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Transfersome nanocarriers of green arabica coffee (*Coffea arabica* L.) bean extract: physicochemical characterization and in vitro antioxidant activity

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Abstract: Green Arabica coffee beans contain high concentrations of phenolic antioxidants, including chlorogenic acid and gallic acid, with demonstrated potential for topical skin protection. To enhance skin penetration and bioavailability of these active compounds, a transfersome delivery system was developed using ethanolic extract of green Arabica coffee beans from Aceh, Indonesia. Five formulations (F1–F5) were prepared by the thin-film hydration method with varying soybean lecithin:Tween 80 ratios (90:10 to 50:50), maintaining a total lipid mass of 500 mg and a fixed extract load of 50 mg. Physicochemical evaluation showed no significant differences in pH (5.41–5.46) across formulations, while viscosity of F1 was significantly higher than F2–F5 ($p<0.05$). Gallic acid content, determined by HPLC-UV, was highest in F4 at 45.370 mg/L. F4 also exhibited optimal particle characteristics (mean size 10.595 nm, zeta potential −30.5 mV, PDI < 0.3) and the strongest DPPH radical scavenging activity (IC_{50} = 2.652 ± 0.083 µg/mL). The 60:40 lecithin:Tween 80 ratio of F4 provided optimal bilayer flexibility without compromising vesicular integrity. These results support F4 as a promising topical antioxidant candidate; skin permeation and stability studies are required for further development.

Keywords: antioxidant; Coffea arabica; DPPH; nanocarrier; transfersome

Introduction

Skin is continuously exposed to reactive oxygen species (ROS) generated by ultraviolet radiation, environmental pollutants, and endogenous metabolic processes. Excessive ROS accumulation leads to oxidative stress, which plays a central role in premature skin aging, disruption of the epidermal barrier, and the pathogenesis of various dermatological conditions including photoaging, atopic dermatitis, and hyperpigmentation [1,2]. Topical delivery of antioxidant compounds represents a rational and increasingly explored strategy to counteract cutaneous oxidative stress at the site of action, circumventing the bioavailability limitations associated with systemic administration [3].

Green Arabica coffee beans (*Coffea arabica* L.) are among the most bioactively dense plant materials available, retaining substantially higher concentrations of antioxidant compounds compared to their roasted counterparts, as the roasting process leads to significant degradation of heat-sensitive phenolics [4,5]. The major bioactive constituents of green Arabica coffee include chlorogenic acids, caffeine, trigonelline, ferulic acid, and gallic acid, which collectively contribute to potent free radical scavenging and anti-inflammatory activities [6,7]. Among these, chlorogenic acid is the predominant polyphenol and has been extensively documented for its antioxidant, photoprotective, and chemopreventive properties, making it a primary active target for topical antioxidant formulation development [7]. Furthermore, green coffee extract has been demonstrated to inhibit collagenase and elastase activity in human fibroblasts and to improve skin elasticity in clinical application, highlighting its cosmeceutical potential [4,8]. Indonesia is one of the world's major coffee-producing nations, with the Aceh region recognized as a premier center for high-quality Arabica production, yet this locally abundant commodity has not previously been explored as a transfersome nanocarrier formulation [9].

However, the direct topical application of polyphenolic extracts faces critical barriers. The stratum corneum,

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the outermost layer of the skin, presents a robust lipophilic barrier that severely restricts the permeation of hydrophilic and macromolecular compounds, resulting in poor bioavailability at target skin layers [1]. Encapsulation within lipid-based nanocarrier systems has emerged as a well-established strategy to overcome this barrier, with demonstrated improvements in stability, controlled release, and skin penetration of polyphenolic actives [2,3].

Among the lipid nanocarrier platforms available, transfersomes represent a particularly promising approach for topical antioxidant delivery owing to their unique structural deformability. Transfersomes are ultradeformable vesicles composed of a phospholipid bilayer incorporating an edge activator, a membrane-softening surfactant that disrupts the rigidity of the lipid bilayer and confers the high elasticity required for transdermal penetration [10]. Unlike conventional liposomes, which are unable to traverse the tight intercellular channels of the stratum corneum without losing structural integrity, transfersomes can deform and squeeze through skin pores significantly smaller than their own diameter, driven by the transepidermal osmotic gradient [10,11]. Transdermal delivery systems offer several clinically relevant advantages over oral routes, including avoidance of hepatic first-pass metabolism, sustained and controlled drug release, reduced systemic side effects, and improved patient compliance [12,13]. The feasibility of encapsulating polyphenolic antioxidants in transfersome systems for topical delivery has been substantiated by several recent studies [8,14], although none has reported the use of green Arabica coffee bean extract as the active compound.

