



## ***Curcuma caesia* Extract-Based Nanoparticle Formulation in Antibacterial Shampoo Against *Staphylococcus aureus* and *epidermidis* Bacteria**

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### **Abstract**

*Curcuma caesia* shows promise as a medicinal plant due to its bioactive compounds, including flavonoids, phenols, and alkaloids, which contribute to its antibacterial activity. Based on this antibacterial effect, the shampoo formulation was created with variations in the concentration of *C.caesia* nanoparticles at 10%, 15%, 20%. The shampoo preparation was tested for physical-chemical characteristics, including organoleptic properties, pH levels, viscosity, foam height, and a physical stability test using the cycling-test method. Antibacterial activity tests were then conducted on *S.aureus* and *S.epidermidis*, followed by preliminary dermal toxicity tests on test animals, such as mice. The results of the physical-chemical properties test indicated that the preparation met the criteria for a good shampoo. The particle size test of *C.caesia* extract revealed a size of 229.3 nm. The variation in *C.caesia* nanoparticle showed differences, as the concentration increased, the color produced was more intense organoleptically, the pH level rose, the viscosity value decreased, and the foam production increased. *C.caesia* extract nanoparticles, used as an active ingredient in shampoo, demonstrate antibacterial properties. The inhibition zones measured against *S.aureus* were F1 at 8.41 mm (moderate), F2 at 9.10 mm (moderate), and F3 at 11.16 mm (strong). Meanwhile, the diameters of the inhibition zones formed against *S.epidermidis*, namely F1, F2 and F3, were 10.19 (strong), 10.45 (strong), and 11.00 (strong) respectively.

## **INTRODUCTION**

Dandruff is a common scalp disorder characterised by the excessive shedding of dead skin cells, often accompanied by itching and inflammation. The causes of dandruff include overproduction of sweat by the glands or the presence of microorganisms on the scalp that produce metabolites capable of inducing dandruff formation (Maimunah et al., 2019). Dandruff can also be caused by bacteria, including *S. aureus* and *S. epidermidis*. *Staphylococcus epidermidis* bacteria are commonly found on the human scalp (Mishra et al., 2021).

Many people use chemical shampoos to eliminate dandruff. However, these anti-dandruff shampoos are less effective, as dandruff may recur when the hair and scalp are dirty and oily. Moreover, the scalp can absorb chemicals found in shampoos. Therefore, using shampoos made from natural ingredients as an alternative form of medicine is essential for ensuring safety and preventing side effects when addressing scalp issues. Black turmeric is an herb with active compounds that could serve as a medicine for dandruff caused by bacteria. It is a natural alternative for formulating shampoo preparations.

Black turmeric (*Curcuma caesia* Roxb.) has the potential to be a medicinal plant due to its

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bioactive compounds, including flavonoids, phenols, and alkaloids. These bioactive compounds exhibit antibacterial properties. Antimicrobials derived from plants have significant therapeutic potential and can achieve the desired outcomes without the side effects frequently linked to synthetic antimicrobials (Pandey & Gupta, 2014).

This study utilized black turmeric obtained by squeezing fresh rhizomes. The squeezing method still retains inhibitory properties against bacterial growth, even in the absence of a specific solvent to bind desired substances and remove unwanted ones, as in the extract. Moreover, the squeezing method has economic value when compared to other methods, such as extraction. *C. caesia* extract is then simplified into nanoparticles to enhance its bioavailability. Nanoparticle technology has been widely developed for use in drug delivery systems.

Nanoparticles are a technology with sizes ranging from 10 to 1000 nm (Yusuf et al., 2023). They consist of materials that function as drug carriers, encapsulating and dissolving active compounds (Rojas et al., 2024). Nanoparticles address the solubility issues of poorly soluble active ingredients, enhance low bioavailability, improve the stability of active ingredients against environmental degradation (such as enzymatic decomposition, oxidation, and hydrolysis), modify the drug delivery system to target therapeutic sites directly, increase the absorption of macromolecular compounds, and mitigate the irritating effects of active ingredients on the digestive tract (Liu et al., 2024).

