

Formulation of Gold (Au) Nanoparticles Serum Containing Sunflower (*Helianthus annuus* L.) seed extract from Indonesia as an antibacterial: in vitro and skin irritation assessments

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Abstract

Serum is a cosmetic preparation containing active ingredients and is widely used to maintain skin health. Sunflower (*Helianthus annuus* L.) seed extract contains flavonoid compounds that can reduce and stabilize gold ions into nanoparticles, offering a green synthesis approach with potential antibacterial applications. This study aimed to formulate a gold nanoparticle serum synthesized using sunflower seed extract and to evaluate its physicochemical characteristics and antibacterial activity. Sunflower Seed Extract (SFSE) was obtained through maceration using 96% ethanol. Gold nanoparticles derived from the SFSE were synthesized by combining 70% ethanolic SFSE with 1400 μ L of chloroauric acid (HAuCl_4) and 50 μ L of polyvinyl alcohol, following a modified standard protocol. Three serum formulations (F1, F2, and F3) were then produced by incorporating SFSE at varying concentrations: 1,525% for F1, 1,650% for F2, and 1,775% for F3 into a serum base consisting of carbopol, triethylamine, propylene glycol, propylparaben, Na benzoate, glycerin, and EDTA. Each formulation was assessed for organoleptic characteristics, pH, viscosity, spreadability, homogeneity, and irritation. Antibacterial activity was examined against *Staphylococcus aureus* ATCC 25923 using the disc diffusion technique.

The characterization of the gold nanoparticles showed absorbance values of 0.948, 1.049, and 1.422 at wavelengths of 493, 521, and 559 nm, respectively. The particle sizes obtained for each formulation were 276.2 nm, 661.1 nm, and 897.2 nm. The finalized preparations showed distinct appearances: F1 was purple, F2 was light purple, and F3 was light purple. All exhibited slight viscosity, uniform texture, and pH values ranging from 5.54 to 5.77. All formulations demonstrated inhibitory activity against *Staphylococcus aureus*, with F3 producing the strongest response, as reflected by an inhibition zone measuring 12.6 mm. The results indicated that the gold nanoparticles serum exhibits excellent stability, significant antibacterial activity, and is safe for topical application, with no irritant effects observed during skin irritation assessments.

Keywords: Gold nanoparticles; Serum; Sunflower seed extract; Antibacterial; *Staphylococcus aureus* ATCC 25923.

1. Introduction

Acne vulgaris is one of the most prevalent skin disorders worldwide and is associated with excessive sebum

production, follicular hyperkeratinization, inflammation, and bacterial colonization. Among the microorganisms involved in skin infections, *Staphylococcus aureus* has

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been reported to contribute to the aggravation of acne lesions and other skin disorders. In addition to causing physical discomfort, acne may negatively affect an individual's appearance and self-confidence [1]. Therefore, the development of effective antibacterial skincare products remains an important area of research.

Recent advances in nanotechnology have created new opportunities for developing innovative pharmaceutical and cosmetic products. Nanotechnology involves the design, characterization, and manipulation of materials at the nanoscale, typically below 100 nm, to improve their physicochemical and biological properties [2]. Due to their unique characteristics, nanoparticles have attracted considerable attention for biomedical and cosmetic applications, including drug delivery and antimicrobial formulations. Among various nanomaterials, gold nanoparticles (AuNPs) have demonstrated significant potential due to their excellent biocompatibility, low toxicity, and ability to enhance the delivery of bioactive compounds [3].

Cosmetic products function to protect the skin and modulate its biological activities through the active ingredients they contain [4]. Among cosmetic formulations, serums are widely used to address skin concerns [5]. Serums are particularly appealing for cosmetic applications because they contain higher concentrations of active compounds, making them quicker and more effective at improving skin conditions [6]. Furthermore, incorporating active ingredients into nanoparticle-based serum formulations may enhance their stability, skin penetration, and therapeutic efficacy.

Natural products have attracted increasing interest as sources of bioactive compounds for antibacterial applications. Sunflower (*Helianthus annuus* L.) is a plant known to contain a wide range of biologically active constituents. Previous studies have reported that sunflower seeds contain proteins, fatty acids, phenolic compounds, flavonoids, alkaloids, phytosterols, and various oils that contribute to their biological activities [7-9]. In addition, several phenolic compounds, including caffeic acid, chlorogenic acid, and dicaffeoylquinic acid, have been identified in sunflower seeds and are recognized for their antioxidant and other beneficial biological properties [10-12]. Previous studies have demonstrated that sunflower seed extracts possess antibacterial activity against both Gram-positive and

Gram-negative bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi* [12-13].

These findings suggest that sunflower seed extract may serve as a valuable natural source for the development of novel antibacterial formulations. Furthermore, the use of gold nanoparticles as carriers of plant-derived bioactive compounds may enhance their stability, bioavailability, and antibacterial activity. In the present research, gold nanoparticles will be incorporated with sunflower seed oil extract (*Helianthus annuus* L.) and formulated into a serum preparation intended for antibacterial application.

