

Harnessing the bioactive potential of coffee extracts: comparative analysis of green and roasted coffee-based semisolid formulations for antioxidant and antimicrobial skin care applications

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Abstract. Significant interest in plant-derived bioactive compounds has been fueled by the demand for effective, sustainable, and natural skincare solutions. Coffee and caffeine stand out as particularly promising ingredients, thanks to their well-established antioxidant, anti-inflammatory, and antimicrobial characteristics. This study investigates the formulation and assessment of innovative semisolid products enhanced with extracts from coffee beans and caffeine, responding to the increasing need for natural, sustainable, and effective options in both pharmaceutical and cosmetic skincare. The formulations utilized aqueous extracts from both green and roasted coffee beans (Arabica and Robusta), alongside synthetic caffeine, to facilitate a comparative analysis. These formulations demonstrated enhanced sensory properties and optimal compatibility with skin pH levels. Rheological analysis revealed thixotropic and pseudoplastic behavior with variable hysteresis loops. Four formulations with optimal characteristics were further evaluated for antioxidant activity (using photo-chemiluminescence) and antimicrobial properties (using the diffusion method). This study highlights the impact of coffee species and processing methods on the antioxidant activity of pharmaceutical formulations. Composites containing green Arabica coffee aqueous extract obtaining at room temperature (e.g., C11) exhibited slightly higher antioxidant activity compared to those with roasted Arabica coffee aqueous extract obtaining by hot water infusion (e.g., C2), indicating a processing-related enhancement (C11 > C2). Similarly, formulations with green Robusta coffee aqueous extract obtaining at room temperature (e.g., C12) showed superior antioxidant activity compared to those with roasted Robusta coffee aqueous extract obtaining by hot water infusion (e.g., C3), emphasizing the influence of coffee type and preparation on the bioactive properties (C12 > C3). Notably, two formulations (C2 and C3) containing roasted coffee extracts demonstrated antimicrobial activity against reference strains *Staphylococcus aureus* 25923 (Gram-positive) and *Escherichia coli* ATCC 25922 (Gram-negative), forming inhibition zones of 11 mm and 15 mm. These findings highlight the potential of caffeine-based formulations with coffee extracts for skin protection and care. Overall, this study highlights the potential of caffeine-based formulations enriched with coffee extracts for dermatological applications. The influence of coffee species, processing methods, and extraction techniques on antioxidant and antimicrobial properties underscores their significance in the development of effective and multifunctional skincare solutions.

Keywords: caffeine; antioxidant capacity; antimicrobial activity; cosmetic formulations; skin care.

1. Introduction

The coffee tree (*Coffea* spp.) is a tropical evergreen plant from the *Rubiaceae* family, native to the highlands of Ethiopia and cultivated in warm, well-drained regions worldwide. Of the over 100 species, *Coffea arabica* (Arabica) and *Coffea canephora* (Robusta) dominate global production [1, 2]. Arabica, known for its delicate flavor and lower caffeine content, thrives at higher altitudes but is more vulnerable to pests and climate

changes. In contrast, Robusta, with its bold taste and higher caffeine concentration, is more resilient and grows in lower-altitude regions. The variety of coffee species leads to distinct aromatic characteristics and bioactive compounds, rendering them valuable across multiple domains. [3, 4].

Coffee, one of the most widely consumed beverages, has gained prominence in cosmetic and pharmaceutical formulations due to its rich bioactive profile, including phenolic acids, flavonoids, and alkaloids [5-10].

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Notably, chlorogenic acids exhibit potent antioxidant, anti-inflammatory, and antimicrobial properties, making coffee-derived ingredients valuable in dermatological applications [11, 12]. Their ability to penetrate the skin barrier enhances their efficacy in addressing concerns such as cellulite and premature aging. Caffeine, with its lipolytic, vasoconstrictive, and microcirculation-enhancing properties, is widely used in anti-cellulite formulations [13, 14]. Additionally, coffee extracts help mitigate skin aging by protecting against oxidative stress, improving hydration, and stimulating dermal repair. Green coffee extracts, rich in chlorogenic acids, offer superior antioxidant and anti-inflammatory benefits, while roasted coffee extracts contribute UV-protective and anti-glycation properties [5, 15, 16, 18]. The multifunctionality of coffee-derived compounds underscores their potential as key components in modern skincare solutions.

Coffee extracts, rich in phenolic compounds, enhance hydration, elasticity, and reduce inflammation, while clinical evaluations confirm their efficacy in improving skin texture, minimizing fine lines, and addressing hyperpigmentation and cellulite [19-22]. Additionally, their antimicrobial properties support skin barrier health and protection against infections [23-25].

Previous studies involving Arabica and Robusta green coffee beans revealed that water extracts, particularly from Robusta species, demonstrated greater total phenolic content (TPC) and antioxidant capacity, along with enhanced bioactive and mineral levels [26]. These results emphasize the role of extraction methods in the release of bioactive compounds and showcase the versatile applications of coffee-derived ingredients in both cosmetic and therapeutic fields.

The objective of this study is to create and evaluate novel cosmetic creams that integrate extracts from both green and roasted coffee beans, as well as synthetic caffeine, emphasizing stability, safety, and effectiveness. From twelve initial formulations, four were chosen for detailed examination due to their elevated levels of antioxidant compounds derived from Arabica and Robusta coffee extracts. This choice facilitates a thorough evaluation of their antioxidant, antimicrobial, and cosmetic properties.

This research enhances scientific literature by expanding our understanding of bioactives derived from coffee in skincare, aiding in the creation of effective, natural, and sustainable cosmetic formulations.