## MATERIALS AND METHODS

### Equipment

The equipment used in this study included an analytical balance (Precisa, Switzerland), water bath (Memmert®, Germany), viscometer (Rion

Viscotester, Japan), pH meter (Hanna Instruments, Romania), and particle size analyzer (Malvern Panalytical, United Kingdom).

### Materials

The materials used were fresh black turmeric (*Curcuma caesia*) obtained from a local herbal market in Indonesia; Texapon® (BASF, Germany); Na-CMC (Sigma-Aldrich, USA); glycerin (Merck, Germany); Iselux® (Innospec, USA); phenoxyethanol (Sigma-Aldrich, USA); grape essence (IFF, USA); sodium powder (Merck, Germany); Staphylococcus aureus bacteria (American Type Culture Collection, USA); Staphylococcus epidermidis ATCC (American Type Culture Collection, USA); distilled water (Aqua Dest, Indonesia); Mayer's reagent (Merck, Germany); concentrated HCl (Merck, Germany); and FeCl<sub>3</sub> (Sigma-Aldrich, USA).

### Formula Design

The formula design is shown in Table 1.

### Preparation of Fresh Black Turmeric Juice Extract

#### *Fabrication of C. caesia Extract Nanoparticles*

Fresh black turmeric rhizomes were washed under running water until clean, then drained.

The drained black turmeric rhizomes were then grated until smooth. The grated results were placed in a filter cloth and squeezed to extract the juice.

A total of 6 mL of aquabides and 1 drop of VCO were added to 10 mg of *C. caesia*. This mixture was then subjected to ultrasonication using an ultrasonic processor at a frequency of 40 KHz for 30 minutes. Nanoparticle analysis was performed using a Particle Size Analyzer (psa) (Vifta et al. 2022).

Table 1. Shampoo formulation.

| Material                              | Formula 1 (%) | Formula 2 (%) | Formula 3 (%) |
|---------------------------------------|---------------|---------------|---------------|
| <i>C.caesia</i> extract nanoparticles | 10            | 15            | 20            |
| Iselux ultra mild                     | 20            | 20            | 20            |
| Carbopol                              | 1             | 1             | 1             |
| Glycerin                              | 5             | 5             | 5             |
| Phenoxyethanol                        | 0.5           | 0.5           | 0.5           |
| Citric acid                           | qs            | qs            | qs            |
| Essence                               | Qs            | qs            | qs            |
| Aquadest                              | Ad 100        | Ad 100        | Ad 100        |

***Making shampoo preparations***

Carbopol, weighed in a mortar, was then dissolved in 20 ml of hot water. It was left for a few minutes until it expanded, and stirred vigorously (mixture 1). A total of 10 ml of Aquadest was placed into a beaker, Iselux was added, and the mixture was stirred until it was dissolved (mixture 2). Phenoxyethanol and glycerin were dissolved and stirred until homogeneous (mass 3). Phenoxyethanol and glycerin (mixture 2) were added gradually to mixture 1 while stirring slowly until a homogeneous mixture was formed. Fresh black turmeric juice extract was added to mixtures 1 and 2, and stirred until homogeneous. Iselux (mixture 2) was slowly mixed and stirred until homogeneous. Grape essence was added and stirred until a homogeneous mixture was obtained.

***Organoleptic Test***

Organoleptic testing was conducted by observing the appearance, odor, and color of the shampoo preparation at various concentrations of fresh black turmeric juice extract (Sjamsiah et al., 2025). According to SNI 06-2692-1992, the ideal shampoo standard has a liquid consistency, alongside a distinctive odor and color.

***Homogeneity Test***

The homogeneity test was conducted visually by taking samples of the shampoo preparation from the upper, middle, or lower parts, and then observing them on a glass slide (Wijayanti et al., 2025).