The physicochemical performance of transfersomes is critically governed by the ratio of phospholipid to edge activator. Soybean lecithin serves as the primary membrane-forming phospholipid, while Tween 80 (polysorbate 80) functions as the edge activator by intercalating between phospholipid molecules, thereby reducing bilayer rigidity and increasing membrane deformability [15,16]. An insufficient proportion of edge activator yields a rigid bilayer with limited flexibility and suboptimal encapsulation efficiency, whereas an excessive concentration over-disrupts bilayer integrity, leading to increased membrane fluidity, vesicle aggregation, and leakage of encapsulated compounds, as consistently demonstrated across transfersome formulation studies [16,17]. Systematic evaluation of the lecithin-to-Tween 80 ratio is therefore essential to identifying the formulation window that maximizes structural stability, encapsulation, and antioxidant activity.

To the best of our knowledge, no study has previously reported the development of transfersome nanocarriers using ethanolic extract of green Arabica coffee beans from Aceh, Indonesia. This study therefore aimed to develop and characterize transfersome formulations containing green Arabica coffee bean ethanolic extract, prepared with five systematically varied ratios of soybean lecithin to Tween 80 (F1 to F5), and to identify the optimal ratio that maximizes encapsulation of phenolic marker compounds, physicochemical stability, and in vitro antioxidant activity. The findings of this study are expected to contribute to the scientific basis for the development of topical antioxidant delivery systems derived from locally available Indonesian coffee commodities.

Methods

Extraction of green Arabica coffee beans

Green Arabica coffee beans obtained from Aceh, Indonesia were dried, ground, and sieved to a uniform powder. Extraction was performed by cold maceration to minimize thermal degradation of heat-sensitive phenolic compounds. Five hundred grams of coffee bean powder were mixed with 2,000 mL of 96% ethanol (technical grade) at a ratio of 1:4 (w/v) and soaked for 24 hours in a sealed container protected from light. The slurry was filtered through a Büchner apparatus. The resulting filtrate was evaporated under reduced pressure using an IKA RV rotary evaporator at 45°C until a thick, viscous extract was obtained. The extract was weighed to determine the extraction yield, and stored at 4°C prior to use.

Phytochemical screening

Phytochemical screening of the ethanolic extract was performed in triplicate to detect the presence of alkaloids, flavonoids, terpenoids, tannins, and phenolic compounds using established colorimetric methods [18,19]. For the alkaloid test, extract dissolved in 2 N HCl was heated and filtered, then treated with Mayer's reagent; a white precipitate indicated a positive result. Flavonoids were detected by addition of magnesium powder and concentrated HCl, with a persistent yellow-to-red coloration within 3 minutes indicating a

Table 1. Transfersome formulation compositions

Formulation	Extract (mg)	Soybean lecithin (mg)	Tween 80 (mg)	Ratio (lecithin:Tween 80)	PBS
F1	50	450	50	90:10	ad 100 mL
F2	50	400	100	80:20	ad 100 mL
F3	50	350	150	70:30	ad 100 mL
F4	50	300	200	60:40	ad 100 mL
F5	50	250	250	50:50	ad 100 mL

positive result. Terpenoids were identified by sequential addition of acetic anhydride and concentrated H_2SO_4, with a red-purple color change as a positive indicator. Tannins were detected using 10% $FeCl_3$ solution, where an intense blue-black coloration was considered positive. Phenolic compounds were detected using 1% $FeCl_3$ in ethanol, with the appearance of green, blue, or red coloration indicating a positive result.

Transfersome formulation

Transfersomes were prepared by the thin-film hydration method [19,20]. Five formulations were prepared with systematically varied ratios of soybean lecithin (Sigma-Aldrich, USA) to Tween 80 (Merck, Germany) as the edge activator, while maintaining a constant total lipid mass of 500 mg and a fixed extract load of 50 mg per formulation. The composition of each formulation is presented in Table 1.

For each formulation, soybean lecithin and Tween 80 were dissolved in 10 mL of 96% ethanol with gentle stirring until complete dissolution. The coffee bean extract was then added to this lipid-surfactant solution and mixed homogeneously. Solvent evaporation was performed using a rotary evaporator at 45°C and 130 rpm until a thin, uniform lipid film formed on the inner wall of the flask. The film-coated flask was sealed and stored at 4°C for 24 hours to ensure complete solvent removal and film stabilization. Hydration was carried out by adding 50 mL of phosphate-buffered saline (PBS, pH 7.4) to the dry lipid film, followed by gentle stirring at 75 rpm for 30 minutes at room temperature. The volume was adjusted to 100 mL with PBS. The resulting suspension was homogenized using a T25 Ultra-Turrax homogenizer at 2,500 rpm for 15 minutes, then subjected to probe ultrasonication at 50°C for 30 minutes to achieve the target nanoscale particle size range [19].