2. Experimental

2.1. Materials and work equipment

The chemicals used for the preparation of the formulations were of analytical grade. They were sourced from Sigma-Aldrich (Darmstadt, Germany) for silver sulfadiazine, caffeine ethanol, menthol crystals, Tween 80, vitamin C and triethanolamine; from Ellemental (Bucharest, Romania) for ingredients including lanolin, vaseline, macadamia oil, cocoa butter, vegetable glycerin, cetearyl alcohol, citric acid, emulsifying wax, stearic acid, hyaluronic acid and castor oil. The total antioxidant capacity of the prepared composites was quantified in Trolox Equivalent (TE)

units by comparison to the standard calibration substance, Trolox® - Hoffman-LaRoche (Bern, Switzerland). For antimicrobial activity testing, the following reference strains were selected: *Staphylococcus aureus* ATCC 25923 (Gram-positive bacteria), *Escherichia coli* ATCC 25922 (Gram-negative bacteria), and *Candida albicans* ATCC 900288 (fungal strain) obtained from the Microorganisms Collection of the Department of Microbiology, Faculty of Biology of the University of Bucharest. These microbial species were selected for their presence, as commensal strains, in the normal microbiota of the skin and mucous membranes, but which, under certain conditions of imbalance, following associated diseases (immunocompromising, old age, etc.), can become pathogenic.

Appropriate equipment was employed for the physicochemical analysis methods. The pH values were measured using a multiparameter pH meter from Hanna Instruments (Woonsocket, USA). The spreadability of the composites was evaluated following the method described by P. Ojeda and S. Arbussa [27]. Rheological measurements were performed using an ST-2020 R rotational viscometer from the Spanish manufacturer Laboquimia (Madrid) to obtain rheological parameters such as viscosity, shear rate, rotational speed, and shear stress. To determine the total antioxidant capacity of the composites, the PHOTOCHEM device (Analytik Jena AG, Germany) was used. The antimicrobial activity of the composites against pathogenic strains (*S. aureus*, *E. coli*, and *C. albicans*) was evaluated using a modified Kirby-Bauer disk diffusion method, specifically the well diffusion method.

2.2. Methods

Coffee collection and preparation. Coffee samples, specifically Arabica and Robusta varieties, were acquired from a supplier located in Iasi (Romania). The beans underwent roasting in a wood roaster, achieving consistent roasting at a medium temperature of 209 °C. Both the green and roasted beans were then ground into a fine powder.

Three methods of extraction were employed. The first method involved hot water infusion, where roasted coffee powder from Arabica and Robusta (in a 1:10 ratio) was extracted using hot distilled water heated to over 100 °C for 5 minutes, followed by filtration to eliminate insoluble residues (CA0, CR0). The second method utilized distilled water heated to below 100 °C, into which green coffee was introduced. This infusion was kept in a water bath for 10 minutes at a 1:10 ratio (grams of green coffee to milliliters of distilled water), and the extract was then hot filtered to remove insoluble particles (CA1, CR1).

Maceration involved soaking the green coffee powder in distilled water at room temperature (18-20 °C) for a duration of 24 hours, using a 1:10 ratio. The resulting macerates were stored away from light and moisture before being filtered to eliminate any residues (CA2; CR2).

All extracts were freshly prepared and sterilized by filtration with sterile PES filters (0.22 µm) prior to use,

as previously studied [28], to prevent microbial contamination.

A solution of caffeine standard was prepared following the guidelines of the Romanian Pharmacopoeia ed. X [29]. To achieve this, 0.5 g of caffeine was dissolved in 45 mL of purified water through heating, then cooled and adjusted to a final volume of 50 mL using the same solvent.

2.3. Formulating caffeine composites

The 12 semisolid formulations, incorporating extracts from green and roasted coffee beans as well as pure caffeine solution, were prepared following the methods outlined in Table 1. The preparation process was consistent across all formulations and involved two main phases: the hydrophilic phase (A) and the

lipophilic phase (B). The coffee extracts were incorporated into phase A, where dissolution occurred. Phase B was prepared by weighing and mixing the ingredients in a heat-resistant container, followed by dissolving the components in a water bath at a maximum temperature of 75 ± 2 °C, with continuous stirring. Both phases were brought to the same temperature before emulsification, which was achieved through continuous mixing for 15 minutes, followed by gradual cooling. All formulations were stored at 5 °C for a minimum of 24 hours prior to analyzing their physicochemical properties. Table 2 provides a detailed overview of the essential components used in the creation of these semisolid composites.

Table 1. Composition of caffeine-based topical preparations: C1-C12

Components	Mass (g)						Components	Mass (g)					
	C1	C2	C3	C4	C5	C6		C7	C8	C9	C10	C11	C12
Lanolin	30	30	30	20	20	20	Lanolin	28	28	28	25	25	25
Vaseline	10	10	10	-	-	-	Vaseline	-	-	-	-	-	-
Cocoa butter	5	5	5	5	5	5	Cocoa butter	-	-	-	-	-	-
Emulsifying wax	-	-	-	-	-	-	Emulsifying wax	5	5	5	-	-	-
Castor oil	-	-	-	10	10	10	Castor oil	5	5	5	-	-	-
Vegetable glycerine	-	-	-	-	-	-	Vegetable glycerine	-	-	-	5	5	5
Macadamia oil	5	5	5	-	-	-	Macadamia oil	-	-	-	-	-	-
Ag sulfadiazine	5	5	5	-	-	-	Ag sulfadiazine	2,5	2,5	2,5	5	5	5
Cetearyl alcohol	5	5	5	3	3	3	Cetearyl alcohol	3	3	3	5	5	5
Citric acid	2	2	2	2,5	2,5	2,5	Menthol crystals	-	-	-	-	-	-
Menthol crystals	-	-	-	1	1	1	Citric acid	1,5	1,5	1,5	-	-	-
Stearic acid	-	-	-	-	-	-	Stearic acid	-	-	-	3	3	3
Vitamin C	1	1	1	-	-	-	Vitamin C	-	-	-	2	2	2
Hyaluronic acid	-	-	-	-	-	-	Hyaluronic acid	-	-	-	0,5	0,5	0,5
Tween 80	-	-	-	3	3	3	Tween 80	3	3	3	3	3	3
Triethanolamine	-	-	-	-	-	-	Triethanolamine	-	-	-	3	3	3
Ethanol 70%	-	-	-	5	5	5	Ethanol 70%	-	-	-	-	-	-
Caffeine solution -CS	2,5	-	-	3	-	-	Caffeine solution -CS	3,5	-	-	3	-	-
Roasted Arabica coffee - CA0	-	20	-	-	-	-	Roasted Arabica coffee - CA0	-	-	-	-	-	-
Roasted Robusta coffee - CR0	-	-	20	-	-	-	Roasted Robusta coffee - CR0	-	-	-	-	-	-
Green Arabica coffee - CA1	-	-	-	-	10	-	Green Arabica coffee - CA1	-	10	-	-	-	-
Green Robusta coffee - CR1	-	-	-	-	-	10	Green Robusta coffee - CR1	-	-	10	-	-	-
Green Arabica coffee - CA2	-	-	-	-	-	-	Green Arabica coffee - CA2	-	-	-	-	32	-
Green Robusta coffee - CR2	-	-	-	-	-	-	Green Robusta coffee - CR2	-	-	-	-	-	32
Purified water	until 100 g						Purified water	until 100 g					