***Viscosity Test***

Viscosity was measured using a Rion VT-04 Viscometer by placing the black turmeric shampoo preparation in a beaker ( $\pm 200$  mL), and then placing it under the Rion VT-04 Viscometer with a suitable spindle. The spindle was inserted into the preparation until it was submerged. The spindle used was spindle no. 1 (Saryanti & Prameswari 2024).

***pH Test***

The pH level was measured using a pH meter electrode.

***Foam Height Measurement Test***

A total of 0.1 g of shampoo was dissolved in 10 mL of water, and then put into a test tube, closed and shaken for 20 seconds by inverting the

test tube regularly. Furthermore, the height of the foam formed was measured (Maimunah et al., 2019). The foam height requirement is between 1.3 - 22 cm (Rowe et al., 2009).

***Physical Properties Test of Black Turmeric Shampoo Preparations***

A physical properties test on the preparation was conducted using the cycling test method. This test was carried out by storing the preparation from each formula in a transparent glass container, applying a single cycle treatment by storing the preparation at a temperature of 4 °C for 24 hours, and then transferring it to an oven at a temperature of  $40 \pm 2$  °C for 24 hours. The test was carried out for 6 cycles or 12 days to observe whether there were changes in each preparation. Subsequently, the condition of the preparation was compared with that of the previous preparation during the experiment (Sambodo & Salimah, 2021).

***Antibacterial activity test***

Antibacterial activity tests were conducted on *S. aureus* and *S. epidermidis* bacteria using the agar diffusion method with wells. The clear zone formed around the well was measured with a caliper. Treatment commences with the sterilisation of the tools and materials used in an oven or autoclave (Wijayanti et al., 2022).

***Preliminary dermal toxicity test***

A preliminary acute dermal toxicity test was conducted to determine whether any test animals died after being given the preparation. This study used male Wistar rats of the white strain as test animals. The test mice were healthy, 3 to 4 months old, and weighed between 200 and 250 grams. Observations were conducted on day 1, following the release of the test material, and continued until day 14 to monitor whether any mice had died (Samirana et al., 2018). The hair shaving process is performed the day before the test on the dorsal/flank areas of 6 x 8 cm (at least 10% of the body surface area) to allow for exposure to the test material. Dermal toxicity test treatment is shown in Table 2.

**RESULTS AND DISCUSSION**

Phytochemical screening results showed that black turmeric juice extract contains alkaloids, phenols, saponins, and tannins, which are

Table 2. Test treatment.

| Group                | Treatment                               | Information                             |
|----------------------|-----------------------------------------|-----------------------------------------|
| Normal control       | No treatment was given                  | Sacrificed on the 14th day              |
| Test the shampo base | Given a shampoo base application        | Given on day 1 and sacrificed on day 14 |
| Treatment 1          | Given a smear of F1 shampoo preparation | Given on day 1 and sacrificed on day 14 |
| Treatment 2          | Given a smear of F2 shampoo preparation | Given on day 1 and sacrificed on day 14 |
| Treatment 3          | Given a smear of F3 shampoo preparation | Given on day 1 and sacrificed on day 14 |

Table 3. Phytochemical screening results of black turmeric juice extract.

| Compound  | Parameter                               | Result |
|-----------|-----------------------------------------|--------|
| Alkaloid  | A white/cream precipitate form          | +      |
| Flavonoid | A red color form                        | +      |
| Phenol    | Red, purple color changes are formed    | +      |
| Saponin   | Foam forms                              | +      |
| Tannin    | <b>A blackish green color is formed</b> | +      |

Description:

(+) : contain compounds as secondary metabolites

(-) : does not contain compounds as secondary metabolites

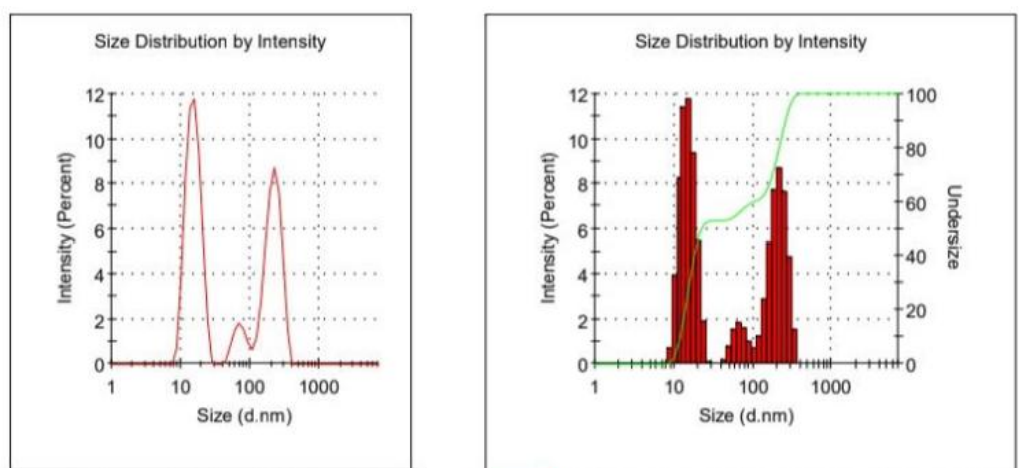


Figure 1. PSA analysis results.

secondary metabolites with antibacterial properties. The test results are shown in Table 3.

Alkaloids, as antibacterials, interfere with the synthesis of bacterial cell peptidoglycan, preventing the formation of cell walls. This condition causes bacterial cells to undergo lysis easily, preventing them from forming colonies and leading to cell death (Sa'diyah et al., 2024). The phytochemicals in the *C. caesia* rhizome extract contain various bioactive substances, including alkaloids, carbohydrates, proteins, amino acids, flavonoids, phenols, steroids, glycosides, and

tannins. These active compounds may be responsible for antibacterial activity (Ranemma & Reddy, 2017).

#### Particle Size Test

Making nanoparticles from juice extract using the sonication method aims to determine the size of the extract that has reached a nanoscale using the PSA tool. The PSA analysis indicates that the Z-Average calculation reveals the average size of *C. caesia* extract nanoparticles to be 229.3 nm, which falls within the nanometer range of 1-1000 nm. PSA Analysis Results are shown in Figure 1.

Table 4. Organoleptic test results

| Formula | Test  | Test results                 |                          |
|---------|-------|------------------------------|--------------------------|
|         |       | <i>Pre cyclnig test</i>      | <i>Post cycling test</i> |
| F1      | Form  | Liquid ++                    | Liquid +                 |
|         | Color | Reddish purple               | Brownish red             |
| F2      | Form  | Liquid +++                   | Liquid ++                |
|         | Color | The red purple is quite dark | Orange red               |
| F3      | Form  | Liquid ++++                  | Liquid +++               |
|         | Color | Deep red purple              | Red a little bit orange  |

### Organoleptic Test

Organoleptic tests on the preparation were carried out through direct observation using eyesight. The results of the preparation test, taken before and after the cycling test, are presented in Table 4.

The most visible change is in the form of the preparation, after the cycling test, the preparation has a thicker consistency, and there is a pretty different color change from reddish purple to brownish red and orange red. This reaction is known as an enzymatic browning reaction, caused by the oxidation of phenol compounds in the extract by the catecholase enzyme, resulting in the formation of brown pigment (Singh et al., 2018). This process occurs because the preparation undergoes six cycling tests, conducted at both cold temperatures (4°C) and high temperatures (40°C), to assess the stability of the shampoo under extreme conditions. It can be concluded that the preparation is unstable following the cycling test treatment.