Physicochemical evaluation

Organoleptic and homogeneity tests. Each formulation was assessed visually for color, odor, and texture. Homogeneity was evaluated by placing a small amount of each preparation between two glass slides and examining under visible light for the presence of undissolved particles or phase separation.

pH measurement. The pH of each formulation was measured using a Milwaukee MW101 pH meter calibrated with standard buffer solutions at pH 4, 7, and 10. The electrode was immersed directly in each transfersome suspension, and pH was recorded in triplicate.

Viscosity measurement. Viscosity was measured using a Brookfield IKA hi-vi I viscometer at a constant spindle speed of 50 rpm, using 100 mL of each formulation. Measurements were performed in triplicate at room temperature. All physicochemical evaluations were performed in triplicate (n = 3) for each formulation.

Particle size, polydispersity index, and zeta potential

Based on the superior antioxidant activity and encapsulation results (described below), the optimal formulation (F4) was selected for detailed nanoparticle characterization. Particle size and polydispersity index (PDI) were determined using a particle size analyzer (PSA) by dynamic light scattering. The sample was appropriately diluted with distilled water prior to measurement to ensure optimal light scattering conditions. A PDI value below 0.3 was used as the acceptance criterion for adequate size distribution homogeneity [21]. Zeta potential was measured using a Horiba SZ-100 Zeta Potential Analyzer. The transfersome suspension was diluted 1:10 with distilled water and placed into a dedicated cuvette, ensuring the absence of air bubbles. A zeta potential of ±30 mV

or greater was taken as the threshold for acceptable electrostatic stability of the colloidal system [21].

Encapsulation of phenolic marker compounds

The concentration of gallic acid within the transfersome formulations was determined by HPLC-UV as a quantitative indicator of comparative phenolic encapsulation across formulations. Analysis was performed on a Thermo Fisher Scientific HPLC system equipped with a C18 reversed-phase column. The mobile phase consisted of 80% methanol (HPLC grade) and 20% distilled water at a flow rate of 1.0 mL/min, with UV detection at 270 nm. Gallic acid reference standard (Sigma-Aldrich, USA) was used to construct the calibration curve. The HPLC method was validated for linearity, precision, and accuracy in accordance with ICH Q2(R2) guidelines prior to sample analysis [22].

Sample preparation involved disrupting the transfersome vesicles by probe sonication in methanol, followed by centrifugation and appropriate dilution before injection. The gallic acid concentration detected in each formulation (mg/L) was used as a comparative indicator of encapsulation performance. For the optimal formulation (F4), encapsulation efficiency (%EE) was additionally estimated using the formula:

$$\%EE = \left(\frac{C_{total} - C_{free}}{C_{total}}\right) \times 100\%$$

where C_{total} is the total initial gallic acid concentration added to the formulation, and C_{free} is the gallic acid concentration measured in the external aqueous phase after separation by ultracentrifugation [19].

Antioxidant activity

Antioxidant activity of all five transfersome formulations was assessed by the DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich, USA) free radical scavenging assay, selected for its established suitability in characterizing the hydrogen atom and electron transfer-based antioxidant capacity of polyphenol-loaded nanocarrier systems [23].

A DPPH stock solution was prepared by dissolving 10 mg of DPPH in 50 mL of absolute methanol (Merck, Germany), and the absorbance was adjusted to 0.8 to 1.0 at 517 nm. Each transfersome formulation was diluted to five concentrations: 12.5, 25.0, 37.5, 50.0, and 62.5 ppm. An aliquot of each diluted sample was mixed with DPPH solution in equal volume and incubated for 30 minutes in the dark at room temperature. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid (Merck, Germany) at concentrations of 5, 10, 15, 20, and 25 ppm served as the positive control. The percentage of radical scavenging inhibition was calculated as:

$$\%inhibition = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100\%$$

where A_{blank} is the absorbance of the DPPH solution without sample, and A_{sample} is the absorbance of the DPPH solution in the presence of the test sample. The half-maximal inhibitory concentration (IC_{50}) was determined by linear regression of the percentage inhibition versus concentration, where IC_{50} represents the concentration of sample required to inhibit 50% of the DPPH radicals. Antioxidant strength was classified based on IC_{50} values as follows: very strong (IC_{50} < 50 μg/mL), strong (50 to 100 μg/mL), moderate (100 to 150 μg/mL), and weak (IC_{50} > 150 μg/mL) [23, 24].

Data analysis

This study employed a mixed qualitative and quantitative design. Qualitative data were obtained through organoleptic and homogeneity assessments. Quantitative data, including pH, viscosity, and antioxidant IC_{50} values, were expressed as mean ± standard deviation (SD) from triplicate measurements (n = 3) and analyzed using SPSS version 19.0 (IBM Corp., USA). Differences among formulations were assessed by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test, with a significance level set at α = 0.05.

