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# Nanocream with Active Ingredients of Cardamom Seeds Oil as an Antibacterial Topical Preparation

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## Abstract

Cardamom is one of the Indonesian herbs that produce essential oil which is known to have various pharmacological properties such as antioxidant, antifungal, antiviral, and antibacterial. Cardamom essential oil (CEO) in this study was obtained by ultrasonic-assisted extraction-hydrodistillation (UAE-HD). For practical topical application, nano

cream formulation was developed. This study aimed to observe the CEO antibacterial activity in nano cream preparation with the variation of Hydrophile-Lipophile Balance (HLB) with values 10, 11, 12 and oil concentrations 2.5%, 5%, 7%, 10%, and 15%. CEO nano cream presented inhibition zone 6.03-15.23 mm against Escherichia coli and 11.13-15.23 mm against Staphylococcus aureus. The antibacterial activity in CEO was due to the main constituent that was 1,8-cineol. The particle size and zeta potential produced by the nano cream formulation ranged from 114,3 – 341,53 nm and 73,43 – 94,70 mV, respectively. The physical properties of CEO nanocream were as follows, pH 4.30-6.45; spreadability 5.1-6.9 cm; and viscosity 5210-6910 cP and showed excellent stability for 4 weeks. The best formulation was the formulation with the highest antibacterial activity in order to provide good efficacy. The formulation with CEO concentration 15%; HLB 12 possessed inhibition zone 10.2 mm and 11.4 mm for E. coli and S. aureus, respectively. The average particle size was 270.40 ± 0.38 nm and zeta potential -88.70 ± 0.20 mV, wherein still meets the nanoemulsion size range that enable penetrated into stratum corneum. This concluded that cardamom essential nano cream can be considered a potential antibacterial topical preparation.

## Keywords

Antibacterial; Cardamom; Essential oil; Nanocream; Nanoemulsion.

## Introduction

Cardamom or cardamon/cardamum is a spice sourced from the seeds of the genus *Elettaria, Amomum*, and *Aframomum* plants from the Zingiberaceae family. In Indonesia, there are two types of cardamom, i.e., local Java cardamom (*Amomum compactum* Soland ex. Maton.) and true cardamom (*Elettaria cardamomum* (L.)

Maton.; syn. *Amomum cardamomum* L.). Cardamom has been used for culinary and traditional medicine applications. Several studies reported that cardamom possesses various pharmacological properties including antioxidant, anticancer, antidiabetic, anti-inflammatory, antifungal, antiviral, and gastroprotective activities [1,2].

Cardamom Essential Oil (CEO) can be obtained by several techniques and one of them is Ultrasonic Assisted Extraction – Hydrodistillation (UAE-HD). UAE-HD is one of the most effective and efficient essential oil extraction methods. This method can fasten the extraction time, reduce the solvent usage and increase the extract yield [3]. The main constituent of CEO is 1,8 Cineol which is responsible for antibacterial activity. Previous studies reported that cardamom oil has strong antibacterial activity against gram-positive and gram-negative bacteria [4,5]. CEO also showed significant anti-proliferative activity against skin cells for topical use. It robustly affected various essential genes and signaling pathways, which support the CEO's anti-inflammatory potential [6].

The potential of CEO as an antibacterial agent further developed into topical preparation to ease the application on the skin. The cream topical preparation is commonly used due to the simple method and comfortable on skin application [7]. The cream preparation is a colloid formulation that is suitable for essential oil because it can protect the active agent, improve the stability, and enhance the dispersion on water. Further, nanotechnology has been developed to enhance the performance of the cream product. The small droplets are expected to enhance the penetration on the skin thus enhancing the efficacy [8]. Nanoemulsion or nanocream is a topical system of oil and water which stabilized by surfactant with average droplet size range 100-500 nm [9]. With this small size, the compounds able to diffuse across the stratum corneum make this system suitable for delivery vehicles to enhance skin penetration [10]. To formulate a stable nano cream, several factors might affect its characteristics such as

surfactant selection, surfactant concentration, and Hydrophile-Lipophile Balance (HLB) value [11].