### Homogeneity Test

Based on the homogeneity test of the preparation before and after the cycling test, the shampoo has homogeneous characteristics. It can be observed that there were no coarse grains in the preparation when it was applied to the object glass. Homogeneity Test Results of Shampoo Preparation are shown in Table 5.

Table 5. Homogeneity test results of shampoo preparation.

| Formula | Test Result             |                          |
|---------|-------------------------|--------------------------|
|         | <i>Pre cyclnig test</i> | <i>Post cycling test</i> |
| F1      | Homogeneous             | Homogeneous              |
| F2      | Homogeneous             | Homogeneous              |
| F3      | Homogeneous             | Homogeneous              |

Homogeneity is defined by the absence of fibers or coarse particles in the formulation (Rohmani & Pangesti, 2024). Therefore, it can be concluded that black turmeric shampoo satisfies the criteria for the homogeneity test of shampoo formulations and demonstrates stability in homogeneity following the cycling test treatment.

### Viscosity Test

The viscosity test aims to measure the viscosity of the preparation, where the resulting viscosity value indicates the liquid's resistance to flow: the higher the viscosity value, the greater the resistance to flow (Rohmani et al., 2021). The viscosity of a good shampoo ranges from 4 - 40 dPas (Ameliana et al., 2019). The results of the viscosity test of the black turmeric shampoo preparation are shown in the following table. Viscosity Test Results of Shampoo Preparations are shown in Table 6.

All formulas have viscosity values in the range of 4-40 dPas, namely 9.8-39 dPas. Thus, it can be concluded that the black turmeric shampoo preparation meets the viscosity requirements of shampoo.

Table 6. Viscosity Test Results of Shampoo Preparations

| Replication | Viscosity (dPas)        |        |         |                          |          |          |
|-------------|-------------------------|--------|---------|--------------------------|----------|----------|
|             | <i>Pre cycling test</i> |        |         | <i>Post cycling test</i> |          |          |
|             | F1                      | F2     | F3      | F1                       | F2       | F3       |
| 1           | 23                      | 16     | 9.8     | 37                       | 30       | 27       |
| 2           | 20                      | 15     | 10      | 39                       | 29       | 28       |
| 3           | 22                      | 17     | 9.9     | 38                       | 35       | 22       |
| Average±SD  | 21.7±1.5                | 16±1.0 | 9.9±0.1 | 38±1.0                   | 31.3±3.2 | 25.7±3.2 |

Table 7. pH Test Results of the Preparation

| Replication | pH               |           |          |                   |           |           |
|-------------|------------------|-----------|----------|-------------------|-----------|-----------|
|             | Pre cycling test |           |          | Post cycling test |           |           |
|             | F1               | F2        | F3       | F1                | F2        | F3        |
| 1           | 5.67             | 5.77      | 5.79     | 5.09              | 5.19      | 5.22      |
| 2           | 5.70             | 5.71      | 5.81     | 5.03              | 5.17      | 5.21      |
| 3           | 5.68             | 5.70      | 5.80     | 5.04              | 5.13      | 5.26      |
| Average±SD  | 5.68±0.01        | 5.73±0.03 | 5.8±0.01 | 5.05±0.03         | 5.16±0.03 | 5.23±0.02 |

Statistical test results were conducted before and after the cycle test using one-way ANOVA. The analysis results showed a significance value  $<0.05$ , namely 0.000 before the cycling test and 0.004 after the cycling test, meaning that each formula, F1, F2, and F3, had a significantly different average viscosity value. Therefore, it can be concluded that variations in the concentration of black turmeric juice extract affect the viscosity of the shampoo preparation. Based on the results of the viscosity test, a higher concentration of active substances added to the shampoo preparation leads to lower viscosity. The treatment involving variations in high black turmeric extract concentration increases the volume of water in the preparation, resulting in a high-water content. Turmeric is a type of tuber plant with a water content in the form of rhizomes ranging from 11% to 20% (Syafutri et al., 2010). Consequently, the high-water content in fresh black turmeric will result in a reduction in solvent concentration and viscosity.