Table 2. Key components of semi-solid formulations.

Components	Characteristics
Lanolin	Oil phase
Vaseline	Oil phase
Cocoa butter	Oil phase
Emulsifying wax	Co-Emulsifiers
Castor oil	Oil phase
Vegetable glycerine	Humectants/Emolient
Macadamia oil	Oil phase
Ag sulfadiazine	Antiseptic
Cetearyl alcohol	Co-Emulsifiers
Menthol crystals	Decongestant
Citric acid	pH modifiers
Stearic acid	Co-Emulsifiers
Vitamin C	Antioxidant
Hyaluronic acid	Humectants/Emolient
Tween 80	Surfactants
Triethanolamine	pH modifiers
Ethanol 70%	Solvent
Caffeine solution -CS	Active ingredient
Roasted Arabica coffee - CA0	Active ingredient
Roasted Robusta coffee - CR0	Active ingredient
Green Arabica coffee - CA1	Active ingredient
Green Robusta coffee - CR1	Active ingredient
Green Arabica coffee - CA2	Active ingredient
Green Robusta coffee - CR2	Active ingredient
Purified water	Aqueous phase

2.4. Determination of composite characteristics

To assess the sensory attributes of composites C1–C12, a comprehensive analysis was conducted. The appearance was evaluated using a magnifier (4.5x) to examine a thin layer of composite spread on a microscope slide. The scent of each composite was determined by applying a thin layer over a 20 cm² surface and assessing the aroma from a distance of 2–4 cm. For color evaluation, a small amount of the sample was spread thinly on white tissue paper under natural daylight to determine its hue. The pH was measured potentiometrically using a Hanna Instruments multiparameter pH meter. Samples were dissolved in a 1:10 ratio with boiled distilled water, and the pH was measured from the aqueous phase that separated after heating the mixture in a water bath at 60 °C, followed by homogenization for 10 minutes. To test thermal resistance, the composites were exposed to two distinct temperature conditions (20 °C and 40 °C) for 8 hours. Five grams of each composite were placed in capped weighing bottles and stored in a refrigerator and an oven at the specified temperatures. The samples were then examined for uniformity and consistency. The spreadability of the semisolid formulations was evaluated 24 hours after preparation by measuring the diameter a 1 g sample could achieve when compressed between two 20x20 cm glass plates. Measurements were taken after 1 minute using the Ojeda Arbussa method

[30, 31]. The upper plate was standardized to a weight of 125 g. Additional weights of 30 g, 50 g, 100 g, 150 g, 200 g, 250 g, 500 g, and 750 g were successively applied, with the spread areas recorded in millimeters. These measurements were repeated 30 days after preparation. Results were expressed as the spread area relative to the applied mass, calculated using Equation (1). All tests were performed in triplicate at 25 °C.

$$S_i = d_i^2 \left(\frac{\pi}{4} \right) \quad (1)$$

where: S_i represents the spreading area (mm²) generated by the applied mass i (g), and d_i is the average diameter achieved by the sample (mm).

2.5. Rheological study

For the 12 newly developed pharmaceutical formulations, a comparative analysis of their rheological behavior was conducted by evaluating key parameters, including apparent viscosity, shear rate, and shear stress [32]. The study involved measuring the apparent viscosity of each sample at increasing rotational speeds (1.5 rpm to 200 rpm) followed by decreasing speeds (200 rpm to 1.5 rpm). Measurements were taken at 10-second intervals, with each reading lasting 10 seconds. Coaxial cylinders R5 and R6, selected based on the viscosity ranges of the formulations, were used for these measurements. The rotational speeds were converted to shear rates using Equation (4). Rheograms and flow curves for composites C2, C3, C11, and C12 were plotted using Equations (2) and (3), respectively. Equation (2) represents the flow behavior of the composites, while equation (3) describes their rheological profile. All experiments were conducted at a controlled temperature of 22 ± 2 °C.

$$\eta = f_{(D)} \quad (2)^1$$

$$\tau = f_{(D)} \quad (3)^2$$

$$D = \omega \times R \quad (4)^3$$

$$\tau = k \times D^n \text{ or } \eta = k \times D^{n-1} \quad (5)$$

¹where η is the apparent viscosity measured in cP and D is the shear rate measured in s⁻¹;

²where: τ is the shear rate and is measured in mPa;

³where: ω is the rotational speed of the coaxial cylinder of the apparatus measured in rpm, and R is a coefficient of the coaxial cylinder as a function of the diameter of the cylinder of the apparatus.

The rheological properties of the formulations were validated through comparison with established rheological models. Among these, the Ostwald-de Waele model, commonly referred to as the power law, is one of the most widely recognized and is described by Equation (5).