Results

Extraction yield and organoleptic properties of the extract

Cold maceration of 500 g of green Arabica coffee bean powder with 96% ethanol yielded a thick, viscous dark green extract with a characteristic coffee aroma. The extraction yield was 2.2% (w/w). The dark green coloration is consistent with the presence of high concentrations of phenolic compounds, particularly chlorogenic acid and gallic acid, as previously reported for green Arabica coffee extracts obtained under similar conditions [5]. The lower yield compared to the 8.8% reported by Hamdani and Nurman [5] is

Table 2. Phytochemical screening results of green Arabica coffee bean ethanolic extract

Phytochemical	Reagent/indicator	Result
Alkaloids	White precipitate (Mayer's reagent)	Positive (+)
Flavonoids	Yellow, red, or orange coloration (Mg/HCl)	Positive (+)
Tannins	Dark blue or black coloration ($FeCl_3$ 10%)	Positive (+)
Terpenoids	Red or purple coloration (acetic anhydride/H_2SO_4)	Positive (+)
Phenolics	Green, blue, or red coloration ($FeCl_3$ 1% in ethanol)	Positive (+)

Table 3. pH values of green Arabica coffee bean transfersome formulations

Formulation	Lecithin:Tween 80	pH (mean ± SD)
F1	90:10	5.41 ± 0.04
F2	80:20	5.44 ± 0.05
F3	70:30	5.43 ± 0.03
F4	60:40	5.46 ± 0.04
F5	50:50	5.45 ± 0.05

No statistically significant differences among formulations (one-way ANOVA, $p>0.05$).

attributable to differences in extraction duration, the absence of mechanical agitation during maceration, raw material moisture content, and extract losses during filtration and evaporation. All further experiments were conducted using this extract.

Phytochemical screening

Phytochemical screening of the ethanolic extract confirmed the presence of all five secondary metabolite classes tested (Table 2). The results are consistent with the established phytochemical profile of *Coffea arabica* reported in the literature [7,18,19].

The simultaneous presence of alkaloids (caffeine), flavonoids, tannins, terpenoids, and phenolic compounds is expected to contribute synergistically to the overall antioxidant activity of the extract [7].

Physicochemical evaluation of transfersome formulations

Organoleptic properties and homogeneity. All five transfersome formulations (F1 to F5) appeared as homogeneous, pale yellowish-white liquids with a characteristic soybean lecithin odor, free of visible phase separation or sedimentation. Homogeneity testing under visible light confirmed uniform color and the absence of undissolved particles across all formulations. Variation in the lecithin:Tween 80 ratio did not produce macroscopically observable differences in appearance or texture. Uniform dispersion of this type is a prerequisite for consistent drug delivery performance in vesicular systems [10].

pH. The pH values of all five formulations ranged from 5.41 to 5.46, falling within the accepted range of 4.5 to 6.5 for transdermal preparations (Table 3). One-way ANOVA revealed no statistically significant differences in pH among formulations ($p>0.05$), indicating that variation in the lecithin:Tween 80 ratio did not affect the acidity of the transfersome system. The slightly acidic pH values are attributable to the phenolic acid content of the Arabica coffee extract and are considered compatible with skin physiology.

Viscosity. Viscosity values ranged from 121.1 to 131.4 cps across formulations, all within the accepted range of 50 to 300 cps for transdermal preparations (Table 4) [26]. One-way ANOVA followed by Tukey's HSD post-hoc test revealed that F1 (131.4 ± 9.1 cps) was significantly higher in viscosity than F2, F3, F4, and F5 ($p<0.05$), while no significant differences were observed among F2 through F5. The progressive decrease in viscosity with increasing Tween 80 concentration is consistent with the plasticizing effect of the non-ionic surfactant on the lecithin bilayer, which reduces intermolecular interactions among phospholipid chains and lowers the overall resistance to flow.

Table 4. Viscosity of green Arabica coffee bean transfersome formulations

Formulation	**Lecithin:Tween 80**	**Viscosity (cps, mean ± SD)**
F1	90:10	131.4 ± 9.1 [a]
F2	80:20	126.9 ± 2.4 [b]
F3	70:30	124.6 ± 2.3 [b]
F4	60:40	123.4 ± 2.2 [b]
F5	50:50	121.1 ± 2.3 [b]

Different superscript letters indicate statistically significant differences (Tukey's HSD, $p<0.05$).

