



Formulation and Antibacterial Activity of Gold (Au) Nanoparticles Serum from Green Coffee (*Coffea canephora var. Robusta*) Bean Extract

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ARTICLE INFO

ABSTRACT

Article history:

Received 10 October 2023

Revised 11 December 2023

Accepted 19 September 2024

Published online 01 October 2024

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Serums are skincare products containing concentrated active substances, often used to enhance skin health and manage specific skin conditions. Green coffee (*Coffea canephora var. Robusta*) beans are rich in flavonoids and possess antibacterial activity. Gold nanoparticles are popular in cosmetics due to their ability to enhance skin elasticity and their anti-aging properties. The present study aims to formulate, characterize, and assess the antibacterial activity of green coffee bean extract gold nanoparticle serum. Green coffee beans were extracted by maceration in 96% ethanol. Gold nanoparticles of green coffee bean extract (GCBE) were prepared by mixing green coffee bean ethanol extract (70%), 1400 µL chloroauric acid (HAuCl₄), and 50 µL polyvinyl alcohol following standard procedure. Different formulations of the serum (F1, F2, and F3) were prepared by combining different concentrations of GCBE; 5% (F1), 10% (F2), and 15% (F3) with serum base comprising carbopol, triethyl amine, propylene glycol, propylparaben, methylparaben, and sodium metabisulfite. The formulations were analyzed for their organoleptic properties, pH, viscosity, stability, spreadability, and adhesion. The antibacterial activity was evaluated against *Staphylococcus epidermidis* ATCC 12228 using the disc diffusion method. The resulting formulations F1 (light yellow), F2 (pink), and F3 (light purple) were slightly viscous, homogenous, and had pH values from 5.84 to 5.87. They were stable on storage at room and cold temperatures, evidenced by unchanged organoleptic properties, pH, and viscosity over a 28-day period. All three formulations exhibited antibacterial activity against *Staphylococcus epidermidis*, with F3 exhibiting the highest antibacterial activity with an inhibition zone diameter of 12.6 mm.

Keywords: Antibacterial, *Coffea canephora var. Robusta*, Gold nanoparticles, Natural products, *Staphylococcus epidermidis*.

Introduction

The human skin serves as the primary barrier against environmental stressors. This necessitates adequate skincare to prevent or slowdown various skin conditions such as aging, dryness of the skin, and skin blemishes. Cosmetic products that possess both antioxidant and antibacterial properties are essential in combatting skin conditions such as acne, which is an inflammatory bacterial infection often caused by *Propionibacterium acne*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*.¹

Extensive research has explored the pharmacological and therapeutic potentials and efficacy of herbal remedies.²⁻⁶ Green coffee, derived from unroasted coffee beans (*Coffea canephora var. Robusta*), exhibits higher antioxidant activity compared to its roasted counterpart. This variation may be attributed to the higher concentration of antioxidants, particularly chlorogenic acid in the green coffee extract.^{7,8} In addition, coffee extract contains phenolic and flavonoid compounds that possess antibacterial effects.

For example, research has highlighted the inhibitory effect of ethanol extract of robusta coffee beans on the growth of *Staphylococcus epidermidis* ATCC 12228.⁹

Nanotechnology deals with the design, production, and use of particles at the nanoscale.^{10,11} Gold nanoparticles (AuNPs), a component of nanomaterials, find application in pharmaceutical formulations owing to their low toxicity, inertness, stability, small size, expansive surface area enabling cell targeting, membrane penetrability, and biocompatibility.^{12,13} In cosmetics, these nanoparticles are extensively utilized for their anti-aging properties and ability to enhance skin elasticity.¹⁴ Traditional methods for synthesizing gold nanoparticles, including top-down (physics) and bottom-up (chemistry) approaches, incur high costs and environmental impact.¹⁵ Hence, there is a need for cost-effective and environmentally friendly biosynthetic methods for these nanoparticles.^{12,15} Cosmetics products from natural ingredients has been widely developed in several cosmetic industries. The development of natural ingredients as cosmetics has a disadvantage of poor absorption of active substances into the skin cells. Therefore, modification of the active compounds is needed to improve their absorption. The green synthetic method can be carried out by using natural materials that have active compounds in the form of proteins, amino acids, polysaccharides, flavonoids, phenolic compounds, organic acids, terpenoids, and polyphenols with reducing, and stabilizing properties, and can play a role in oxidation. Apart from their reducing and stabilizing properties, AuNPs have antioxidant, and antibacterial activities, and can improve skin elasticity.¹

Ginseng gold nanoparticles in cosmetic formulations have been shown to effectively inhibit free radicals.¹ Cosmetic serum formulation of bee venom and aloe vera gel without nanoparticle structures was found to

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Citation: Wardani TS, Aziz YS, Wahid RAH, Setianto R. Formulation and Antibacterial Activity of Gold (Au) Nanoparticles Serum from Green Coffee (*Coffea canephora var. Robusta*) Bean Extract. Trop J Nat Prod Res. 2024; 8(9): 8561 - 8570 <https://doi.org/10.26538/tjnpr/v8i9.45>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

exhibit anti-aging properties and contribute to enhanced skin elasticity.¹⁶ Antibacterial investigation of green coffee bean extract showed that the ethanol extract of robusta coffee beans inhibited bacterial growth. Arising from the above, the present study formulated and investigated the antibacterial activity of gold nanoparticle serum derived from green coffee beans. The selection of green coffee beans stemmed from their antioxidant properties, functioning as stabilizers for gold nanoparticles and demonstrating anti-aging and antibacterial properties. Hence, it was proposed that a serum formulation can deliver a high concentration of active drug through the skin, which is critical in inhibiting bacterial growth on the skin.⁹

Gold nanoparticle formulation of green coffee beans expected to enhance user convenience and maximize its potency, which could be further developed into a cosmetic product of choice for acne treatment.