In the present study, gold nanoparticles (AuNPs) are incorporated with Indonesian sunflower seed extract (*Helianthus annuus* L.) and formulated into a serum preparation intended for antibacterial application. To the best of our knowledge, no previous study has reported the formulation of a gold nanoparticle serum containing Indonesian sunflower seed extract and simultaneously evaluated its antibacterial activity against *Staphylococcus aureus* ATCC 25923 and its skin irritation profile in human volunteers. Therefore, this study aims to develop and characterize a gold nanoparticle serum containing Indonesian sunflower seed extract and to evaluate its antibacterial activity in vitro and its skin safety through a human skin irritation assessment.

2. Materials and Methods

2.1. Chemicals and equipment

The tools and materials used in this research were a collection of glassware, analytical scales (Mettler Toledo), Particle Size Analyzer (PSA/HORIBA), UV-Visible spectrophotometer (HORIBA), ultrasonic (BRASON), pH meter, Brookfield Viscometer DV-I Prim, a magnetic stirrer, Viscometer, (*Helianthus annuus* L.) pure gold powder (Sigma), carbopol (Merck), propylene glycol (Merck), propylparaben (Merck), TEA (Merck), glycerin (Merck), EDTA (Merck), Na Benzoate (Merck), ethanol 96% (Merck), aquadest, and Nutrient Agar (Merck),

2.2. Collection, identification, and preparation of plant material

Sunflower Seeds Extract (SFSE) was collected in March from Semarang, Central Java, Indonesia. The plant material

was identified and verified 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. Wet sorting and oven drying of 2500 g of sunflower seeds were carried out. The dried material was then ground and sieved using a 60-mesh sieve [14]. The resulting simplicia powder was stored at room temperature in a container protected from direct sunlight [15].

2.3. Ethical Approval for Clinical Studies

This study was approved by the Health Research Ethics Committee of Dr. Moewardi Regional General Hospital, Surakarta, Indonesia (Approval No. 1.097/IV/HREC/2024; approved on May 5, 2024). The study was conducted in accordance with the Declaration of Helsinki and the ICH-GCP guidelines.

2.4. Sunflower Seeds Ethanol Extract (SFSE).

Sunflower seeds were finely ground and macerated. 500 grams of sunflower seed powder was soaked in 5 liters of 96% ethanol for 3 days, with gentle stirring every 8 hours. After the maceration period, the mixture was filtered to remove solid residues [16]. The filtrate was then concentrated using a rotary evaporator at 40°C until a thick ethanolic extract was obtained [15]. The extraction yield was calculated using the following equation:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried raw material}} \times 100\%$$

2.5. Preparation of 0.5 mM Gold Solution (HAuCl₄)

A 0.5 mM HAuCl₄ solution was prepared by dissolving 0.0493 g of gold powder in 4 mL of aqua regia, then

transferring the mixture to a 500-mL beaker. The solution was then heated to reduce its volume. After cooling, it was diluted with distilled water to a final volume of 500 mL and thoroughly homogenized [17].

2.6. Synthesis of Gold Nanoparticles (Au) Using a High-Energy Method with Sunflower Seed (*Helianthus annuus* L.) Ethanolic Extract as a Bioreductor and 0.5 mM HAuCl₄ Solution

Prepare 0.125 mL of sunflower seed extract (*Helianthus annuus* L.) and transfer it into a container. Add 1400 µL of 0.5 mM HAuCl₄ solution, then synthesize the mixture on a hotplate for 25 minutes. After this step, subject the solution to ultrasonication for 15 minutes at 30°C. The solution will gradually turn purple, indicating the formation of nanoparticles. The experiments were conducted in triplicate, using three different extract volumes: 125 µL, 250 µL, and 375 µL. The formulation was adapted from the previous study by Vellayanti [18] and modified using different extract volumes and concentrations, in accordance with the original methodology. The wavelength and absorbance results of gold nanoparticles synthesized using sunflower seed extract (*Helianthus annuus* L.) from the three formulations in this study are presented in **Table 1** below.

2.7. Characterization of Gold Nanoparticles

2.7.1. Visual Observation of Color Changes

Visual observation of the color change in gold nanoparticles can be performed by monitoring the color transition during synthesis. Under the specified experimental conditions, the initially clear, yellowish solution gradually turns purple, indicating the formation of gold nanoparticles [19].

Table 1. Formulation of Nanoparticle Synthesis

Formulation	Sunflower Seed Extract (<i>Helianthus annuus</i> L.) (µL)	Gold Solution HAuCl ₄ 0.5 mM (µL)	Time (minutes)	Suhu (Celcius)
F1	125	1400	15	30
F2	250	1400	15	30
F3	375	1400	15	30

2.7.2. Observation of UV-Vis Absorption Wavelengths.

The absorption wavelength of the synthesized gold nanoparticles can be measured using UV-Vis spectrophotometry. Upon nanoparticle formation, the characteristic surface plasmon resonance (SPR) band typically appears in the 400–600 nm wavelength range [20].

2.7.3. Observation of Particle Size Measurement Using a Particle Size Analyzer (PSA).

Particle size measurements can be performed using a particle size analyzer (Horiba Scientific, Nanoparticle Analyzer SZ- 100) [21]. A 5 mL sample was collected and placed into a cuvette, which was then inserted into the cuvette holder for particle size measurement. The particle size analysis was conducted on 0.5 mM gold nanoparticles synthesized using ethanol extract of sunflower (*Helianthus annuus* L.) seeds. Particle measurement was performed at the point of nanoparticle formation. The particle size determination aimed to evaluate differences in particle size formed after the synthesis process [22].