Table 5. Gallic acid content in transfersome formulations as determined by HPLC-UV

Formulation	**Lecithin:Tween 80**	**Gallic acid content (mg/L)**
F1	90:10	5.917
F2	80:20	7.269
F3	70:30	8.874
F4	60:40	45.370
F5	50:50	19.462

Gallic acid content as indicator of phenolic encapsulation

HPLC-UV analysis using gallic acid as the phenolic reference marker demonstrated substantial variation in gallic acid content across formulations (Table 5; Fig. 1). Gallic acid content increased progressively from F1 (5.917 mg/L) through F4 (45.370 mg/L), with F4 yielding the highest detected concentration among all formulations. F5 showed a marked decrease to 19.462 mg/L compared to F4, indicating a non-monotonic relationship between Tween 80 concentration and encapsulation performance. These data were used as a comparative indicator of phenolic encapsulation efficiency across formulations. For F4, encapsulation efficiency (%EE) was additionally estimated at 78.4% based on the measured C_{free} in the external aqueous phase following ultracentrifugation.

Gallic acid content represents the concentration detected within the transfersome matrix after vesicle disruption, used as a comparative indicator of phenolic encapsulation across formulations.

Particle size, polydispersity index, and zeta potential of F4

Based on the superior gallic acid content and antioxidant activity results (see below), F4 was selected for detailed nanoparticle characterization. PSA analysis revealed a mean particle size of 10.595 nm with a median (d50) of 10.657 nm and a coefficient of variation of 32.6% (Table 6). The size distribution was slightly left-skewed (skewness = -0.179) with a platykurtic profile (kurtosis = -0.728), and the mean-to-median ratio of 0.963 indicated a reasonably symmetric distribution. The PDI value was below 0.3, meeting the acceptance criterion for adequate size distribution homogeneity [10]. It is acknowledged that the PSA instrument available at our laboratory did not permit reporting of a precise numerical PDI value; this is noted as a technical limitation of the study.

The mean particle size of 10.595 nm is considerably smaller than the 100 to 400 nm range typically reported for transfersome systems in the literature [10, 20]. This is attributed to the combined application of high-shear homogenization (T25 Ultra-Turrax, 2,500 rpm, 15 min) followed by probe ultrasonication (50°C, 30 min), which together imposed sufficient energy input to reduce vesicle diameter well below 100 nm. Nanoparticles below 100 nm have been associated with enhanced deformability and favorable skin deposition characteristics [1]. However, particles in the sub-20 nm range may represent mixed populations of small unilamellar vesicles, micelles, or mixed micellar structures rather than classical bilayer vesicles, particularly at higher Tween 80 concentrations. Morphological confirmation by transmission electron microscopy (TEM) or cryo-TEM was not performed