Materials and Methods

Chemicals and equipment

The chemicals used comprised HauCl_4 (Sigma), aquadest, aqua pro injection (PT. Ikapharmindo Putramas), polyvinyl alcohol/PVA (Merck), NaOH (Merck), FeCl_3 powder (Merck), carbopol (Bratoco), triethyl amine/TEA (Bratoco), propylene glycol (Dow Chemical Pacific Singapore Private Ltd), propylparaben (UENO Fine Chemical Industry Ltd. Jepang), methylparaben (UENO Fine Chemical Industry Ltd. Jepang), sodium metabisulfite (Bratoco). The equipment employed in this investigation included a set of laboratory glassware, analytical scales (Mettler Toledo, USA), Particle Size Analyzer/PSA (Horiba Scientific, Nanoparticle Analyzer SZ-100, China), UV-Vis spectrophotometer (Hitachi, Japan), pH meter, viscometer (Brookfield DV-I Prime, USA).

Collection, identification, and preparation of plant material

Green coffee beans (*Coffea canephora* var. *Robusta*) were sourced from the Banaran coffee plantation, Semarang Regency, Central Java, Indonesia, in March 2023. The beans were procured from an elevation ranging between 480 and 600 meters above sea level within the Kebun Getas Afdeling Assinan, situated between Semarang and Surakarta (Solo). The plant material was identified and authenticated at the Research and Development Institute of Medicinal Plants and Traditional Medicines in Tawangmangu, Karanganyar, Central Java. The plant material was assigned a voucher number B2P2TOOT. Robusta green coffee beans were sorted wet, weighed using a digital scale, and then dried in the oven at 40°C. The coffee beans were weighed every 24 h until a constant weight was achieved after three consecutive weighings. The dried beans were pulverized with the aid of an electric blender. The powdered coffee beans were stored in a tightly sealed glass jar and kept at room temperature away from direct sunlight.⁷

Preparation of extract

Powdered green coffee beans (300 g) were extracted by maceration in 96% ethanol (3 L) for 9 h on a shaker, and then allowed to stand for 15 h. The extract was filtered, and the residue was remacerated twice using 1.5 L of 96% ethanol. The combined extract was concentrated using a rotary evaporator at 55°C.⁷

Preparation of gold nanoparticles of green coffee bean extract (GCBE)

Gold nanoparticles were synthesized following the formula shown in Table 1. A 10% solution of GCBE was prepared and transferred into a test tube. Subsequently, 1400 μL of HAuCl_4 solution was dispensed using a micropipette and introduced into the test tube containing the 10% green coffee bean extract, followed by 50 μL of a 0.5% PVA solution. The resulting mixture was subjected to ultrasonication at 30°C, employing 20 pulses over approximately 2 h.¹⁷

Characterization of gold nanoparticles

Visual observation of colour change

Colour changes were observed at 0 h, 15 min, 30 min, 1 h, 3 h, 6 h, and 24 h.¹⁷

Measurement of absorbance

The absorbance of the gold nanoparticles was measured at 650 nm using a UV-Vis spectrophotometer.¹⁷

Particle size analysis

Particle size measurements were conducted using a Particle Size Analyzer (Horiba Scientific, Nanoparticle Analyzer SZ-100). Briefly, a 1 mL volume of the sample was transferred into a cuvette, subsequently positioned within a cell holder for particle measurement. GCBE at a concentration of 10% combined with 0.5 mM of chloroauric acid was used for the analysis.¹⁷

Formulation of green coffee bean gold nanoparticle serum

The preparation of green coffee bean gold nanoparticle serum was done using the formula in Table 2. First, mixture A was prepared by dissolving carbopol in distilled water and allowed to stand for 24 hours until it swells. After swelling, triethyl amine was added. Secondly, mixture B consisting of methyl paraben and propyl paraben dissolved in propylene glycol was prepared. Mixtures A and B were combined, and mixed until homogeneous. Sodium metabisulfite was added, and stirred until homogeneous. After preparing the serum base, gold nanoparticles of green coffee beans were added, and stirred until homogeneous.¹⁸

Determination of the physical characteristics of green coffee bean gold nanoparticle serum

The assessment of the physical characteristics was carried out by storing the preparation at room temperature (15-30°C), and in the refrigerator (2-8°C). Observations were made on days 1, 7, 14, 21, and 28. The physical characteristics of the green coffee bean gold nanoparticle serum assessed include; organoleptic properties, homogeneity, spreadability, adhesion, pH, and viscosity. The organoleptic tests included colour, odour, phase separation, and clarity. The homogeneity test was carried out by applying the serum sample onto a piece of glass or other suitable transparent material.¹⁷ The pH test was performed using a pH meter calibrated with a neutral standard pH buffer (pH 7.01) and an acidic pH buffer (pH 4.01). The electrodes were washed with distilled water and then dried with a handkerchief. The electrode was immersed in the sample until the instrument showed a constant pH value.¹⁷ Viscosity test was performed by placing 100 mL of the preparation in a viscometer tube, the viscometer was turned on, and the speed was adjusted. The stable value obtained was taken as the viscosity of the sample.¹⁷ For the spreadability test, 0.5 g of the test sample was placed on a glass, a load of 50 g was applied, and left for 1 min. The dispersion in centimeters (cm) of the sample was measured. The adhesion test was carried out on the adhesion tester by placing 0.5 g of the sample between two glass objects to which a load of 250 g was applied for 5 min.