2.8. The Preparation of Gold Nanoparticle Serum from Sunflower Seed Extract (*Helianthus annuus* L.)

The serum formulation was prepared by heating. The required materials and equipment were assembled prior to processing [23]. A total of 40 mL of distilled water was weighed and heated in a water bath at 70–80 °C. Separately, Disodium EDTA was weighed, dissolved in 20 mL of distilled water, and stirred until completely homogeneous, then incorporated into the heated distilled water. Propyl paraben was subsequently added and mixed thoroughly to obtain solution A. Carbopol and TEA were weighed and dissolved in 10 mL of distilled water, stirred

until fully dispersed, and then added to the Disodium EDTA–propyl paraben mixture. The solution was stirred until uniform, forming solution B. Glycerin and propylene glycol were then added to solutions A and B, and the mixture was continuously stirred until homogeneity was achieved. Sodium benzoate was weighed separately, dissolved in 10 mL of distilled water, and stirred until homogeneous. The solution was added gradually to the mixture while stirring continuously to maintain uniformity. Finally, the gold nanoparticles derived from SFSE were incorporated into the serum base and mixed until a fully homogeneous preparation was obtained. The serum formulation is presented in [Table 2](#).

2.9. Evaluation of sunflower seed extract (*Helianthus annuus* L.) Gold Nanoparticle Serum Preparations

2.9.1. Organoleptic and Homogeneity Analysis

The organoleptic evaluation of the fig leaf gold nanoparticle serum was conducted through visual inspection of its appearance, color, and odor. Furthermore, homogeneity was assessed by spreading a small amount of the serum onto a glass slide or another suitable transparent surface and examining it for the presence of any visible particles, aggregates, or phase separation [24].

2.9.2. pH Measurement

The pH of the formulation was measured using a pH meter calibrated with standard buffer solutions at neutral (pH 7.01) and acidic (pH 4.01) conditions. Prior to measurement, the electrode was rinsed with distilled water, dried carefully, and subsequently immersed in the serum sample until a stable reading was obtained. The constant value displayed by the instrument was documented as the pH of the preparation [24].

Table 2. Serum Formulation Composition

Ingredient	Placebo	F1	F2	F3	Monograph
Gold nanoparticles (%)	–	1.525%	1.650%	1.775%	Active ingredient
Carbopol (%)	0.5%	0.5%	0.5%	0.5%	Thickening agent
Propylene glycol (%)	1%	1%	1%	1%	Humectant
Propyl paraben (%)	0.2%	0.2%	0.2%	0.2%	Preservative
Sodium benzoate (%)	0.5%	0.5%	0.5%	0.5%	Preservative
TEA (%)	1%	1%	1%	1%	Emulsifier
Glycerin (%)	0.5%	0.5%	0.5%	0.5%	Humectant
EDTA	0.1%	0.1%	0.1%	0.1%	Chelating agent
Distilled water ad	100	100	100	100	Solvent

2.9.3. Viscosity Test

Viscosity was evaluated by placing 100 mL of the serum formulation into a cylindrical vessel and installing an appropriate spindle on the viscometer. The spindle was fully immersed in the sample prior to instrument activation, after which the rotational speed was adjusted to the required setting. A stable reading from the viscometer was then recorded as the formulation's viscosity [24].

2.9.4. Daya sebar

A total of 0.5 g of serum was placed at the center of a glass plate and covered with another glass plate. A predetermined load was applied for 1 min, after which the diameter of the spread sample was measured in two perpendicular directions. The average spreadability diameter was calculated using the following equation:

$$D = \frac{d_1 + d_2}{2}$$

where D is the average spreadability diameter (cm), d_1 is the horizontal diameter, and d_2 is the vertical diameter of the spread sample measured after applying the specified load

2.9.5. The irritation test of the sunflower seed extract (*Helianthus annuus* L.) Gold Nanoparticle

A skin irritation test was conducted to evaluate the dermal compatibility of the developed gold nanoparticle serum formulations containing sunflower (*Helianthus annuus* L.) seed extract. The study involved 20 healthy volunteers aged 18–50 years. Skin compatibility was defined as the absence of adverse skin reactions under normal use conditions, including both objective signs (erythema and edema) and subjective symptoms (stinging, burning, or itching) [25]. The inclusion criteria comprised healthy individuals who were not using other cosmetic products on the test area, were willing to apply the test formulation according to the study protocol, and provided written informed consent. Exclusion criteria included acute or chronic skin diseases, dermatological disorders, sensitive skin, known hypersensitivity to any component of the test formulation, and pregnancy or breastfeeding. The serum formulations were applied to the participants' upper backs under occlusive patches and

maintained for 48 h. Skin reactions were evaluated at 1, 24, and 48 h following patch application. Erythema and edema were visually assessed and graded according to severity. The Primary Irritation Index (PII) was calculated by dividing the total erythema and edema scores by the number of grading intervals. This study was approved by the Health Research Ethics Committee of Dr. Moewardi Regional General Hospital, Surakarta, Indonesia (Approval No. 1.097/IV/HREC/2024; approved on May 5, 2024), and was conducted in accordance with the principles of the Declaration of Helsinki [25].