2.6. Determination of antioxidant activity of C2, C3, C11, C12 composites

The total antioxidant capacity (ACL) of lipid-soluble substances in preparations C2, C3, C11 and C12 was determined by photochemiluminescence [33]. For each analysis, 1 g of sample was dissolved in 10 mL of *n*-butanol and filtered through filter paper to obtain a clear solution. The antioxidant capacity was measured for stock solutions prepared with reagent R1 as described in the ACL protocol (Analytik Jena AG) [34]. A 5 μ l

aliquot of the supernatant was exposed to radiation from a phosphorus-coated mercury lamp. The standard ACL kit (Analytik Jena, Germany) was used for this study, which contains R1 (dilution solvent), R2 (buffer reagent), R3 (photosensitive reagent) and R4 (calibrator reagent). The mixture was prepared according to the specifications given in Table 3.

Table 3. Workflow for ACL analysis.

Reagents	R1 (μL)	R2 (μL)	R3 (μL)	R4 (μL)	Sample (μL)
Blank	2300	200	25	0	0
Calibration curve	2300	200	25	5	0
Measurement Sample	2300	200	25	0	5

A calibration curve for the Trolox standard was constructed using a series of Trolox standard solutions with concentrations of 0.5, 1.0, 1.5, 2.0, and 3.0 nmol/sample volume. A minimum of three blank tests were conducted. The resulting equation was $y = 1.59160x + 1.50449$, with a correlation coefficient of $R^2 = 0.9948$, demonstrating a linear relationship.

2.7. Determination of antimicrobial activity of C2, C3, C11, C12 composites

The antimicrobial activity of the four composites was evaluated against reference strains: *Staphylococcus aureus* ATCC 25923 (Gram-positive), *Escherichia coli* ATCC 25922 (Gram-negative), and the yeast species *Candida albicans* ATCC 10231. Biological tests were conducted by cultivating bacterial strains on Plate Count Agar (PCA) medium at $37\text{ }^{\circ}\text{C} \pm 0.5$ for 22 ± 2 hours. Inoculum preparation involved the direct homogenization of 3–5 microbial colonies from an 18-hour stationary phase culture plate into sterile saline (AFS) to achieve a standardized turbidity of 0.5 McFarland. A PCA plate was seeded with 1 mL of this bacterial suspension (1.5×10^8 CFU/mL) via incorporation. After solidification, wells of varying diameters were made in the agar, and different amounts of composite were added under aseptic conditions to achieve varying concentrations of active principles. The variants tested were as follows: $\varnothing = 9$ mm with 0.25 g of composite, $\varnothing = 11$ mm with 0.30 g of composite, and $\varnothing = 15$ mm with 0.60 g of composite. Samples were incubated at $37\text{ }^{\circ}\text{C}$ for 22 ± 2 hours. After incubation, the diameter of the microbial growth inhibition zone (in mm) was measured using a graduated ruler. Results were interpreted qualitatively following NCCLS guidelines [35].

2.8. Statistical analysis

The data collected during the study were analyzed using statistical methods to ensure accuracy and reliability of the results. Descriptive statistics, including mean and standard deviation (SD), were calculated for all measured parameters to summarize the data distribution. The rheological, antioxidant, and antimicrobial properties of the formulations were compared using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test to identify significant differences among the formulations. A p-value < 0.05 was considered statistically significant [36, 37].

All statistical analyses were conducted using Microsoft Excel 2016 and GraphPad Prism version X.X. The rheological parameters were analyzed based on triplicate measurements, and the hysteresis loops were calculated for thixotropic behavior evaluation. For antioxidant capacity, the photochemiluminescence results were interpreted as total antioxidant capacity (TAC) values, with comparisons made between formulations (C2, C3, C11, and C12). Antimicrobial activity was analyzed based on inhibition zone diameters (in mm) for Gram-positive *Staphylococcus aureus* ATCC 25923 and Gram-negative *Escherichia coli* ATCC 25922, with results averaged over three replicates.

The statistical approach ensures that the observed differences in the formulations' properties can be confidently attributed to the variations in coffee extract type, preparation methods, and inclusion of active ingredients.

2.9. Quality control for analytical measurements

To ensure the reliability and reproducibility of the analytical results, strict quality control measures were implemented. Instrument calibration was performed before each experimental session using certified standards. All reagents were from reputable suppliers and validated for purity before use. Analytical procedures were performed in triplicate to minimize variability, and results were expressed as mean \pm standard deviation. Negative and positive controls were included in the microbial assays to confirm the validity of the assay. Data integrity was maintained through rigorous documentation and adherence to Good Laboratory Practice (GLP) guidelines.

3. Results and discussion

3.1. Physicochemical characteristics. The results of the physicochemical analyses for the 12 composites are presented in Tables 4 and 5 and the most stable formulations are illustrated in Figure 1, including in terms of compatibility with the skin's pH.