Determination of Antibacterial Activity of green coffee bean gold nanoparticle serum

The antibacterial activity of the formulation was evaluated using the disc diffusion method. The antibacterial activity was assessed based on the inhibition zone diameter (IZD) against *Staphylococcus epidermidis* ATCC 12228. Clindamycin (10 mcg) was used as the positive control.

Statistical analysis

Data was analyzed using SPSS version 26. The normality of the data was determined using the Shapiro-Wilk test. Data is normally distributed if $p \geq 0.05$ and were subjected to one-way analysis of variance (ANOVA), whereas, $p < 0.05$ suggest that the data is not distributed normally, and were further analyzed using the Kruskal-Wallis test.

Results and Discussion

Extraction yield and phytochemical constituents of green coffee beans

The percentage yield of green coffee beans was 18.07%, which indicated that the extraction method was efficient. The extract meets the requirements outlined in the Indonesian herbal pharmacopeia which

recommends an extract yield of <9.2% for green coffee bean extract. The solvent used for the extraction (96% ethanol) has a good ability to penetrate the lipophilic and hydrophilic matrix as well as cell membranes to interact with plant metabolites. The moisture content of green coffee beans was found to be 6.14%. Moisture content determination was carried out to determine the amount of water contained in the extract because too high moisture content encourages microbial growth, and this can cause changes in the chemical composition of the extract. The ash content of green coffee bean extract was obtained as 2.21%, which met the requirements outlined in the Indonesian herbal pharmacopeia. Phytochemical screening was conducted to identify the secondary metabolites present in green coffee beans. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and terpenoids in green coffee bean extract. The phytoconstituents of the different formulations (F1, F2, and F3) are shown in Figure 1.

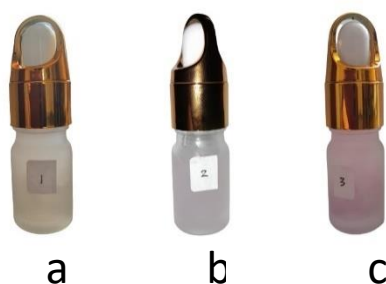


Figure 1: Serum Preparation of Gold Nanoparticles of Green Coffee Bean Extract (a) Formula 1 active ingredient 5%; (b) Formula 2 active ingredient 10%; (c) Formula 3 active ingredient 15%.

Visual characteristics of green coffee bean extract gold nanoparticles
Visual examination was carried out to qualitatively assess the formation of gold nanoparticles. The formation of gold nanoparticles was manifested by a colour change of the solution, transitioning from its initial colour to different shades of yellow, pink, and purple.¹⁹

Ultrasound treatment of the different formulations (F1, F2, and F3) of green coffee bean extract gold nanoparticles at various time intervals; 0 hours, 15 minutes, and 30 minutes did not cause any noticeable colour alterations. However, after 30 minutes, all the formulations assumed a purple shade.

Subsequent observations at 1 hour, 3 hours, 6 hours, and 24 hours indicated that formulation 1 maintained a stable yellow colour without any aggregation. Nevertheless, after a week of storage, aggregation, signified by the presence of black dots, was observed, indicating instability. In contrast, Formulations 2 and 3 exhibited stable gold nanoparticles, displaying pink and purple hues without any aggregation (Table 3). The potential cause of aggregation in gold nanoparticles might stem from the interactions between ions and proteins in the formulation's biological matrix, leading to increased particle size and instability.²⁰ The discoloration of gold nanoparticles of green coffee bean extract is shown in Figure 2.

We argued that gold nanoparticles will aggregate or destabilize if the extract used in the preparation is too concentrated, whereas, less concentrated extract results in no aggregation of the sample. Concentrated extracts may result from prolonged drying in the oven.

UV-Vis spectrophotometric absorption

The formation of gold nanoparticles occurs when a reduction reaction occurs in the presence of plant extract. In this case, the reduction of gold (Au) from Au^{3+} to Au^0 is facilitated by flavonoids which act as bioreductors. The change in colour of the formulation can be used as an indicator of the formation of gold nanoparticles. The formation of gold nanoparticles is considered successful when the colour of the sample solution changes from yellow to pink to purple (depending on the

particle size). In addition, UV-Vis spectrophotometers can also be used to monitor the formation and stability of gold nanoparticles by measuring the absorbance of the sample in the wavelength range of UV (180 – 380 nm) and visible (380 – 780 nm) spectrum. The formation of gold nanoparticles is confirmed by the display of maximum absorption in the wavelength range of 500-550 nm. The stability of gold nanoparticles must be monitored because gold nanoparticles tend to aggregate. The presence of strong interparticle forces in gold nanoparticles causes these particles to fuse and cluster into larger groups over time. However, in the present study, formulations 2 and 3 did not form aggregates on storage. This may be attributed to the addition of polyvinyl alcohol in the synthetic process of the gold nanoparticles. Polyvinyl alcohol functions as a stabilizer of gold nanoparticles to reduce the occurrence of aggregation in gold nanoparticles.¹⁷

The formulations exhibited transmittance values ranging from 90 to 100%, indicating clear and transparent nanoparticles.²¹ High percentage transmittance corresponds to smaller droplet sizes, reflecting an enhanced surfactant efficacy in the emulsification process.²² Table 4 presents the absorption wavelengths and percentage transmittance of gold nanoparticles derived from green coffee bean extract. The values obtained for both wavelength and transmittance of green coffee bean extract gold nanoparticles falls within the expected range, affirming the stability of the formulations.