Eligible participants consisted of healthy individuals aged 18–50 years who were not applying other cosmetic products on the test site, agreed to follow the application instructions, and permitted regular observation of the treated area according to the study protocol. Volunteers were excluded if they had acute or chronic dermatological disorders, sensitive skin, known hypersensitivity to the product components, or if they were pregnant or lactating. For the evaluation, serum was applied to the dorsal skin under occlusive patches for 48 hours, followed by examinations at 1, 24, and 48 hours post-application. The presence and degree of erythema were visually graded, while redness intensity was also quantified instrumentally. The Primary Irritation Index (PII) was then computed by dividing the cumulative erythema and edema score by the number of observation intervals [25]. The comprehensive assessment outcomes are displayed in [Table 3](#).

Table 3. Average Irritation Index [26]

Average Irritation Index	Category
0	No erythema or edema
1	Slight erythema
2	Well-defined erythema
3	Moderate erythema with edema
4	Severe erythema with edema

The Primary Irritation Index (PII) was calculated by dividing the total erythema and edema scores recorded during the observation period by the total number of observations. The PII was determined using the following equation:

$$PII = \frac{\sum \text{Erythema} + \text{Edema}}{\text{Number of observations}}$$

The obtained PII value was subsequently used to classify the formulation's irritation potential.

2.10. Determination of Antibacterial Activity of Sunflower Seed (*Helianthus annuus* L.) Gold Nanoparticle Serum

The antibacterial efficacy of the serum formulation was investigated using the disc diffusion assay. Antimicrobial performance was determined by measuring the inhibition zone diameter (IZD) formed against *Staphylococcus aureus* ATCC 25923. Clindamycin (10 µg) served as the reference (positive) control for comparative evaluation.

2.11. Statistical analysis

Prior to the comparative analysis, the data were assessed for normality using the Shapiro–Wilk test, as each formulation was evaluated with a limited number of replicates ($n = 3$). The results indicated that the data were normally distributed ($p > 0.05$), thereby satisfying the assumptions required for parametric analysis. Consequently, one-way ANOVA was employed to determine whether significant differences existed among the formulations. Since the ANOVA results revealed statistically significant differences ($p < 0.05$), a Tukey HSD post hoc test was subsequently performed to identify the specific formulation pairs responsible for these differences. The use of Tukey HSD allowed a more detailed interpretation of the effect of sunflower seed extract concentration on the physicochemical properties of the gold nanoparticle serum formulations.

3. Results and Discussion

3.1. Extraction yield and phytochemical constituents of Sunflower Seed

The SFSE yielded 16.6%, indicating that the extraction process effectively recovered soluble constituents from the plant material. The relatively high yield may be attributed to the extraction efficiency of 96% ethanol, which is capable of extracting a broad range of phytochemical constituents. The use of 96% ethanol as the extraction solvent was appropriate, as it efficiently penetrates both lipophilic and hydrophilic matrices and cell membranes, enabling effective interaction with plant metabolites. Phytochemical screening was performed to identify the secondary metabolites present in the

sunflower seed extract. The analysis confirmed the presence of alkaloids, phenolics, flavonoids, tannins, saponins, and terpenoids. The phytoconstituents of the different formulations (F1, F2, F3, and Placebo) are shown in **Figure 1**.



Figure 1. Gold Nanoparticle Serum Preparation.

3.2. Visual characteristics of Sunflower Seed extract gold nanoparticles.

Auric chloride, whose concentration was reduced by dilution using 4 mL of aqua regia and subsequently adjusted with 500 mL of distilled water through a heating process at 70 °C. Visual observation of the gold nanoparticles was conducted to qualitatively assess the color change associated with nanoparticle formation, characterized by a purple hue (Wildan, 2019). In this study, formulations 1-3 showed evidence of gold nanoparticle formation, as indicated by a color transition to purple. The color change of the gold nanoparticles synthesized using the ethanol extract of sunflower seeds (*Helianthus annuus* L.) is presented in **Figure 2**.



Figure 2. Visual characteristics of Sunflower Seed extract gold nanoparticles.

The results above confirm the formation of gold nanoparticles. The sunflower seed (*Helianthus annuus* L.) bioreductor contains oil, as reported in a previous study by Aisyah Meisya Putri (2020), which stated that sunflower seeds are the second-largest source of vegetable oil globally. The presence of flavonoid compounds in sunflower seeds enables them to function as bioreductors, facilitating the reduction of Au^{+3} ions to Au^0 nanomaterial. However, the oil content in sunflower seeds (*Helianthus annuus* L.) significantly influences the size of the resulting nanoparticles, leading to a turbid solution. Consequently, the synthesis process produces an emulsion characterized by the dispersion of oil droplets within the aqueous phase (O/W). The nanoparticles are considered unstable or aggregated when the solution contains black specks. This instability is affected by several factors, including the volume of the bioreductor and the temperature. Excessively concentrated bioreductors may result in an unstable solution, and similarly, low temperatures can decrease the system's stability [18].