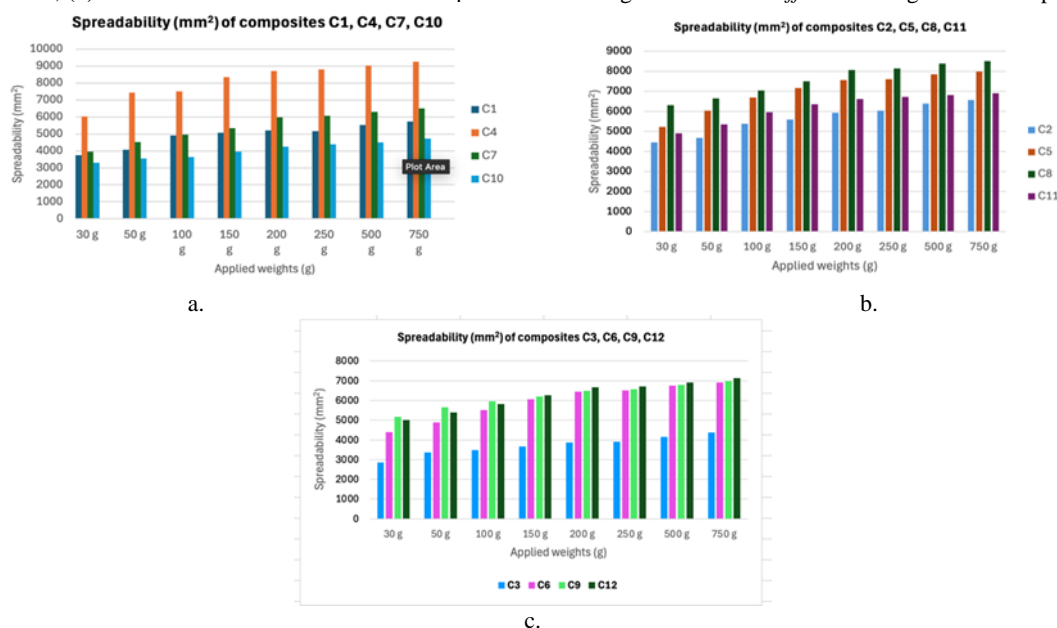
Table 4. Characteristics of topical caffeine-based formulations: C1-C6.

Characteristics	C1	C2	C3	C4	C5	C6
Initial organoleptic characteristics	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; odor characteristic	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor
Organoleptic characteristics after 30 days	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form

Characteristics	C1	C2	C3	C4	C5	C6
Initial pH	5.9	5.5	5.4	6	7	7.5
pH after 30 days	5.6	5.4	5.3	5.7	6.6	6.9
Initial thermal stability	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product
Thermal stability after 30 days	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product

Table 5. Characteristics of topical caffeine-based formulations: C7-C12.

Characteristics	C7	C8	C9	C10	C11	C12
Initial organoleptic characteristics	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor	Appearance: homogeneous; color: white; characteristic odor
Organoleptic characteristics after 30 days	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form	Maintaining the initial form
Initial pH	5.9	6.4	7.0	5.8	5.4	5.5
pH after 30 days	5.7	6.1	6.7	5.6	5.2	5.3
Initial thermal stability	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product
Thermal stability after 30 days	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product	Stable, homogeneous product

**Figure 1.** Appearance, colour and consistency of the composite (a) C2 - Semi-solid formulation with aqueous extract of roasted *Arabica coffee* obtaining by hot water infusion; (b) C3 - Semi-solid formulation with aqueous extract of roasted *Robusta coffee* obtaining by hot water infusion; (c) C11 - Semi-solid formulation with aqueous extract of green *Arabica coffee* obtaining at room temperature; (d) C12 - Semi-solid formulation with aqueous extract of green *Robusta coffee* obtaining at room temperature.**Figure 2.** Spreadability of composites C1-C12 after 24 hours: (a) C1, C4, C7, C10 - Topical preparations with synthetic caffeine; (b) C2, C5, C8, C11 - Topical preparations with aqueous extract of *Arabica coffee*; (c) C3, C6, C9, C12 - Topical preparations with aqueous extract of *Robusta coffee*.

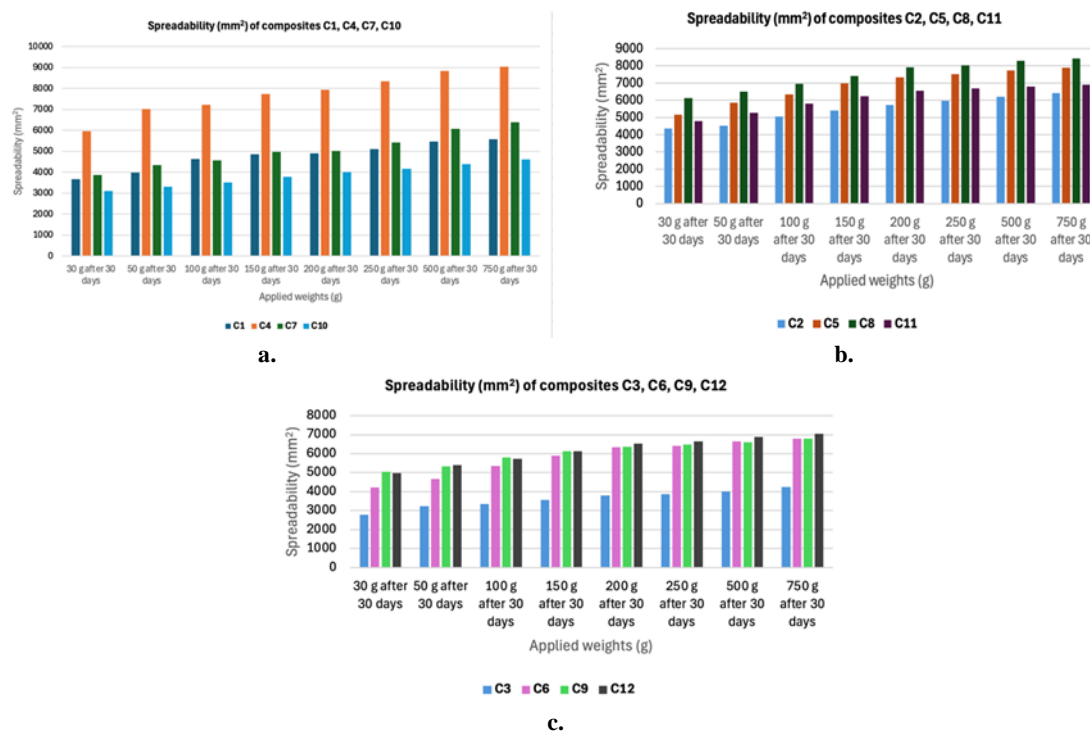


Figure 3. Spreadability of composites C1-C12 after 30 days: (a) C1, C4, C7, C10 - Topical preparations with synthetic caffeine; (b) C2, C5, C8, C11 - Topical preparations with aqueous extract of *Arabica coffee*; (c) C3, C6, C9, C12 - Topical preparations with aqueous extract of *Robusta coffee*.