The decrease in viscosity at room temperature may result from the shampoo preparation containing glycerin, which possesses hygroscopic properties due to its three hydroxyl groups, enabling the formation of hydrogen bonds with water molecules.

The hygroscopic nature of glycerin enables it to absorb water vapor from the surrounding environment, thereby increasing the water content in the preparation (Rowe et al., 2009). This reduction in viscosity can occur due to an increase in particle diameter, which decreases the surface area and results in lower viscosity. The factors influencing the viscosity value include temperature, material concentration, and the chemical reactions that occur during accelerated storage. Under accelerated storage conditions (cycling test treatment), the viscosity of the shampoo preparation increased. This is because the particles in the shampoo preparation tend to combine or become closer to each other to form a tighter bond

structure at low temperatures, increasing the viscosity of the black turmeric shampoo (Auliah et al., 2020).

### pH Test

The pH measurement seeks to examine the acidity level in the shampoo preparation. The measurement used a pH meter. The results of the pH test on black turmeric extract shampoo are presented in the following Table 7.

The pH test results in the table indicated that shampoo with a 10% concentration of black turmeric extract (F1) produces a lower pH value (more acidic) compared to shampoos with concentrations of 15% and 20%. The pH value of the shampoo ranges from 5.05 to 5.8. The standard pH value for shampoo set by SNI (1992) is between 5 and 9, while the pH of the scalp is between 4 and 6. Therefore, the black turmeric extract shampoo preparation complies with the standard and passes the pH test. A good shampoo has the same pH acidity as the skin's pH to minimize allergies or irritation to the skin when using the shampoo. The pH value is related to hair growth; a pH of less than 5 or more than 7.4 in the bioreactor can lead to depletion, meaning microorganisms may die (Sulhatun et al., 2022). Meanwhile, at a pH of 5 to 6.7, microorganisms are in an unstable state. Microorganisms in this unstable state can grow if they receive additional substances.

The one-way ANOVA statistical test showed a significance value of  $<0.05$ , namely 0.001 (before the cycle test) and 0.003 (after the cycle test), meaning that the pH values for all formulas, namely F1, F2, and F3, showed significant differences. It can be concluded that the variation in black turmeric extract concentration affects the pH value of the shampoo preparation, and there is a significant average difference in the treatment before and after the cycling test. The concentration variation of black turmeric juice indicates a change in pH value, demonstrating an increase or a slight change to become more alkaline. This alteration is

Table 8. Foam Height Test Results of the Preparation

| Repetition       | Foam height (cm) |               |               |                   |               |             |
|------------------|------------------|---------------|---------------|-------------------|---------------|-------------|
|                  | Pre cycling test |               |               | Post cycling test |               |             |
|                  | F1               | F2            | F3            | F1                | F2            | F3          |
| 1                | 5                | 7             | 8             | 7                 | 6.5           | 8           |
| 2                | 4.3              | 6             | 6.5           | 6.5               | 7.5           | 7.5         |
| 3                | 4.5              | 5.5           | 7             | 6                 | 8             | 8.5         |
| Average $\pm$ SD | 4.6 $\pm$ 0.3    | 6.2 $\pm$ 0.7 | 7.5 $\pm$ 0.7 | 6.5 $\pm$ 0.5     | 7.3 $\pm$ 0.7 | 8 $\pm$ 0.5 |

attributed to the compounds present in black turmeric juice.

The basic alkaloid compounds in the preparation influence the resulting acidity level (pH) (Purwanto et al., 2019). Alkaloids are classified as compounds that contain at least one nitrogen atom, which typically possesses basic properties (Nugrahani et al. 2020). The pH acidity level of the black turmeric shampoo preparation increased after the cycling test. The pH changes of the preparation are influenced by the environment and the length of storage time. Changes in pH value will affect the effectiveness and safety of the preparation.