3.3. UV-Vis spectrophotometric absorption

A UV-Vis spectrophotometer is an analytical instrument used to monitor the formation time of gold nanoparticles [18]. The formation of gold nanoparticles is indicated by a visible color change from pale yellow to purple [27]. In this study, the UV-Vis spectrophotometric absorbance values for all three formulations were within the range of 400–600 nm, with the expected absorbance for gold nanoparticle formation falling between 0.2 and 1.2 [28]. This phenomenon occurs because nanoparticle absorbance increases proportionally with storage time; the longer the storage time, the higher the sample's absorbance [18]. The wavelength and absorbance results of gold nanoparticles synthesized using sunflower seed (*Helianthus annuus* L.) extract from the three formulations in this study are presented in Table 4 below:

Based on UV-Vis spectrophotometric observations, formulation 3 exhibited a markedly high absorbance, exceeding the typical absorbance range for gold nanoparticles (0.2–1.2). (Vellayanti, 2020). The absorbance of gold nanoparticles synthesized using sunflower seed (*Helianthus annuus* L.) extract is influenced by the amount of bioreductor added during

the synthesis process. Bioreductors containing oil tend to increase the nanoparticles' absorbance.

Table 4. Absorbance Results of Gold Nanoparticles Synthesized from Sunflower Seed Extract.

Formula (F)	Volume of Sunflower Seed Extract (<i>Helianthus annuus</i> L.) (μL)	Volume of HAuCl_4 (μL)	Wavelength (nm)	Absorbance
1	125	1400	493	0.948
2	250	1400	521	1.049
3	375	1400	559	1.422

3.4. Observation of Gold Nanoparticles Synthesized Using Sunflower Seed (*Helianthus annuus* L.) Extract as a Bioreductor via Particle Size Analyzer (PSA)

Particle size analysis of gold nanoparticles synthesized using sunflower seed (*Helianthus annuus* L.) extract as a bioreductor was conducted using a Particle Size Analyzer to determine nanoparticle size. The particle size values and formulation index values are presented in Table 5 below :

Table 5. Particle Size and Polydispersity Index Values.

Formula (F)	Volume Ekstrak (μL)	Volume HAuCl_4 0,5 Mm (μL)	Ukuran Partikel (nm)	Polidispersi Indeks (Đ)
F1	125	1400	276.2	0.188
F2	250	1400	661.1	0.860
F3	375	1400	897.2	0.678

Based on the particle size analysis, the synthesized gold nanoparticles exhibited hydrodynamic diameters ranging from 276.2 to 897.2 nm, which are larger than the conventional nanoparticle size range of 1–100 nm [29]. Nevertheless, these values remain within the colloidal size range commonly reported for phytochemical-mediated nanoparticle systems. The relatively large particle size may be attributed to the adsorption of bioactive compounds present in sunflower seed extract onto the nanoparticle surface. Particle size

measurements obtained by dynamic light scattering (DLS) represent the hydrodynamic diameter, which includes not only the metallic gold core but also the surrounding solvation layer and adsorbed phytochemicals. Consequently, the measured particle sizes may be substantially larger than the actual dimensions of the gold nanoparticle cores.

Among the three formulations, F1 exhibited the smallest particle size (276.2 nm), whereas F2 and F3 showed progressively larger particle sizes of 661.1 and 897.2 nm, respectively. The increase in particle size with increasing extract volume suggests that higher concentrations of phytochemical constituents influenced nanoparticle growth and stabilization. In addition to flavonoids, which are considered the primary reducing agents responsible for converting Au^{3+} to Au^0 , sunflower seed extract contains other bioactive compounds that may function as capping and stabilizing agents. At higher extract concentrations, these compounds may form thicker organic layers around the nanoparticles, leading to interparticle bridging or aggregation and larger hydrodynamic diameters. These findings indicate that the extract-to-gold precursor ratio plays a key role in determining nanoparticle size and colloidal properties.

The polydispersity index (PDI) values obtained for F1, F2, and F3 were 0.188, 0.860, and 0.678, respectively. The PDI reflects the uniformity of the particle size distribution within a nanoparticle system; lower values indicate greater homogeneity. F1 demonstrated the most uniform particle distribution, as evidenced by its low PDI value (<0.3), suggesting a relatively monodisperse system. In contrast, F2 exhibited a PDI value greater than 0.7, indicating a broad particle size distribution and a higher degree of heterogeneity [30]. Although F3 showed a lower PDI value than F2, its value remained substantially higher than that of F1, indicating a less uniform particle population. A tendency was observed whereby formulations with larger particle sizes also exhibited higher PDI values, suggesting that increasing extract concentration not only promoted particle growth but also reduced the uniformity of nanoparticle formation. Therefore, F1 was considered the optimal formulation, exhibiting both the smallest particle size and the most homogeneous particle size distribution among the tested formulations.

Although the synthesized particles exceeded the

conventional nanoparticle size range, the results confirm the successful formation of phytochemical-stabilized gold colloids. Further optimization of the extract concentration and synthesis parameters may be required to obtain smaller particle sizes and narrower particle size distributions suitable for biomedical and pharmaceutical applications.