Figures 2 and 3 show the results of the spreadability analysis for the 12 formulations. All dermatocosmetic formulations demonstrated macroscopic homogeneity and stability, maintaining consistent properties over time. The pH values were compatible with skin, and viscosity showed minimal fluctuations over the testing period. Figures 2 and 3 further emphasize the excellent spreadability of the composites, with only minor variations noted among similar formulations.

In the case of coffee-based formulations, the careful balance between aqueous and oil phases, as well as the proper dispersion of coffee extracts, contributes to achieving both good stability and smooth spreadability, resulting in a product that is not only effective but also pleasant to use.

3.2. Rheological characteristics. The rheological study using the ReoViskostar R viscometer confirmed the non-Newtonian behavior of all formulations, with shear stress increasing with shear rate (Figure S1). Apparent viscosity measurements, recorded in triplicate, demonstrated consistency, ensuring reproducibility. The thixotropic and pseudoplastic properties support ease of application while maintaining structural integrity.

Tables 6 and 7 summarize the rheological properties, highlighting variations influenced by coffee extract type and caffeine concentration, emphasizing the importance of optimizing flow behavior for stability and user experience.

Table 6. Rheological parameters obtained for caffeine composites.

Sample	Shear speed D (s ⁻¹)	Viscosity η (cP)	Shear stress π (mPa)
C1	4.08-68	8102-49800	201960-551060
C2	1.075-43	2.500-57569	61565-107590
C3	0.51-34	28206-783857	399720-959600
C4	2.04-68	1650-18350	4.20-4624
C5	2.04-68	980-6750	13668-66980
C6	2.04-68	1300-8520	17340-89760
C7	1.7-34	5838-40000	66096-201240
C8	1.7-34	1862-19235	32700-63380
C9	1.7-20.4	6314-26900	45700-128908
C10	2.04-34	10094-74590	151960-343200
C11	2.04-68	3500-33519	68030-23800
C12	1.7-17	22429-63700	106950-384200

Table 7. The coefficient values of Ostwald de Waele rheological model.

Sample	K Consistency coefficient	n -Flow index	R Correlation coefficient Ostwald de Waele
C1	11.627	0.3455	0.9982
C2	11.324	0.1525	0.9999
C3	13.2406	0.2239	0.9999
C4	10.3296	0.3267	0.9990
C5	9.2660	0.4646	0.9981

Sample	K Consistency coefficient	n-Flow index	R Correlation coefficient Ostwald de Waele
C6	9.4301	0.4731	0.9950
C7	10.8520	0.3853	0.9960
C8	10.3340	0.2320	0.9949
C9	10.5273	0.4187	0.9980
C10	11.7650	0.3042	0.9900
C11	10.9986	0.3503	0.9977
C12	11.2940	0.5519	0.9997

3.3. Antioxidant activity. The results of the antioxidant capacity evaluation using the photo-chemiluminescence technique provide valuable insights into the effectiveness of the selected composites. Among the four formulations tested - C2, C3, C11, and C12 - significant variations in antioxidant activity were observed, highlighting the influence of the type of coffee extract used in the formulations. The values obtained for the total antioxidant capacity of caffeine pharmaceutical composites with caffeine from natural sources are shown in Table 8.

Table 8. Total antioxidant capacity (TEAC) obtained for C2, C3, C11, C12 composites solubilized in *n*-butyl alcohol.

Sample	Volume (μ L)	Max. inhibition	TEAC mg TE/100 g sample
C2	5	0.568	281.383 \pm 2.55
C3	5	0.208	132.746 \pm 1.33
C11	5	0.559	287.476 \pm 1.67
C12	5	0.756	447.618 \pm 2.84

The C12 composite, which incorporates an aqueous extract of green Robusta coffee, demonstrated the highest antioxidant activity at 447.618 \pm 2.84 mg Trolox per 100 g sample. This result underscores the potent antioxidant properties of green Robusta coffee, likely attributable to its high content of bioactive compounds such as chlorogenic acids. In contrast, the C3 composite, containing an aqueous extract of roasted Robusta coffee, exhibited the lowest antioxidant activity at 132.746 \pm 1.33 mg Trolox per 100 g sample. This lower performance may be due to the thermal degradation of

antioxidant compounds during the roasting process, which is known to reduce the concentration of phenolic compounds and other antioxidants.

3.4. Antimicrobial activity. The aim of the present analysis was to evaluate the antimicrobial effect of pharmaceutical composites containing two types of coffee: Arabica and Robusta, both in green and roasted state. Testing of the antimicrobial activity of C2, C3, C11 and C12 composites, using the orifice method, revealed the antimicrobial activity of C2 and C3 against Gram-positive bacteria (*Staphylococcus aureus*) with diameters of 11 mm and 15 mm, respectively, and against Gram-negative bacteria (*Escherichia coli*) with diameters of 11 mm and 15 mm. This was demonstrated by the presence of an inhibition area around the preparation, indicating bacterial sensitivity to it. The results obtained by the diffusimetric method for the evaluation of the antimicrobial activity of the composites are shown in Table 9. In the case of formulations C12, a weak antimicrobial activity on the reference bacterial strains *Staphylococcus aureus* 25923 (Gram-positive) and *Escherichia coli* ATCC 25922 (Gram-negative) is observed as shown in Figure 5a, which is evidenced by the existence of a reduced inhibition area at 11 mm and 15 mm diameter around the preparation, which indicates the bacterial sensitivity to the preparation. This antimicrobial activity of C12 is probably due to the presence of the silver sulfadiazine component in their composition. *Candida albicans* showed the greatest resistance to the action of C2, C3, C11 and C12 (Figure 5 a and b right).

Table 9. Inhibitory activity of composites (C2, C3, C11, C12) on *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* strains (mm inhibition zone) (mean \pm SD).