#### Foam Height Test

The purpose of the foam height test is to assess the foam power produced by the shampoo. The results demonstrate that a higher concentration of black turmeric extract in the shampoo formulation leads to increased foam production. This is due to the presence of saponin compounds in black turmeric, which can dissolve in water and generate a stable foam when shaken (Indrawati et al., 2020). Foam Height Test Results of the Preparation are shown in Table 8.

The average foam height of shampoo preparations F1, F2, and F3 ranges from 4.6 to 8 cm. The standard foam height lies between 1.3 and 22 cm (Rowe et al., 2009). Therefore, it can be concluded that the black turmeric extract shampoo meets the requirements for the foam height test of shampoo preparations. The One-Way ANOVA statistical test observed that the average foam height values of shampoo formulas F1, F2, and F3 were significantly different before the cycle test, showing a significance value of 0.009 ( $<0.05$ ). However, they did not show a significant difference or were nearly the same after the cycle test, with a significance value of 0.059 ( $>0.05$ ).

Saponins form a stable foam when shaken, are classified as a group of complex natural compounds, have large molecular masses, and are

useful in many applications. The structure of saponins causes them to act like soap or detergent, so they are called natural surfactants (Minarno, 2016).

#### Antibacterial Activity Test

The antibacterial activity test was conducted using the well method with Mueller Hinton Agar (MHA) as the agar medium, erythromycin antibiotic as a positive control for *S. aureus*, and vancomycin antibiotic as a positive control for *S. epidermidis*. The bacteria used in this test are ATCC pure strain bacteria. The test bacteria used are pure cultures or those from the American Type Culture Collection (ATCC), not bacteria originating from the environment or patients. Data on the diameter of the inhibition zone produced by the black turmeric shampoo preparation against *S. aureus* and *S. epidermidis* bacteria are presented in the Table 9 and 10.

The test results conducted on shampoo preparations with variations in black turmeric concentrations of 10%, 15%, and 20% revealed a clear inhibition zone against *S. aureus* and *S. epidermidis* bacteria, similar to that seen in the positive antibiotic control. The largest inhibition zone is owned by the positive control (antibiotic). The test results conducted on shampoo preparations with variations in black turmeric concentrations of 10%, 15%, and 20% revealed a clear inhibition zone against *S. aureus* and *S. epidermidis* bacteria, similar to that seen in the positive antibiotic control. Black turmeric contains alkaloid compounds that exhibit antibacterial activity. The effectiveness of alkaloid compounds is significantly influenced by the biological activity of the compound, which arises from the presence of nitrogen-containing base groups. When this base group encounters bacteria, it reacts with the amino acid compounds that constitute the cell walls and DNA of the bacteria.

Changes in the amino acid chain structure of DNA will lead to alterations in genetic balance, resulting in damage to bacterial DNA. Damage to

the DNA will also harm the bacterial cell nucleus,

Table 9. Inhibition Zone Diameter Against *S. aureus* Bacteria

| Repetition       | Inhibition Zone Diameter (mm) |               |                 |                          |
|------------------|-------------------------------|---------------|-----------------|--------------------------|
|                  | F1                            | F2            | F3              | Streptomycin control (+) |
| 1                | 8.4                           | 9.2           | 11.4            | 28.9                     |
| 2                | 8.6                           | 8.8           | 10.9            | 27.9                     |
| 3                | 8.2                           | 9.4           | 11.2            | 29.2                     |
| Average $\pm$ SD | 8.41 $\pm$ 0.2                | 9.1 $\pm$ 0.3 | 11.16 $\pm$ 0.2 | 28.67 $\pm$ 0.6          |