3.5. Physical Quality Evaluation of Gold Nanoparticle Serum Using Sunflower Seed (*Helianthus annuus* L.) Extract as a Bioreductor

3.5.1. The Organoleptic Test

The organoleptic evaluation of the gold nanoparticle serum formulated with sunflower seed (*Helianthus annuus* L.) extract was conducted visually by assessing its appearance, color, and odor.

The color produced by each formulation of the gold nanoparticle serum ranged from transparent purple to purple, with variations among formulations. Formulation 0, which served as the placebo/basic serum formulation prior to extract incorporation, had a slightly cloudy, transparent, white color. Formulation 1 (1.525% sunflower seed gold nanoparticles) displayed a faint purple color, formulation 2 (1.650% sunflower seed gold nanoparticles) exhibited a light transparent purple color, and formulation 3 (1.755% sunflower seed gold nanoparticles) presented a clear transparent purple color. The differences in serum color intensity were attributed to the varying amounts of gold nanoparticles synthesized from sunflower seed (*Helianthus annuus* L.) extract incorporated into each formulation. The greater the concentration of nanoparticles added, the more intense the resulting purple coloration. The gold nanoparticle serum exhibited a characteristic odor derived from the extract used in the formulation, and its consistency was slightly viscous.

3.5.2. pH, Viscosity, Homogeneity, and Spreadability of Serum Formulations ($n=3$) Test

The homogeneity test was performed by applying the serum sample onto a glass plate or another suitable transparent surface, followed by tactile assessment through gentle rubbing. This outcome is attributed to the materials used, which underwent dilution and were synthesized using a bioreductor containing flavonoid compounds that reduced Au^{3+} to Au^0 , resulting in smaller particles (Table 6).

Table 6. pH, Viscosity, Homogeneity, and Spreadability of Serum Formulations (n=3).

Formulation	pH (mean)	Viscosity (mean)	Homogeneity	Spreadability (mean)
F1	5.77±0,30	841.333±0,06	Homogeneous	38.6±0,30
F2	5.64±0,05	1037.333±0,05	Homogeneous	44.9±0,30
F3	5.54±0,01	1155.000±0,04	Homogeneous	30.8±0,30

Although one-way ANOVA revealed statistically significant differences among the formulations ($p = 0.043$), the observed pH differences were relatively small, ranging from 5.54 to 5.77. Importantly, all formulations remained within the acceptable pH range for topical facial preparations (4.5–6.5) [31]. Therefore, these differences are unlikely to be clinically significant and are not expected to adversely affect skin compatibility, product safety, or user comfort during application. The slight decrease in pH observed with increasing concentrations of sunflower seed extract may be attributed to the extract's acidic phytochemicals. Similar findings have been reported in previous studies, in which increasing concentrations of plant extracts resulted in modest reductions in formulation pH, while remaining within acceptable limits for topical use. Although differences in mean pH values were observed among formulations, post hoc Tukey HSD analysis indicated that none of the pairwise comparisons were statistically significant ($p > 0.05$). Furthermore, all formulations exhibited pH values within the acceptable range for topical facial preparations (4.5–6.5). Therefore, the observed variations are unlikely to have a clinically meaningful impact on product safety, skin compatibility, or user comfort. These findings indicate that although overall pH variations were observed among the formulations, the magnitude of the differences was relatively small and insufficient to yield statistically significant pairwise comparisons.

The results showed that viscosity increased with increasing extract concentration, with mean values of 841.33 cP, 1037.33 cP, and 1155.00 cP for F1, F2, and F3, respectively. This trend suggests that higher SFSE concentrations contributed to increased resistance to flow within the formulation matrix. Similar observations have been reported in herbal serum formulations, where increasing concentrations of plant-derived constituents enhanced intermolecular interactions, thereby increasing

viscosity. Viscosity is an important physical parameter that affects the stability, spreadability, and overall performance of topical serum preparations [32]. Serum formulations with appropriate viscosity provide adequate retention on the skin surface while maintaining ease of application and user comfort. According to the recommended standards for serum preparations (230–1150 cP) [33], F1 and F2 were within the acceptable range, whereas F3 exhibited a viscosity value slightly above the upper limit (1155.00 cP). Although the deviation was minimal, the higher viscosity of F3 may reduce spreadability and affect application characteristics.

Nevertheless, increased viscosity may also enhance physical stability by minimizing phase separation and sedimentation during storage. Post hoc Tukey HSD analysis demonstrated significant differences in viscosity among all formulations ($p < 0.05$). Formulation F3 exhibited significantly higher viscosity than both F1 and F2, while F2 also showed significantly higher viscosity than F1. These findings indicate that increasing the concentration of sunflower seed (*Helianthus annuus* L.) extract significantly increased the viscosity of the gold nanoparticle serum formulations. The observed increase in viscosity may be attributed to the higher concentration of phytochemical constituents, which enhanced intermolecular interactions within the formulation matrix and increased resistance to flow.