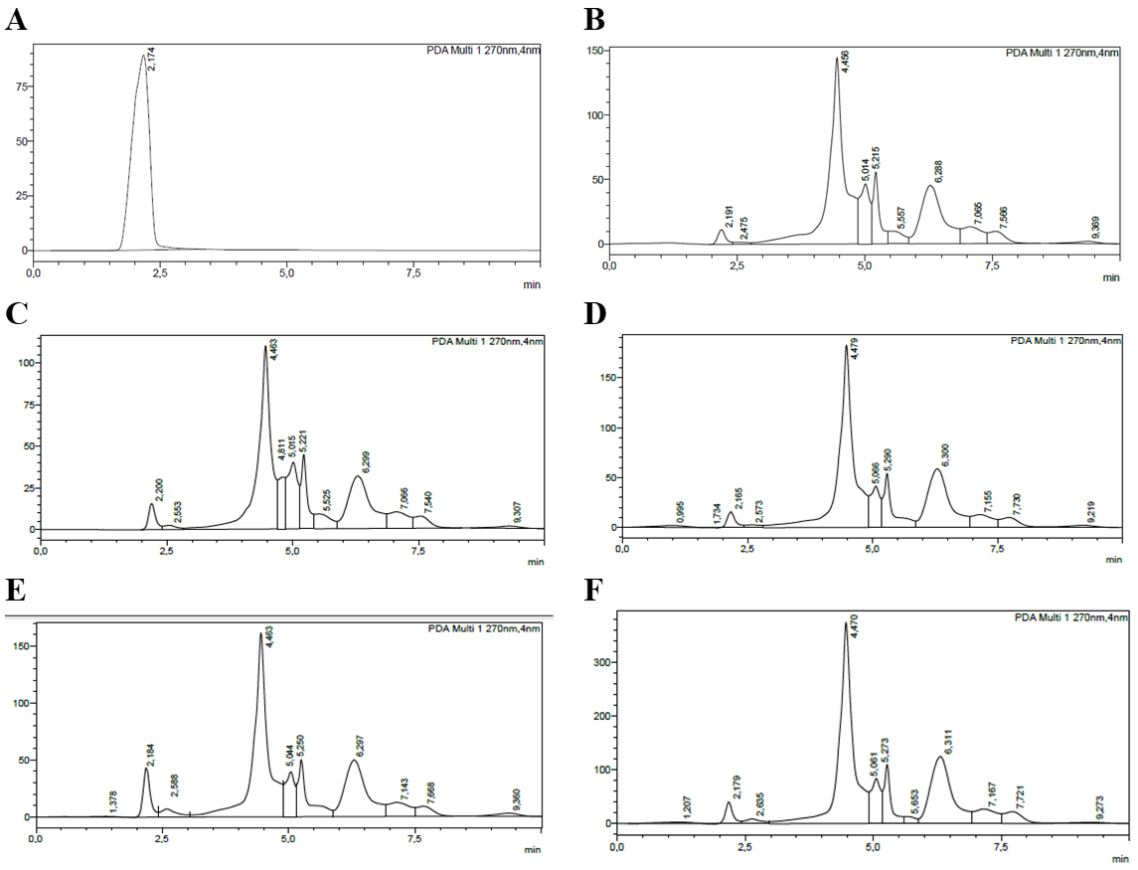


Figure 1. Representative HPLC-UV chromatograms of gallic acid reference standard and transfersome formulations F1 to F5. (A) Gallic acid reference standard. (B) F1. (C) F2. (D) F3. (E) F4. (F) F5. Detection wavelength: 270 nm. The peak corresponding to gallic acid is indicated by an arrow. The progressive increase in peak area from F1 to F4 and the decrease in F5 reflect differences in gallic acid content across formulations.

Table 6. Particle size characteristics of the optimal transfersome formulation (F4)

Parameter	Value
Mean particle size	10.595 nm
Median particle size (d50)	10.657 nm
Coefficient of variation (C.V.)	32.6%
Polydispersity index (PDI)	< 0.3 (homogeneous distribution)
Skewness	-0.179 (slightly left-skewed)
Kurtosis	-0.728 (platykurtic)
Mean/median ratio	0.963

in this study due to instrument unavailability, and this is acknowledged as a limitation that prevents definitive structural verification of the nanoparticles obtained.

The zeta potential of F4 was -30.5 mV, meeting the threshold of ±30 mV considered indicative of adequate electrostatic stability in colloidal dispersions [10,27]. This value indicates sufficient interparticle repulsion to resist aggregation and maintain suspension stability over time, and is comparable to the -30.4 mV reported by Squittieri et al. [14] for antioxidant-loaded transfersomes prepared by a supercritical CO_2 process.

Table 7. DPPH radical scavenging activity (IC_{50}) of transfersome formulations

Formulation	Lecithin:Tween 80	IC_{50} DPPH (μg/mL, mean ± SD)	Classification
Ascorbic acid (positive control)	—	1.052 ± 0.031	Very strong
F1	90:10	14.306 ± 0.412[a]	Very strong
F2	80:20	12.052 ± 0.358[a]	Very strong
F3	70:30	7.784 ± 0.241[b]	Very strong
F4	60:40	2.652 ± 0.083[c]	Very strong
F5	50:50	5.844 ± 0.197[b]	Very strong

Different superscript letters indicate statistically significant differences among transfersome formulations (Tukey's HSD, $p<0.05$). IC_{50}: half-maximal inhibitory concentration. Classification follows Molyneux [25] as referenced in Munteanu and Apetrei [24]: very strong, IC_{50} < 50 μg/mL.

Table 8. Summary of key performance parameters across all transfersome formulations

Parameter	F1	F2	F3	F4	F5
Lecithin:Tween 80	90:10	80:20	70:30	60:40	50:50
pH	5.41 ± 0.04	5.44 ± 0.05	5.43 ± 0.03	5.46 ± 0.04	5.45 ± 0.05
Viscosity (cps)	131.4 ± 9.1[a]	126.9 ± 2.4[b]	124.6 ± 2.3[b]	123.4 ± 2.2[b]	121.1 ± 2.3[b]
Gallic acid content (mg/L)	5.917	7.269	8.874	45.370	19.462
IC_{50} DPPH (μg/mL)	14.306 ± 0.412[a]	12.052 ± 0.358[a]	7.784 ± 0.241[b]	2.652 ± 0.083[c]	5.844 ± 0.197[b]
Particle size (nm)	—	—	—	10.595	—
Zeta potential (mV)	—	—	—	-30.5	—

Different superscript letters within the same row indicate statistically significant differences (Tukey's HSD, $p<0.05$). Particle size and zeta potential were measured for F4 only.

Antioxidant activity

The DPPH radical scavenging activity of all five transfersome formulations and the positive control (ascorbic acid) is presented in Table 7. The positive control ascorbic acid yielded an IC_{50} of 1.052 ± 0.031 μg/mL. All five formulations demonstrated IC_{50} values below 50 μg/mL, classifying them as very strong antioxidants according to established IC_{50} classification criteria [24, 25].