Sample	Mean inhibition zone diameter (mm) as a function of well diameter (9 mm, 11 mm, 15 mm) in which the test sample was placed \pm SD								
	$\varnothing = 9$ mm (0.25 g cream)			$\varnothing = 11$ mm (0.3 g cream)			$\varnothing = 15$ mm (0.6 g cream)		
	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>C. albicans</i>
C2	0	0	0	11.0 \pm 1.4	12.5 \pm 1.3	0	14.0 \pm 1.7	15.3 \pm 1.8	0
C3	0	0	0	12.9 \pm 1.5	14.0 \pm 1.7	0	15.5 \pm 1.2	16.4 \pm 1.6	0
C11	0	0	0	0	0	0	0	0	0
C12	0	0	0	0	0	0	7.0 \pm 1.7	5.1 \pm 1.3	0

SD- standard deviation

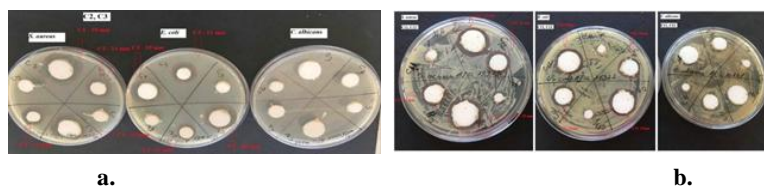


Figure 5. Results obtained for the determination of antimicrobial activity for the studied composites: (a) C2 and C3 composites on the three microbial strains tested (*S. aureus* - left, *E. coli* - middle, *C. albicans* - right) by the well method practiced in medium; (b) C11 and C12 composites on the three mycobacterial strains tested (*S. aureus* - left, *E. coli* - middle, *C. albicans* - right) by the well method practiced in medium.

The evaluation of the physicochemical characteristics of the 12 pharmaceutical composites highlights their suitability for dermatological applications, particularly in terms of pH, homogeneity, and spreadability. These properties are critical for ensuring stability, ease of application, and compatibility with the skin. All 12 formulations were observed to be homogeneous with a consistent appearance and characteristic odor, indicating successful formulation without phase separation or instability. The pH range of the composites is 5.2–7.5. The importance of pH in topical formulations is well-documented, as it influences not only skin tolerance but also factors such as the stability of active ingredients, viscosity, and permeability [38, 39]. The optimal pH for dermatocosmetic preparations is typically within the range of 4.5 to 5.5, aligning with the physiological pH of healthy skin. This slightly acidic range helps maintain the skin's natural barrier, also known as the acid mantle, which is essential for protecting against microbial invasion, preventing moisture loss, and supporting enzymatic activity involved in skin renewal and hydration [40].

Formulations with a pH outside this range, especially those that are too alkaline (above 7), can disrupt the acid mantle, leading to dryness, irritation, and increased susceptibility to infections. Conversely, excessively acidic formulations (below 4) may cause irritation or sensitivity in some individuals. By formulating products within this optimal pH range, dermatocosmetic preparations can ensure compatibility with the skin, promote comfort, and enhance product efficacy [38, 41].

The four selected formulations, C2, C3, C11, and C12 demonstrated the most optimal pH and rheological characteristics among the tested samples, attributed to their composition and the careful balance of active ingredients and excipients (Figure S1). C2 exhibited a well-balanced thixotropic and pseudoplastic behavior, with a moderate apparent viscosity that allowed for easy application and uniform spreading on the skin. The inclusion of roasted Arabica extract contributed to its stable structure, with a consistent decrease in viscosity upon increasing shear stress, ensuring user comfort during application. Upon resting, the formulation regained its initial structure, indicating good stability and resistance to phase separation. C3 displayed slightly higher viscosity than C2, reflecting the influence of roasted Robusta extract, known for its higher concentration of bioactive compounds such as melanoidins. This formulation demonstrated strong thixotropic properties, making it ideal for controlled application without dripping or excessive flow. Its rheological profile suggested excellent stability under

both storage and use conditions, enhancing its functionality for cosmetic purposes. C11 showed the most favorable flow properties among the green coffee-based formulations. Its lower viscosity, coupled with a highly pseudoplastic nature, allowed for smooth and effortless spreading. The chlorogenic acid-rich green Arabica extract contributed to its lightweight texture, making it particularly suitable for sensitive or oily skin types. The rapid recovery of its viscosity after shear stress further confirmed its structural integrity. C12 exhibited the highest apparent viscosity among the four formulations, attributed to the robust phenolic content of green Robusta extract. Despite its higher viscosity, the formulation maintained excellent thixotropic behavior, ensuring good spreadability without feeling overly heavy on the skin. The strong structural recovery observed after shearing made C12 highly stable, reducing the risk of phase separation or textural changes over time.

Overall, the rheological profiles of C2, C3, C11, and C12 demonstrated optimal properties for dermatocosmetic use, balancing ease of application, stability, and user comfort. These findings support the potential of these formulations to deliver effective and pleasant skin care solutions tailored to different consumer needs.

Good spreadability is associated with enhanced patient compliance and the uniform distribution of the product on the skin surface, which is vital for achieving desired therapeutic outcomes [42, 43]. Rheological studies revealed that the formulations exhibited non-Newtonian, pseudoplastic behavior, conforming to the Ostwald de Waele power law. This behavior is characterized by a decrease in viscosity with increasing shear rate, which is advantageous for topical applications. Pseudoplasticity ensures that the formulations are easy to spread during application (low viscosity under shear) while maintaining stability and adhesion on the skin when at rest (high viscosity under low shear conditions) [44]. Additionally, the thixotropic nature of these composites indicates their ability to recover viscosity after the removal of shear stress, which contributes to their consistency and usability. These properties are essential for ensuring that the formulation remains effective and aesthetically pleasing during use [45].