One-way ANOVA revealed a statistically significant difference in spreadability among the formulations ($p = 0.010$), indicating that formulation composition influenced the spreading characteristics of the gold nanoparticle serum. Numerically, F2 exhibited the highest mean spreadability (44.9 cm²), followed by F1 (38.6 cm²), whereas F3 showed the lowest value (30.8 cm²). These findings suggest that the intermediate extract concentration may offer better spreading properties than either lower or higher concentrations. The observed trend may be attributed to differences in the internal

structure and consistency of the formulations. Increasing the concentration of sunflower seed extract may alter intermolecular interactions within the system, thereby affecting its flow behavior and spreadability. However, post hoc multiple-comparison analysis is required to determine which specific formulations differ significantly from one another. Therefore, while F2 appeared to exhibit the most favorable spreadability profile based on the mean values, definitive conclusions regarding pairwise differences should be based on post hoc statistical testing.

3.5.3. Irritation Test of Gold Nanoparticle Serum Using Sunflower Seed (*Helianthus annuus L.*) Extract as a Bioreductor

Irritation testing was conducted to assess the cosmetic product's skin compatibility in a study involving 20 human volunteers. Skin compatibility was defined as the absence of irritation when the product was used under normal conditions, considering both objective reactions, such as redness or swelling, and subjective sensations, including stinging, burning, or itching [34]. The inclusion criteria for the study included healthy volunteers aged 18–50 years who were not using other cosmetic products on the test area, were willing to apply the test product as instructed, and consented to the observation of the application area as per the study protocol. Exclusion criteria included individuals with acute or chronic skin diseases, dermatological conditions, sensitive skin, known

allergies to any components of the test product, and those who were pregnant or breastfeeding. The serum was applied to the participants' backs under occlusive patches for 48 h, with evaluations at 1, 24, and 48 h post-application. Redness was visually assessed, graded for severity, and its intensity was also objectively measured. The primary irritation index (PII) was calculated by dividing the total sum of erythema and edema scores by the number of grading intervals. The detailed results are presented in [Table 7](#).

All three gold nanoparticle serum formulations synthesized using sunflower (*Helianthus annuus L.*) seed extract exhibited excellent skin compatibility, with no evidence of irritation observed among the 20 volunteers during the study period. Consequently, the formulations were classified as non-irritating and may be considered safe for topical facial application. Although aqua regia (4 mL) was used during the synthesis of the gold nanoparticles, subsequent heating at 70 °C and dilution with 500 mL of distilled water likely reduced the concentration of residual acidic constituents. The absence of erythema, edema, or any other adverse skin reactions following application of the formulations reflected this. No erythema, edema, itching, burning sensation, or any other adverse skin reactions were observed in any volunteer at baseline, 1 h, 24 h, or 48 h after patch removal. Consequently, all subjects received an irritation score of 0, yielding a Primary Irritation Index (PII) of 0.00, indicating the formulation is non-irritating.

Table.7. Irritation Test Results of Facial Serum.

No.	Subject Code	Age (Years)	Before	1 H	24h	48h	Score	Remarks
1	ZAR	23	0	0	0	0	0	Non-irritant
2	DOW	28	0	0	0	0	0	Non-irritant
3	ARD	21	0	0	0	0	0	Non-irritant
4	SAA	27	0	0	0	0	0	Non-irritant
5	JDK	21	0	0	0	0	0	Non-irritant
6	IKY	23	0	0	0	0	0	Non-irritant
7	TSW	28	0	0	0	0	0	Non-irritant
8	RNA	25	0	0	0	0	0	Non-irritant
9	IAD	21	0	0	0	0	0	Non-irritant
10	MNM	21	0	0	0	0	0	Non-irritant
11	MUY	20	0	0	0	0	0	Non-irritant
12	FIN	19	0	0	0	0	0	Non-irritant
13	SNF	21	0	0	0	0	0	Non-irritant
14	TWD	21	0	0	0	0	0	Non-irritant
15	TJU	21	0	0	0	0	0	Non-irritant
16	RPH	22	0	0	0	0	0	Non-irritant
17	SDW	24	0	0	0	0	0	Non-irritant
18	AZR	22	0	0	0	0	0	Non-irritant
19	HMA	21	0	0	0	0	0	Non-irritant
20	ADR	21	0	0	0	0	0	Non-irritant

Representative photographs of the skin irritation test are presented in **Figure 3**. Since no erythema, edema, itching, burning sensation, or other adverse skin reactions were observed in any of the 20 volunteers, only representative images are shown. The photographs confirm the absence of visible skin irritation following serum application.

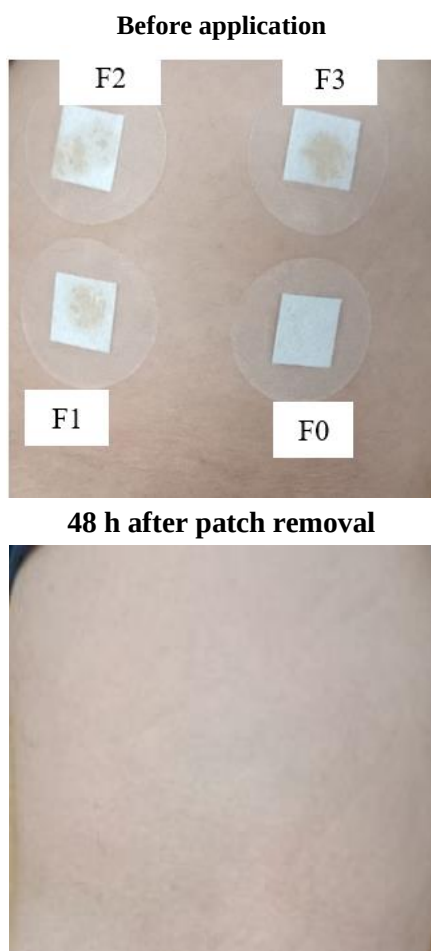


Figure 3. Representative skin irritation test results.