IC_{50} values decreased progressively from F1 (14.306 ± 0.412 μg/mL) to F4 (2.652 ± 0.083 μg/mL) with increasing Tween 80 concentration, and increased again in F5 (5.844 ± 0.197 μg/mL). One-way ANOVA revealed statistically significant differences in IC_{50} values among formulations ($p<0.05$). Post-hoc Tukey's HSD analysis confirmed that F4 was significantly different from all other formulations ($p<0.05$), while F1 and F2 were not significantly different from each other ($p>0.05$). The non-monotonic pattern, with F4 outperforming both F3 and F5, reflects the balance between adequate bilayer flexibility and structural integrity as a function of edge activator concentration.

Summary of formulation performance

Taken together, F4 (lecithin:Tween 80 = 60:40) demonstrated the optimal combination of physicochemical properties, phenolic encapsulation, and antioxidant activity among all formulations tested (Table 8). F4 was therefore identified as the most optimal formulation and selected for detailed particle characterization and discussion.

Discussion

This study successfully developed transfersome formulations of green Arabica coffee bean ethanolic extract using five systematically varied ratios of soybean lecithin to Tween 80, and identified F4 (60:40) as the optimal formulation based on phenolic encapsulation,

physicochemical stability, and antioxidant activity. The findings are discussed below in the context of the physicochemical mechanisms governing transfersome performance, the interpretation of antioxidant data within a nanocarrier system, and the limitations that define the scope of conclusions that can be drawn from the current dataset.

The pH values of all formulations (5.41 to 5.46) were consistent across varying lecithin:Tween 80 ratios ($p>0.05$), confirming that the edge activator concentration does not alter the acidity of the system. This is an expected outcome, as neither soybean lecithin nor Tween 80 contributes meaningfully to the pH buffering capacity of the PBS-hydrated formulation. The slightly acidic pH is attributable to the phenolic acid content of the Arabica coffee extract and is considered appropriate for skin compatibility, as it falls within the range of 4.5 to 6.5 accepted for transdermal preparations and approximates the physiological pH of the skin surface [13]. The progressive decrease in viscosity from F1 to F5 (131.4 to 121.1 cps) reflects the well-established plasticizing effect of Tween 80 on phospholipid bilayers. As a non-ionic surfactant, Tween 80 intercalates between lecithin molecules, disrupting intermolecular hydrogen bonding and van der Waals interactions within the bilayer and reducing resistance to flow [15, 16]. Although the absolute differences in viscosity were modest and only F1 was statistically distinct from the remaining formulations, all values remained within the accepted range for topical transdermal preparations [26], indicating that the Tween 80 gradient did not compromise flow characteristics relevant to practical application.

The gallic acid content data demonstrated a non-monotonic relationship with Tween 80 concentration, with F4 achieving markedly higher gallic acid content (45.370 mg/L) than all other formulations, followed by a substantial decrease in F5 (19.462 mg/L). This pattern is mechanistically consistent with the dual role of Tween 80 as an edge activator in transfersome systems. At intermediate concentrations, Tween 80 increases bilayer deformability and membrane permeability in a controlled manner, facilitating the incorporation of hydrophilic and amphiphilic phenolic compounds into or between the lipid leaflets of the vesicle [15,16]. At the higher Tween 80 concentration used in F5 (50% of total lipid mass), the bilayer is over-disrupted, transitioning from a structured vesicular system toward mixed micelles or disorganized lipid assemblies that are less capable of retaining encapsulated compounds [16,17]. The abrupt decrease in gallic acid content between F4 and F5 is therefore attributable to increased membrane fluidity and structural destabilization rather than to changes in extraction efficiency or analytical artifacts. A comparable non-monotonic encapsulation pattern with Tween 80 as edge activator has been reported in transfersome systems for other active compounds [16,17,20]. It is noted that gallic acid served as a reference marker rather than the primary active compound of the formulation; the predominant phenolics in Arabica coffee, including chlorogenic acid and ferulic acid [7], are expected to follow the same encapsulation trend, though this should be confirmed using chlorogenic acid as an additional HPLC marker in future work.

The particle size of F4 (10.595 nm mean) is substantially smaller than the 100 to 400 nm range most commonly reported for transfersome systems [10, 20]. This reduction is attributed to the combined application of high-shear homogenization and probe ultrasonication, which together impart sufficient mechanical energy to fragment vesicular structures into the sub-20 nm range. Particles below 100 nm have been associated with favorable skin deposition and deformability characteristics in nanocarrier literature [1,2]. However, the sub-20 nm size range raises the legitimate question of whether the particles retain a classical phospholipid bilayer architecture or represent a heterogeneous population that includes small unilamellar vesicles, micellar structures, or mixed micellar-vesicular intermediates, particularly at the relatively high Tween 80 concentration of F4. In the absence of TEM or cryo-TEM confirmation, which was not feasible with available instrumentation, the structural identity of the particles cannot be definitively established, and this constitutes a recognized limitation of the current study [10,20]. The zeta potential of -30.5 mV meets the conventional threshold of ±30 mV for adequate electrostatic stability of colloidal dispersions [10,27], and is comparable to the -30.4 mV reported by Squittieri et al. [14] for antioxidant transfersomes prepared by supercritical CO_2 processing, suggesting that the surface charge characteristics are consistent with stable transfersome-type systems regardless of preparation method.

