptacosane	1
9	Nanocosane	1
10	Neophytadiène	28
11	Phytol	33
12	Tonghaosu, (Z)-	19
13	(E)-Tibetin spiroether	2

Figure 6. (a) Phytocompounds-Targets Network. The number of validated and predicted targets for each of the eleven selected phytocompounds is mentioned. **(b)** Selection of computed pathways with enrichment score.

3.4 *In vitro* validation of predicted activities and preliminary experimental results

Experimental results provided preliminary evidence of the extract's biological potential. *Matricaria recutita* Lutea extracts 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 7a)**.

The antioxidant capacity of the hydroalcoolic extracts of control *Matricaria* Lutea and biostimulated *Matricaria* Lutea were evaluated using the DPPH assay, through two indicators **(Figure 7a)**: the percentage of antioxidant activity and the Trolox equivalent antioxidant capacity (TEAC), expressed in µg of Trolox equivalents per mL of extract. 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. Two concentrations, 5% and 10%, of the control and the biostimulated extract were tested across all assays.

At a concentration of 5%, the biostimulated *Matricaria* Lutea extract exhibited slightly higher values than the conventional extract across all measured parameters: antioxidant activity (34.17% vs. 31.50%), Trolox equivalents (36.05 µg Trolox equivalents/mL vs. 33.45 µg Trolox equivalents/mL), and gallic acid equivalents (37.56 µg gallic acid equivalents/mL vs. 33.98 µg gallic acid equivalents/mL), with statistically significant differences ($p < 0.05$ or $p < 0.01$).

At 10%, this trend became more pronounced: biostimulated *Matricaria* Lutea extracts showed higher antioxidant activity (76.74% vs. 70.23%), as well as increased levels of Trolox equivalents (82.08 µg Trolox equivalents/mL vs. 75.08 µg Trolox equivalents/mL) and gallic

acid equivalents (75.86 µg gallic acid equivalents/mL vs. 70.28 µg gallic acid equivalents/mL), with highly significant differences ($p < 0.01$ or $p < 0.005$) **(Figure 7a)**.

These results suggest that biostimulated *Matricaria* Lutea extract possess enhanced antioxidant potential and a greater abundance of total phenolic compounds compared to control *Matricaria* Lutea extract, particularly at higher concentrations.

In addition, enzyme inhibition assays showed a dose-dependent inhibitory effect on collagenase **(Figure 7b)**.

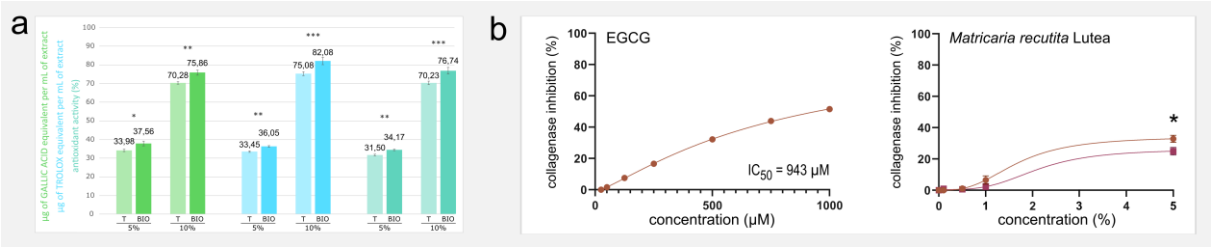


Figure 7. (a) Correlation between antioxidant capacity and total phenolic content of control *Matricaria* Lutea extract et biostimulated *Matricaria* Lutea extract at two different concentrations (5% and 10%). Antioxidant capacity was expressed both in percent of antioxidant activity and in µg Trolox equivalents/mL, whereas total phenolic content was expressed in µg gallic acid equivalents/mL. **(b)** Biostimulated *Matricaria* Lutea extract showed higher colagenase inhibitory activity at 5% concentration, compared to control, with a significant difference (student t test, $p<0.05$).

4. Discussion and conclusions

This study highlights the value of an integrated approach combining controlled-environment plant cultivation, advanced analytical techniques, and *in silico* bioactivity prediction to develop innovative botanical extracts for dermo-cosmetic applications. The use of a rotating indoor farming system enabled precise modulation of environmental conditions and elicitation strategies, which significantly influenced the phytochemical profiles of *Calendula officinalis* and *Matricaria recutita*.

The cultivation experiments demonstrated that UV-C treatment and cold stress in *C. officinalis* could modulate biomass yield and potentially influence secondary metabolite production. Similarly, biostimulation of *M. recutita* with *Pseudomonas fluorescens* led to detectable variations in the chemical composition of the extracts, particularly in the Lutea genotype, as shown by high-resolution analytical profiling.

In silico analysis of twelve major phytocompounds from the biostimulated *M. recutita* Lutea extract identified a large network of validated and predicted molecular targets involved in pathways related to oxidative stress, inflammation, skin aging, and senescence modulation. This network pharmacology approach supported the hypothesis that these extracts may exert multifunctional effects relevant to skin health.

The preliminary *in vitro* validations confirmed the antioxidant potential of the biostimulated extract, as demonstrated by increased DPPH radical scavenging activity, higher Trolox equivalent antioxidant capacity, and elevated total phenolic content compared to control extracts.

Furthermore, enzyme inhibition assays revealed that the biostimulated *M. recutita* Lutea extract exhibited a higher inhibitory effect on collagenase activity than the control extract. These results suggest enhanced anti-aging potential, particularly with respect to extracellular matrix protection.

As a perspective, extracts will also be developed for *Calendula officinalis*, and tested similarly to those obtained from *Marticaria recutita,* in order to characterize the differences induced by the various cultivation conditions.

Altogether, these findings provide a strong rationale for the use of CEA and targeted elicitation to enhance the dermo-cosmetic value of plant extracts. The results support the development of standardized, reproducible, and effective botanical ingredients. Future work will include mechanistic studies, expanded *in vitro* testing on more complex skin models, and formulation trials to validate the performance of these extracts in finished dermo-cosmetic products.

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