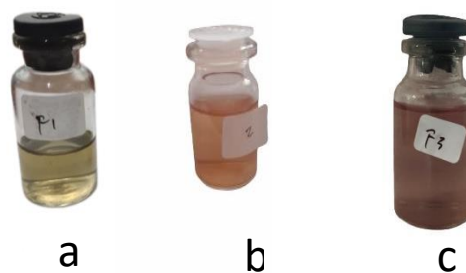


Figure 2: Visual Observation of Gold Nanoparticles of Green Coffee Bean Extract (a) Formula 1; (b) Formula 2; (c) Formula 3

Particle size distribution of green coffee bean extract gold nanoparticles

The particle size distribution of the three formulations is presented in Table 5. In all three formulations, the results showed promising outcomes as they were all within the size range typical for gold nanoparticles, specifically between 1 and 100 nm, accompanied by a polydispersion index value ranging from 0 to 1.2.²⁴

Notably, in Formulation 1, the particle size exhibited the smallest dimension (67.10 ± 16.63 nm) compared to the other formulations. Additionally, the polydispersion index of 0.21 ± 0.07 for formulation 1, denotes a state of polydispersion. This index characterizes the uniformity of size distribution within a nanoparticle system, where a lower polydispersion index value signifies better and more uniform particle size distribution within the sample.²⁵ An acceptable range for polydispersion index is 0 to 1. If the polydispersion index is < 0.7, then the nanoparticle system is monodispersed, while a polydispersion index > 0.7, indicates a polydispersed nanoparticles. An acceptable polydispersion index also indicates the long-term stability of the formulation. Formulation 1 with polydispersion index > 1 was unstable, forming aggregation after 1 week of storage, while formulations 2 and 3 remained stable with no aggregation. The increased polydispersion index in formulation 1 is likely because the aggregates formed are almost the same size or more homogeneous in terms of size. Aggregation occurs if the particles contained in the polydispersion system have opposite charges such that there is incompatibility within the particles.²⁵

Table 1: Formulation of Green Coffee Bean Extract Gold Nanoparticles

Formulation	Green Coffee Bean Extract (μL)	HAuCl_4 Solution (μL)	Polyvinyl Alcohol Solution (μL)
F1	50	1400	50
F2	60	1400	50
F3	70	1400	50

Table 2: Formulation of Green Coffee Bean Extract Gold Nanoparticle Serum

Material	Placebo	F1	F2	F3	Monograph
Gold Nanoparticle (%)	-	5	10	15	Active substance
Carbopol (%)	0.45	0.45	0.45	0.45	Gelling agent
TEA (%)	0.2	0.2	0.2	0.2	pH stabilizer
Propylene glycol (%)	10	10	10	10	Moisturizer
Methylparaben (%)	0.18	0.18	0.18	0.18	Preservative
Propylparaben (%)	0.02	0.02	0.02	0.02	Preservative
Sodium meta bisulfate (%)	0.075	0.075	0.075	0.075	Microbial preservative
Distilled water	to 100	to 100	to 100	to 100	Additional ingredients

Table 3: Visual attributes of Green Coffee Bean Extract Gold Nanoparticles

Formulation	Time (hours)				
	0	1	3	6	24
F1	Yellowish green to purple	purplish	Stable yellow colour, no aggregation	Formed aggregation (black dots)	Formed aggregation (black dots)
F2	Yellowish green to purple	Stable pink colour, no aggregation	Stable pink colour, no aggression	Stable pink colour, and no aggression	Stable pink colour, no aggregation
F3	Yellowish green to purple	Stable purple colour, no aggregation	Stable purple colour, no aggregation	Stable purple colour, no aggregation	Stable purple colour, no aggregation

Table 4: Wavelength and Transmittance of Green Coffee Bean Extract Gold Nanoparticles

Formulation	Wavelength (nm)	Transmittance (%)
F1	650	89.8
F2	650	86.1
F3	650	94.8

Table 5: Particle Size Distribution and Polydispersity Index of Green Coffee Bean Extract Gold Nanoparticles

Formulation (F)	Particle Size (nm)	Polydispersity Index (D)
F1	67.10 \pm 16.63	1.36 \pm 1.99
F2	71.30 \pm 42.00	0.21 \pm 0.07
F3	83.77 \pm 35.93	0.16 \pm 0.17

Physical characteristics of green coffee bean extract gold nanoparticles
Solubility and organoleptic properties of green coffee bean extract gold nanoparticles

The solubility of the extracts is a crucial factor in the formulation of face serums and to improve the solubility of the extracts, co-solvents

such as carbopol are added. Carbopol is preferred due to its ability to increase the solubility of coffee bean extract gold nanoparticles. Additionally, humectants like glycerin were added to enhance skin hydration and aid extract solubility. Improved skin hydration also facilitates the penetration of active ingredients through the stratum

corneum. TEA was added to achieve the desired pH range of 4.5-5.5.¹⁸ The organoleptic properties of the gold nanoparticle serum preparations are documented in Table 6. Evaluation of the organoleptic properties of gold nanoparticle serum preparations derived from green coffee bean extract stored at room temperature and cold temperature revealed sustained stability, absence of phase separation, or formation of crystals, and absence of coarse grains. These observations indicate the homogeneity and fine texture of the serum preparation. However, there was a change in terms of texture. The serum derived from gold nanoparticles of green coffee bean extract produced in this study demonstrated ease of application, uniform distribution upon application to the skin, and rapid drying, which agrees with the requirements for serum preparations.²⁶

The purpose of stability testing on the physical characteristics of preparation is to determine the quality of the product over a long period under the influence of various environmental factors such as temperature and humidity.¹⁷ A cosmetic product is considered stable if the physical characteristics is still within acceptable limits during storage.