The photochemiluminescence evaluation of antioxidant capacity revealed notable differences among the tested formulations, C2, C3, C11, and C12, underscoring the impact of coffee extract type and preparation method. Formulations containing green coffee extracts (C11 and C12) demonstrated higher antioxidant activity compared to those with roasted

coffee extracts (C2 and C3), aligning with the richer chlorogenic acid content in green beans. Specifically, C12 (green Robusta extract) exhibited the highest antioxidant capacity, followed by C11 (green Arabica extract), reflecting the robust phenolic profile of Robusta beans. In contrast, C2 and C3 (roasted Arabica and Robusta extracts, respectively) showed slightly reduced antioxidant values, likely due to the thermal degradation of chlorogenic acids during roasting, though melanoidins may have contributed some antioxidant effects. These findings, detailed in Table 8, emphasize the importance of selecting appropriate coffee extracts to optimize the antioxidant potential of caffeine-based cosmetic and pharmaceutical formulations.

The comparative results suggest that the degree of roasting and the coffee species are critical factors influencing the antioxidant potential of these formulations. The green coffee extracts, particularly from Robusta, appear to retain higher antioxidant capacities compared to roasted counterparts, making them more suitable for formulations targeting oxidative stress and skin protection. These findings align with prior research indicating that green coffee extracts generally exhibit superior antioxidant activity due to their higher content of polyphenols and other bioactive compounds. The superior performance of green Robusta in C12 may be attributed to its higher polyphenol and chlorogenic acid content compared to Arabica coffee, which aligns with previous studies on the antioxidant capacity of coffee species [26, 45]. The roasting process appears to amplify the antioxidant activity in Arabica, likely due to the formation of Maillard reaction products that contribute to antioxidative effect [46, 47]. Interestingly, C3 showed the lowest antioxidant activity at the standard 10 μ L volume 132.746 ± 1.33 (mg Trolox /100 g sample). This indicates that the antioxidant capacity is significantly influenced by the concentration of active extracts and the overall composition of the formulations. These findings align with prior research on the total antioxidant capacity of green and roasted coffee extracts, where the CR2 extract (derived from green Robusta coffee) and the CA0 extract (from roasted Arabica coffee) demonstrated the highest values.

The antimicrobial activity assessment revealed differential effectiveness based on the type of coffee extract and added components: C2 and C3, containing roasted coffee extracts, demonstrated superior antimicrobial activity against both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), as evidenced by inhibition diameters of 11 mm and 15 mm, respectively 11.0 ± 1.4 ; 12.5 ± 1.3 ; 14.0 ± 1.7 ; 15.3 ± 1.8 for C2 and 12.9 ± 1.5 ; 14.0 ± 1.7 ; 15.5 ± 1.2 ; 16.4 ± 1.6 for C3 (Table 9). The enhanced activity may be attributed to the presence of bioactive compounds generated during the roasting process, which exhibit antimicrobial properties [48, 49]. C12, containing green coffee extracts and silver sulfadiazine, displayed weaker antimicrobial activity, as indicated by smaller inhibition zones (11 mm and 15 mm), respectively 7.0 ± 1.7 and 5.1 ± 1.3 . The diminished efficacy could be due to the comparatively lower antimicrobial properties of green coffee extracts, compounded by reliance solely on the silver sulfadiazine

component for antimicrobial effects. While silver sulfadiazine is a well-known antimicrobial agent, its activity may not be sufficient to compensate for the weaker bioactivity of green coffee extracts in these formulations [50]. Additionally, the tested formulations were less effective against *Candida albicans*, indicating limited antifungal activity.

3.5. Future perspectives and industrial applications

This result underscores the need for incorporating additional antifungal agents when targeting fungal infections. To ensure the practical applicability of these formulations, further research should focus on their large-scale production. Key considerations include formulation scalability, stability during manufacturing, and industrial processing challenges. Additionally, aspects such as cost-effectiveness, regulatory compliance, and quality control measures must be evaluated to facilitate the transition from laboratory development to commercial skincare products. Investigating preservation strategies and shelf-life stability will be essential to maintaining the bioactive properties of coffee extracts and caffeine in mass production. Addressing these factors will contribute to the development of sustainable, effective, and market-ready dermatological formulations.

4. Conclusions

The present study highlights the potential of coffee-derived components and complementary pharmacological agents in creating effective dermatocosmetic formulations with dual anti-cellulite and anti-aging properties.

It was successfully developed and evaluated 12 innovative pharmaceutical formulations designed as water-in-oil (W/O) and oil-in-water (O/W) emulsions, integrating aqueous extracts from green and roasted coffee beans (Arabica and Robusta) alongside synthetic caffeine solutions. The formulations were further enriched with pharmacologically active ingredients, including silver sulfadiazine (for antimicrobial preservation), hyaluronic acid (for tissue regeneration), and vitamin C (as an antioxidant), to enhance their therapeutic potential. Out of the 12 formulations, four (C2, C3, C11, and C12) were selected for advanced analysis based on their physicochemical properties and promising preliminary results, focusing on formulations with optimal compositions and bioactive efficacy.

The study demonstrated that the antioxidant capacity of the formulations was significantly influenced by the type of coffee extract and its preparation method. In Arabica-based formulations, the roasted coffee extract (C2) exhibited slightly higher antioxidant activity than the green coffee extract (C11), suggesting a roasting-related enhancement of bioactive compounds. Conversely, in Robusta-based formulations, the green coffee extract (C12) outperformed the roasted coffee extract (C3), emphasizing the impact of coffee type and preparation conditions on the antioxidant potential.

Antimicrobial assessments revealed superior activity in formulations containing roasted coffee extracts (C2 and C3), attributed to the inclusion of silver sulfadiazine

and the bioactive components generated during roasting. These results highlight the importance of robust antimicrobial properties in formulations intended for anti-cellulite and anti-aging applications.

Supplementary material: Figure S1. Rheograms and flow curves of composites C1-C12.

Conflict of interest

The authors declare that there is no conflict of interest concerning the publication of this research article.

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