Table 10. Inhibition Zone Diameter Against *S. Epidermidis* Bacteria

| Repetition | Inhibition Zone Diameter (mm) |                 |              |                        |
|------------|-------------------------------|-----------------|--------------|------------------------|
|            | F1                            | F2              | F3           | Vancomycin control (+) |
| 1          | 9.8                           | 10.4            | 11.1         | 19.1                   |
| 2          | 10.3                          | 10.7            | 10.7         | 18.6                   |
| 3          | 10.5                          | 10.2            | 11.2         | 18.9                   |
| Average    | 10.19 $\pm$ 0.3               | 10.45 $\pm$ 0.2 | 11 $\pm$ 0.2 | 18.87 $\pm$ 0.2        |

as DNA is its primary component. Such damage to the bacterial cell nucleus will ultimately induce lysis within it. The antibacterial activity of polyphenol and flavonoid compounds in black turmeric is thought to occur by complexing with bacterial walls, thereby inhibiting microbial growth. Additionally, the presence of glycosides and tannins is also believed to contribute to this antibacterial effect. The cell wall of Gram-positive bacteria is characterized by multiple layers of peptidoglycan, creating a thick and rigid structure that also includes teichoic acid. This composition makes it resistant to physical shocks, like exposure to antibiotics or other bacterial agents. The results of the activity test indicate that the extract from black turmeric rhizome exhibits antibacterial potential (Nyoman, 2022).

The criteria for antibacterial strength are as follows: an inhibition zone diameter of 5 mm or less is classified as weak; an inhibition zone of 5-10 mm is classified as moderate; an inhibition zone of 10-20 mm is classified as strong; and an inhibition zone of 20 mm or more is classified as very strong (Sanam et al., 2022).

Based on these criteria, the antibacterial efficacy of black turmeric extract in shampoo formulations against *S. aureus* bacteria is classified as moderate for F1 (8.41 mm), moderate for F2 (9.10 mm), and strong for F3 (11.16 mm). The diameter of the inhibition zone formed against *S. epidermidis* indicated that all formulas, F1, F2, and F3, fell into the strong category, with the resulting

inhibition zone diameters measuring 10.19 mm, 10.45 mm, and 11.00 mm, respectively. Furthermore, as positive controls, streptomycin is classified in the very strong category against *S. aureus* bacteria, while vancomycin is categorized as strong against *S. epidermidis* bacteria.

### Toxicity Preliminary Test

Preliminary toxicity tests are conducted for further testing. Toxicity refers to the ability of a molecule or chemical compound to cause harm to vulnerable areas within or outside the body of a living organism. A chemical compound is considered a poison if its reaction with an object can result in a harmful effect.

The resulting toxic effects indicate a disruption of the morphological and physiological processes of the test biota (Duo et al., 2025). Preliminary tests were conducted without repetition. The results showed that all test animals, which were mice given the treatment, did not exhibit any irritation or edema on the skin. Observations from the last day of testing, which was day 14, indicated that all mice survived and began to develop fine hair. It can be concluded that the preliminary test that has been conducted is suitable for further dermal toxicity testing.

### CONCLUSION

The variation of *C. caesia* extract nanoparticle concentration at 10%, 15%, and 20%

has a significant effect on the physical and chemical properties of the shampoo preparation. The test results on the physical and chemical properties of the black turmeric extract shampoo preparation have met the requirements for a good shampoo. The results of the stability test show that organoleptically, there are changes in color, odor, and viscosity of the preparation before and after the cycling test. As the concentration increases, the pH level rises, the foam production increases, but the viscosity decreases.

*C. Caesia* nanoparticle shampoo has been shown to inhibit the growth of *S. aureus* and *S. epidermidis* bacteria. The black turmeric extract shampoo formulation exhibiting the most effective antibacterial activity against *S. aureus* and *S. epidermidis* is the one with the highest concentration, specifically Formula 3, which contains a 20% concentration of black turmeric extract. The antibacterial efficacy of black turmeric extract falls within the moderate to strong category.

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## CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest in this research.

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