In the early stages, the physical quality of the preparation is carried out to determine the stability of the preparation, especially briefly at high temperatures and humidity. The stability of the serum of green coffee bean gold nanoparticles was assessed for 28 days, and monitored weekly. The stability test was carried out at room temperature (15-30°C), and at cold temperature (2-8°C). Based on these results, it can be concluded that the gold nanoparticle serum preparation of green coffee bean extract has good stability. Changes in texture, a potential indicator of diminishing quality, can be influenced by various factors, including fluctuations in temperatures.

pH test of gold nanoparticle serum preparations of green coffee bean extract

Measurement of the pH in the gold nanoparticle serum preparations from green coffee bean extract was conducted on day 1 and replicated three times for each formulation. Figures 3 and 4, and Table 7 show the pH values of the gold nanoparticles derived from green coffee bean extract with values ranging from 5.84 to 5.87. These results indicate that the pH of the serum obtained from the three different formulae conforms to the optimal pH range of the skin (4.5 - 6.5). This suggests that the preparation is safe upon skin application.^{16,27} One of the requirements for cosmetic preparations for skin application is that the pH should not be too acidic or too alkaline. If the pH is too alkaline, it can cause the skin to become dry and sensitive. On the other hand, too acidic pH can cause inflammation of the skin, which can result in acne. Carbopol solutions are stable at pH 6 -11, while at pH less than 3 or more than 12, there is reduced viscosity. The more the carbopol added, the lower the pH, while the fewer the carbopol added, the higher the pH of the formulation. This is because carbopol is a gelling agent that has an acidic pH of 2.5-4.0. The pH of the formulation can also be affected by other components such as triethyl amine (TEA), and ionic contaminants.

The pH charts presented in Figures 3 and 4 show an increase in pH values across cycles 1 to 5 for formulations F1, F2, and F3. However, on completion of the fifth cycle, a decline in the serum's pH value from the initial point was observed. Such fluctuations may arise due to hydrogen ion release or ion contamination within the serum preparations stored at extreme temperatures. Shapiro-Wilk test on the pH values of serum preparation gave a p-value of 0.06 at both room temperature and cold temperature, which means that both treatments have normally distributed pH values. Subsequently, a homogeneity test was carried out using the Levene Variance Homogeneity Test to determine whether the test parameter is homogeneous or not. The results of the homogeneity test at room temperature showed a value of 0.95 and at cold temperature had a value of 0.99, hence, the data could be said to be homogeneous ($p > 0.05$). Results of the One-Way ANOVA suggest that the serum preparation has a stable pH at room temperature and at cold temperature.

Viscosity of green coffee bean extract gold nanoparticle serum

Table 8 illustrates the viscosity of the three formulations of gold nanoparticles derived from green coffee bean extract. The values range from 934.6 to 987.8 cP. These results align with the optimal viscosity

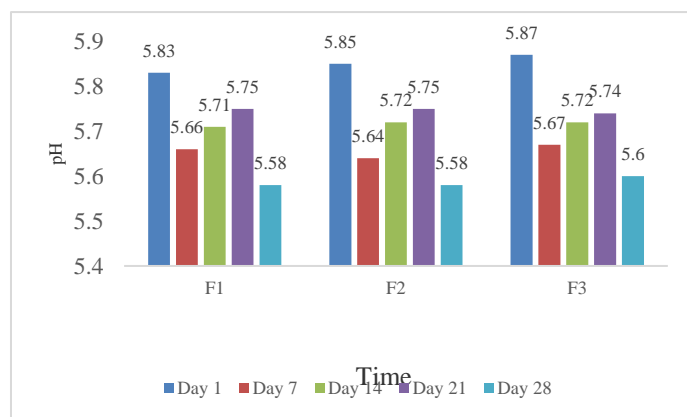


Figure 3: Cold Temperature pH of Green Coffee Bean Extract Gold Nanoparticle Serum.

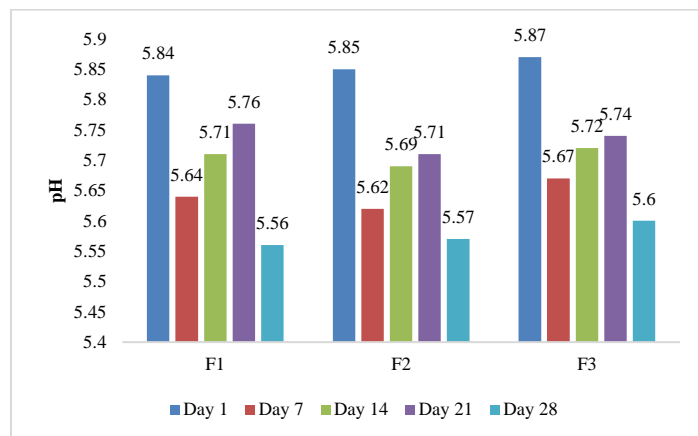


Figure 4: Room Temperature pH of Green Coffee Bean Extract Gold Nanoparticle Serum

range of gel-based serum preparations, typically falling within 800 – 3000 cP.