3.6. Antibacterial activity

The antibacterial performance of gold nanoparticle serum is presented in **Table 8**. All formulations exhibited inhibitory effects against *Staphylococcus aureus*, indicating antibacterial potential. Among the tested formulations, F3 showed the highest activity with an inhibition zone diameter of 12.67 mm, whereas F1 and F2 produced relatively smaller inhibition zones. These results suggest that higher SFSE concentrations may enhance the formulation's antibacterial efficacy.

The antibacterial activity of the gold nanoparticle serum containing *Helianthus annuus* L. seed extract was evaluated against *Staphylococcus aureus* ATCC 25923 using the disc diffusion method. The results demonstrated a concentration-dependent increase in antibacterial activity, as evidenced by the progressively larger inhibition zone diameters observed from F1 to F3. The negative control (F0) showed a negligible inhibition zone (0.17 ± 0.15 mm), indicating that the serum base lacked intrinsic antibacterial activity. This finding confirms that the observed antibacterial effect originated from the active ingredients rather than the formulation excipients.

Increasing the concentration of gold nanoparticles in the serum significantly enhanced inhibition of *S. aureus*. F1 produced a mean inhibition zone of 9.27 ± 0.06 mm, which increased to 10.20 ± 0.10 mm in F2 and reached 12.67 ± 0.06 mm in F3. Statistical analysis using one-way ANOVA revealed a highly significant difference among all treatment groups ($p < 0.001$), while Tukey's HSD test confirmed significant differences between pairs of formulations ($p < 0.05$). These findings indicate that increasing the formulation concentration significantly improved its antibacterial efficacy.

Table 8. Antibacterial Activity of Sunflower Seed (*Helianthus annuus* L.) Extract Gold Nanoparticle Formulations.

Treatment	Inhibition Zone Diameter (mm)			Mean (mm)
	Replicate			
	I	II	III	
F0	0.0	0.2	0.3	0.17 ± 0.15
F1	9.3	9.2	9.3	9.27±0.06
F2	10.1	10.3	10.2	10.20±0.10
F3	12.7	12.6	12.7	12.67±0.06
Control (+) (Repeated)	20.3	20.0	20.5	20.27 ± 0.25

The enhanced antibacterial activity may be attributed to the synergistic interaction between gold nanoparticles (AuNPs) and the bioactive phytochemicals present in *H. annuus* seed extract. Sunflower seeds contain various phenolic compounds, flavonoids, tannins, and other secondary metabolites that can disrupt bacterial cell membranes, inhibit essential enzymes, and interfere with nucleic acid synthesis. Meanwhile, AuNPs possess unique physicochemical properties, including a high surface area-to-volume ratio, which facilitates close interaction with bacterial cells. Gold nanoparticles have been reported to increase membrane permeability, induce oxidative stress through reactive oxygen species (ROS) generation, and enhance the penetration of phytochemicals into bacterial cells, thereby amplifying antibacterial activity.

Despite the concentration-dependent increase observed among the formulations, the positive control exhibited the greatest antibacterial activity, with a mean inhibition zone of 20.27 ± 0.25 mm. This result was expected because the positive control contained a standard antibacterial agent with a well-established mechanism of action and optimized antimicrobial potency. Nevertheless, the inhibition zone produced by F3 (12.67 ± 0.06 mm) indicates that the gold nanoparticle serum possesses promising antibacterial potential against *S. aureus*. According to commonly used inhibition zone classifications, inhibition diameters of approximately 10–20 mm are generally considered to represent **strong antibacterial activity**, suggesting that the highest-concentration formulation exhibited a biologically meaningful inhibitory effect.

Overall, these findings demonstrate that incorporating gold nanoparticles into *H. annuus* seed extract serum enhanced antibacterial activity against *S. aureus* in a concentration-dependent manner. Further investigations, including minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), biofilm inhibition assays, and mechanistic studies, are recommended to elucidate the antibacterial mechanism better and to evaluate the clinical potential of this nanoformulation.

4. Conclusion

A face serum has been prepared using active gold nanoparticles of Sunflower Seed (*Helianthus annuus* L.)

extract. The most optimal formulation contains 96% ethanol SFSE, 1400 μ L HAuCl₄, 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 5.54–5.77, the viscosity ranged from 800 to 3000 cP, and the spreadability ranged from 5.60 to 5.93 cm, resulting in a stable serum preparation. Formulation F3 consisting of 15% SFSE exhibited the highest antibacterial activity against *Staphylococcus aureus* ATCC 25923.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Authors Contributions

Tatiana Siska Wardani: Conceptualization, methodology, supervision, data analysis, writing – original draft, writing – review and editing, and project administration.

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Belinda Arbitya Dewi: Investigation, laboratory experiments, data collection, and data analysis.

Susanti Erikani: Methodology, validation, supervision, and writing – review and editing.

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Using artificial intelligence chatbots

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