The antioxidant activity data revealed a clear and statistically significant non-monotonic pattern across formulations (F1 to F4: decreasing IC_{50}; F5: increased IC_{50}), mirroring the gallic acid content trend and

confirming the mechanistic link between edge activator concentration, encapsulation performance, and radical scavenging activity. F4 demonstrated the lowest IC_{50} (2.652 ± 0.083 µg/mL), which is substantially lower than that of the crude green Arabica coffee ethanolic extract reported in comparable studies [5,9], despite the paradox that encapsulation is generally expected to reduce the immediate availability of antioxidant compounds to react with DPPH radical in solution. This apparent enhancement in IC_{50} performance relative to reported crude extract values may be partly attributable to the high encapsulation efficiency of F4, which concentrates phenolic compounds within the nanoparticle and releases them progressively during the incubation period of the DPPH assay. It has been demonstrated that phenolic compounds encapsulated in lipid nanocarriers may exhibit lower apparent antioxidant activity than their free counterparts in standard in vitro assays due to restricted accessibility of the radical to the encapsulated compound [28]. The IC_{50} values observed in this study therefore likely represent a partial expression of the total antioxidant capacity of the formulation, and the relationship between in vitro DPPH activity and in vivo antioxidant performance following skin delivery cannot be inferred without permeation data.

The absence of a blank transfersome control (formulated without extract) is acknowledged as a limitation of this study, as it prevents formal exclusion of any contribution of soybean lecithin to the measured DPPH activity. Soybean lecithin contains minor amounts of tocopherols and phospholipid oxidation products that possess low intrinsic antioxidant capacity [2]; however, the marked differences in IC_{50} values across formulations with identical lecithin and excipient inputs but varying only in Tween 80 concentration strongly suggest that the observed activity is predominantly attributable to the coffee extract rather than the lipid matrix. Formal confirmation using blank transfersome controls is recommended in future work. Similarly, the absence of accelerated and long-term stability data limits conclusions regarding shelf-life and physical stability of the formulations under storage conditions. Monitoring of particle size, zeta potential, and gallic acid content over time under controlled temperature and humidity conditions would be necessary to assess the practical suitability of F4 for further development [14].

The critical gap in this study is the absence of in vitro skin permeation data. The central premise of a transfersome formulation, namely superior transdermal penetration relative to conventional vesicular systems, has not been experimentally tested in the current work. Franz diffusion cell studies using excised skin membrane would provide direct evidence of whether the deformability conferred by the 60:40 lecithin:Tween 80 ratio translates into measurable permeation enhancement, as has been demonstrated for Tween 80-based transfersome gels loaded with other natural compounds [28]. Without permeation data, the claim that this formulation system improves transdermal delivery of coffee-derived antioxidants remains a mechanistic hypothesis supported by physicochemical characterization rather than experimental demonstration. This represents the most important direction for future research.

Taken together, the results of this study demonstrate that the lecithin:Tween 80 ratio critically governs the physicochemical and functional performance of green Arabica coffee bean transfersomes, and that F4 at a 60:40 ratio represents the optimal balance between bilayer flexibility and structural integrity within the range of formulations investigated. This transfersome nanocarriers formulated from green Arabica coffee bean ethanolic extract contributes a novel application of an abundant Indonesian commodity to the growing body of literature on plant-derived antioxidant nanocarrier systems.

Conclusion

Transfersome formulations of green Arabica coffee bean ethanolic extract were successfully prepared across five lecithin:Tween 80 ratios. Formula F4 (60:40) was identified as optimal, demonstrating the highest phenolic encapsulation (gallic acid content 45.370 mg/L), acceptable nanoparticle characteristics (10.595 nm, -30.5 mV, PDI < 0.3), and strongest antioxidant activity (IC_{50} DPPH = 2.652 ± 0.083 µg/mL). These results support the feasibility of green Arabica coffee bean transfersomes as a topical antioxidant delivery platform. Skin permeation studies, accelerated stability testing, and morphological characterization by TEM are required to confirm the transdermal delivery potential of this formulation.

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Declaration of interest

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Author contributions

Conceptualization: NA; Methodology: NA, AL, YO, TLN, Investigation: AL, YO, TLN; Formal analysis: NA, AL; Data curation: NA, YO, Writing – original draft: NA, AL, YO, TLN; Writing – review & editing: NA; Visualization: AL, YO; Supervision: NA; Project administration: NA.

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