The viscosity of a product plays a pivotal role in its stability; higher viscosity signifies better stability, albeit making application to the skin more challenging, whereas lower viscosity enhances skin spreadability.³¹

The viscosity chart for the green coffee bean extract nanoparticle serum in Figures 5 and 6 illustrates that, in cycle 5, the viscosity values for F1, F2, and F3 increased at cold temperatures. Conversely, at room temperature, in cycle 5, the viscosity values for F1, F2, and F3 decreased. Such fluctuations in viscosity could be attributed to the influence of temperature on the structure of the polymer base, resulting in a denser or looser consistency, making the green coffee extract serum in cycle 5 thicker than the initial preparation.³² The differences in viscosity values of the three formulations observed at room temperature and cold temperature may be due to the differences in the concentration of active ingredients in the preparations. The viscosity value of the product is influenced by several factors such as changes in temperature, pH, changes in manufacturing conditions, as well as the quality and concentration of raw materials.³² The concentration of the gelling agent in the preparation can increase the viscosity. The use of a high amount of gelling agent increases the viscosity of the preparation, such that the preparation becomes too viscous, making it difficult to spread evenly on the skin.

Shapiro-Wilk test on the viscosity of the preparation resulted in p-values of 0.06, and 0.07 at room temperature, and cold temperature, respectively, which indicate a normally distributed viscosity over the temperature range. The homogeneity test gave p-values of 0.51 and 0.79, at room temperature and cold temperature, respectively, while the One-Way ANOVA indicated that the preparation has a stable viscosity at room temperature and cold temperature.

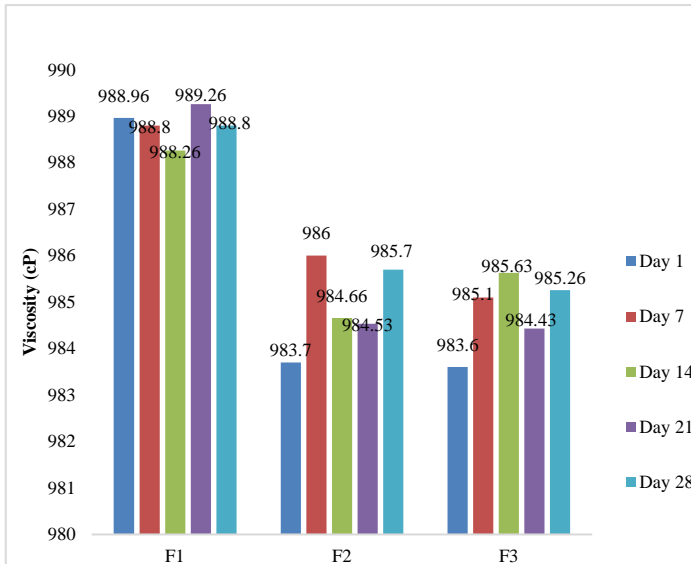


Figure 5: Cold Temperature Viscosity of Green Coffee Bean Extract Gold Nanoparticle Serum

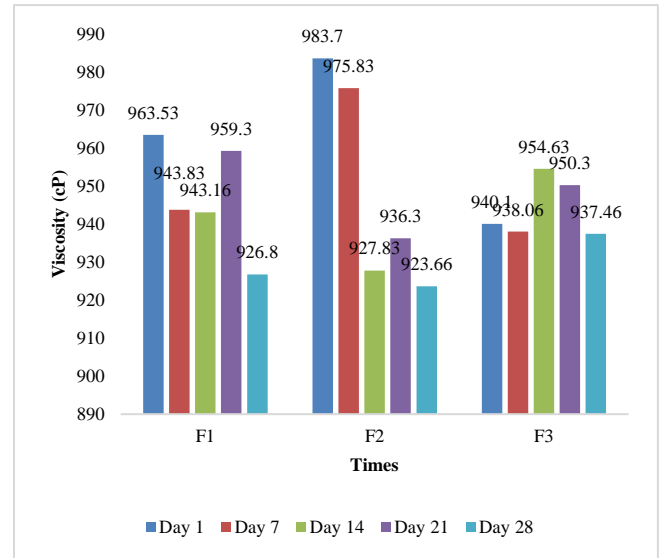


Figure 6: Room Temperature Viscosity of Green Coffee Bean Extract Gold Nanoparticle Serum

Table 6: Organoleptic Properties of Green Coffee Bean Extract Gold Nanoparticle Serum

Formulation	Organoleptic Properties			
	Room temperature (15-30°C)		Cold temperature (2-8°C)	
	Start (Day-1)	End (Day-28)	Start (Day-1)	End (Day-28)
F1	Transparent, light yellow, weak odour, slightly viscous	Transparent, yellow, weak odour, slightly viscous, no crystals form	Transparent, light yellow, weak odour, slightly viscous	Transparent, weak odour, viscous, no crystals form
F2	Transparent, pink, weak odour, slightly viscous	Transparent, pink, weak odour, slightly viscous, no crystals form	Transparent, pink, weak odor, slightly viscous	Transparent, pink, weak odour, viscous, no crystals form
F3	Transparent, light purple, weak odour, slightly viscous	Transparent, light purple, weak odour, slightly viscous, no crystals form	Transparent, light purple, weak odor, slightly viscous	Transparent, light purple, weak odour, viscous, no crystals form

Table 7: pH of Green Coffee Bean Extract Gold Nanoparticle Serum

Formulation	Time	pH	
		Room temperature (15-30°C)	Cold temperature (2-8°C)
F1	Day-1	5.84 ± 0.95	5.83 ± 0.09
	Day-7	5.64 ± 0.27	5.66 ± 0.26
	Day-14	5.71 ± 0.16	5.71 ± 0.15
	Day-21	5.76 ± 0.24	5.75 ± 0.26
	Day-28	5.56 ± 0.17	5.58 ± 0.17
F2	Day-1	5.85 ± 0.08	5.85 ± 0.08
	Day-7	5.62 ± 0.26	5.64 ± 0.25
	Day-14	5.69 ± 0.14	5.72 ± 0.14
	Day-28	5.57 ± 0.16	5.58 ± 0.14
F3	Day-1	5.87 ± 0.09	5.87 ± 0.09
	Day-7	5.67 ± 0.25	5.67 ± 0.25

Day-14	5.72 ± 0.15	5.72 ± 0.15
Day-21	5.74 ± 0.27	5.74 ± 0.27
Day-28	5.60 ± 0.15	5.60 ± 0.15

Values are Mean ± Standard Deviation (SD)

Table 8: Viscosity of Green Coffee Bean Extract Gold Nanoparticle Serum

Formulation	Time	Viscosity (cP)	
		Room temperature (15-30°C)	Cold temperature (2-8°C)
F1	Day-1	963.53 ± 26.90	988.9 ± 0.73
	Day-7	943.83 ± 38.85	988.80 ± 1.83
	Day-14	943.16 ± 23.08	988.26 ± 1.00
	Day-21	959.30 ± 37.23	989.26 ± 0.55
	Day-28	926.80 ± 10.15	988.80 ± 1.34
F2	Day-1	983.70 ± 1.70	983.70 ± 1.41
	Day-7	975.83 ± 20.39	986.00 ± 2.85
	Day-14	927.83 ± 5.64	984.66 ± 1.07
	Day-21	936.30 ± 17.52	984.53 ± 1.44
	Day-28	923.66 ± 6.39	985.70 ± 0.95
F3	Day-1	940.10 ± 13.02	983.60 ± 1.30
	Day-7	938.06 ± 31.65	985.10 ± 2.04
	Day-14	954.63 ± 18.84	985.63 ± 1.25
	Day-21	950.30 ± 21.25	984.43 ± 2.21
	Day-28	937.46 ± 16.35	985.26 ± 1.05

Values are Mean ± Standard Deviation (SD)

Spreadability and adhesion of green coffee bean extract gold nanoparticle serum

The spreadability test was carried out to determine the ability of the preparation to disperse serum nanoparticles when applied on the skin. A good serum gel preparation should have spreadability in the range of 5 - 7 cm.³³ The ability of a preparation to spread is an important characteristic in formulation because it affects the ease of use, and the transfer of active ingredients to the target site in the right dose. The results of the spreadability test showed that there were differences in the spreadability of the formulation at the different storage temperatures (room temperature and cold temperature) on days 1, 7, 14, 21, and 28 (Table 9). All the formulations had spreadability values within the normal range. The slight differences in the values might be due to the viscosity of the preparation. Spreadability is inversely proportional to viscosity; the higher the viscosity of the preparation, the lower the spreadability. Shapiro-Wilk normality test showed a p-value of 0.643 at room temperature and 0.443 at cold temperature, which indicates a normally distributed spreadability over the temperature range. Similarly, the homogeneity test also indicates homogeneous data with p-values of 0.082 and 0.187 at room temperature and cold temperature, respectively. One-Way ANOVA indicated a significant influence of temperature on the spreadability of the preparation ($p < 0.05$).

Adhesion of Green Coffee Bean Extract Gold Nanoparticle Serum

For the adhesion test, results showed that all the preparations had adhesion over 1 second during the 28-day test (Table 10). Although, there are no specific requirements regarding the adhesion of semi-solid preparations, it is recommended that the adhesion of semi-solid preparations should be more than 1 second.³⁴ This showed that the formulations met the requirements for a good gel adhesion. The adhesion of preparation is very closely and directly related to the viscosity, where an increase in adhesion indicates a thicker or more viscous preparation, whereas, a decrease in adhesion indicates a thinner

or less viscous preparation. Serum preparations with longer skin adhesion provide a longer therapeutic effect, because the absorption of drugs through the skin is greater, resulting in optimal therapeutic effect.³⁴

Shapiro-Wilk test showed a normally distributed adhesion over the temperature range with p-values of 0.353 at room temperature and 0.163 at cold temperature. Furthermore, the homogeneity test at room temperature and cold temperature showed p-values of 0.072 and 0.087, respectively, suggesting homogeneous data. Like the spreadability, one-way ANOVA indicates an influence of temperature on the adhesion of the preparation ($p < 0.05$).

Antibacterial activity

The antibacterial activity of green coffee bean extract gold nanoparticle formulations is shown in Figure 5 and Table 9. All three formulations exhibited antibacterial activity by inhibiting the growth of *Staphylococcus epidermidis*. Formulation 3 (F3) demonstrated the most significant antibacterial activity with an inhibition zone diameter of 12.5 mm. Formulations 1 and 2 (F2 and F3) displayed lower antibacterial activity, suggesting a correlation between coffee concentration and antibacterial activity.

The antibacterial activity of the formations can be attributed to the active metabolites like flavonoids, alkaloids, tannins, and saponins present in green coffee beans. Alkaloids have been shown to disrupt the peptidoglycan component of bacterial cell walls, leading to incomplete cell wall formation and subsequent cell death.²⁸ Tannins exert antibacterial effects by interacting with cell membranes, enzyme inactivation, and disruption or inactivation of genetic material. Flavonoids exhibit antibacterial properties by impeding nucleic acid synthesis, interfering with cytoplasmic membrane function, and affecting bacterial energy metabolism. Saponins contain hydrophilic and lipophilic molecules that reduce cell surface tension, ultimately contributing to bacterial destruction.^{35,36}

Table 9: Spreadability of Green Coffee Bean Extract Gold Nanoparticle Serum

Formulation	Spreadability (cm)		
	Time	Room temperature (15-30°C)	Cold temperature (2-8°C)
F1	Day-1	5.63 ± 0.15	5.83 ± 0.09
	Day-7	5.86 ± 0.15	5.66 ± 0.26
	Day-14	5.93 ± 0.15	5.71 ± 0.15
	Day-21	5.83 ± 0.11	5.75 ± 0.26
	Day-28	5.83 ± 0.20	5.58 ± 0.17
F2	Day-1	5.86 ± 0.15	5.85 ± 0.08
	Day-7	5.93 ± 0.15	5.64 ± 0.25
	Day-14	5.83 ± 0.20	5.72 ± 0.14
	Day-21	5.83 ± 0.15	5.93 ± 0.15
F3	Day-28	5.83 ± 0.20	5.83 ± 0.11
	Day-1	5.93 ± 0.20	5.83 ± 0.20
	Day-7	5.93 ± 0.15	5.86 ± 0.15
	Day-14	5.83 ± 0.15	5.72 ± 0.15
	Day-21	5.86 ± 0.05	5.74 ± 0.27
	Day-28	5.96 ± 0.15	5.60 ± 0.15

Values are Mean ± Standard Deviation (SD)

Table 10: Adhesion of Green Coffee Bean Extract Gold Nanoparticle Serum

Formulation	Adhesion (second)		
	Time	Room temperature (15-30°C)	Cold temperature (2-8°C)
F1	Day-1	5.6 ± 0.06	6.0 ± 0.02
	Day-7	5.7 ± 0.03	5.7 ± 0.03
	Day-14	5.9 ± 0.02	5.6 ± 0.06
	Day-21	5.6 ± 0.05	5.6 ± 0.05
	Day-28	5.5 ± 0.06	5.5 ± 0.06
F2	Day-1	5.6 ± 0.05	5.6 ± 0.05
	Day-7	5.7 ± 0.04	5.6 ± 0.01
	Day-14	6.0 ± 0.02	5.8 ± 0.05
	Day-21	5.7 ± 0.03	5.7 ± 0.04
F3	Day-28	5.6 ± 0.06	5.6 ± 0.05
	Day-1	5.8 ± 0.04	5.5 ± 0.06
	Day-7	5.9 ± 0.02	5.6 ± 0.05
	Day-14	5.6 ± 0.01	5.8 ± 0.04
	Day-21	5.8 ± 0.05	5.9 ± 0.02
	Day-28	5.7 ± 0.04	5.6 ± 0.01

Values are Mean ± Standard Deviation (SD)

Table 11: Antibacterial Activity of Green Coffee Bean Extract Gold Nanoparticle Formulations

Storage	Treatment	Inhibition Zone Diameter (mm)			Mean (mm)
		Replicate			
		I	II	III	
Cold temperature	Control +	20.0	20.0	20.0	20.0
	F0	0	0	0	0
	F1	9.3	9.2	9.3	9.27
	F2	10.1	10.3	10.2	10.2
	F3	12.7	12.6	12.7	12.67
	Control +	20.0	20.0	20.0	20.0

Room temperature	F0	0	0	0	0
	F1	9.3	9.2	9.5	9.33
	F2	10.1	10.2	10.2	10.17
	F3	12.7	12.2	12.7	12.53

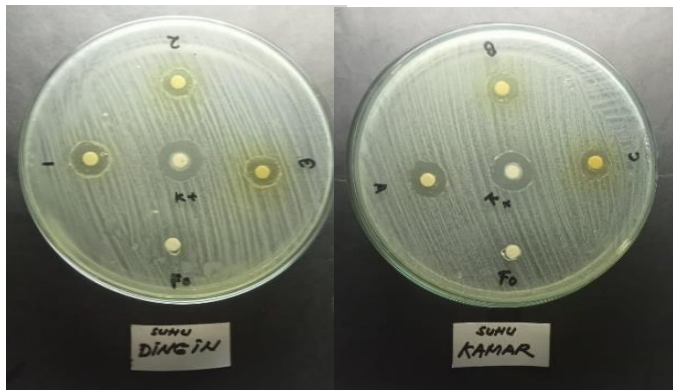


Figure 7: Antibacterial Activity of Green Coffee Bean Extract Gold Nanoparticle Serum

Conclusion

A face serum has been prepared using active gold nanoparticles of green coffee (*Coffea canephora* var. Robusta) bean extract. The most optimal formulation contains 70% ethanol extract of green coffee beans, 1400 μ L H₂O, and 50 μ L polyvinyl alcohol. The formulations have a mild, non-greasy, semi-transparent, brittle, and homogeneous texture. The pH of the serum was between 5.84 – 5.87, the viscosity varied between 800 – 3000 cP, and the spreadability ranged from 5.60 - 5.93 cm, resulting in a stable serum preparation. Formulation F3 consisting of 15% Robusta green coffee bean extract exhibited the highest antibacterial activity against *Staphylococcus epidermidis*.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors would like to acknowledge Universitas Duta Bangsa Surakarta for their facility and financial support; under grant number 050/UDB.LPPM/A.34-HK/VIII/2023).

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