Although CEO is widely used in skin care for topical purposes, no study has been done on preparing CEO nanocream with its antibacterial properties. Therefore, based on its potential, the objective of the study was to prepare cardamom seed essential oil in nanocream formulation by varying HLB value and oil concentration. The formulation was observed for its stability, physical properties, and antibacterial activity.

# Results and Discussion

## Cardamom Essential Oil (CEO) Extraction and GC-MS Characterization

Pretreatment techniques aim to disrupt the cell matrix and facilitate mass transfer and in this study, it was done by wave energy such as sonication and microwave [12,13]. Ultrasonic assistance was able to enhance the leaching rate of the active compound. The yield of CEO obtained from the UAE-HD process was 6.59% (w/w). This value was in range within the average yield in the previous study which was between 4.5-9.5% (w/w) [14].

**Table 1:** Cardamom Essential Oil Chemical Constituent (Rf values were reused from [15]

| Compound | Rf | % Abundance |
|---|---|---|
| α-pinene | 8.486 | 3.08 |
| β-pinene | 9.872 | 10.01 |
| p-cymene | 11.372 | 0.56 |
| D-limonene | 11.498 | 4.65 |
| 1,8-cineole | 11.687 | 73.67 |
| γ-terpinene | 12.405 | 1.69 |
| α-terpeniol | 16.564 | 3.27 |
| Terpinyl acetate | 20.773 | 1.34 |

| | | |
|---|---|---|
| Minor components | | 1.73 |

The primary compound of CEO was 1,8 cineole which covers up to 70% of total compositions as seen in Table 1, higher than several studies reported [16–19]. The oxygenated monoterpenes and monoterpene hydrocarbons are the two major class compounds of *A. cardamomum* essential oils [19]. According to several studies, many factors contribute to the difference in chemical composition, namely raw materials and process treatment. Raw materials are affected by exogenous and endogenous factors [20]. Endogenous factors are related to the site of production and accumulation of the EOs in the plant, such as the age and genetic characteristics that regulate the secondary metabolism. Exogenous factors, such as light, precipitation, growing site (altitude, latitude), seasonal variation, and nature of the soil (pH, constituents), are generally related to the environment. Some studies investigated endogenous factors [21–24] and exogenous factors [25–27] which affect chemical constituents of essential oil.

## CEO Nanocream Particle Size and Zeta Potential

The nano cream droplet size for all formulations is shown in Table 2. The smallest droplet size was F3 (2.5%), HLB 12 with a droplet size of 114.30 nm and the largest droplet size was F1 (15%), HLB 10 with a droplet size of 341.53 nm. In addition to droplet size, the Polydispersity Index (PI) value shows information about the physical stability of a dispersion system and the uniformity of the droplet size. A well-accepted polydispersity (PI) is between 0 (monodispersive particles) to 1.0 (large particle size distribution) [28]. A small Polydispersity Index (PI) value indicates that the formed dispersion system tends to be more stable for a long time [29]. Table 2 shows that the Polydispersity Index (PI) value in each formula produces a PI value of less than 1.0, meaning that the level of droplet size uniformity is acceptable.

Hydrophilic Lipophilic Balance (HLB) is a value that corresponds to the balance of the chemical bonds between hydrophilic and lipophilic groups from each surfactant. The HLB value 1-8 tends to form water in an oil emulsion while the HLB value 9-18 tends to form oil in a water emulsion [30]. The formulation in this study used Tween 20 and Span 80 as surfactants due to their non-toxic and non-irritant properties. The combination of these two surfactants produces the emulsion with HLB 10-12 which is oil in water emulsion type.

**Table 2:** Cardamom Essential Oil Chemical Constituent

| CEO Concentration | HLB | Particle size (nm) | PI | Zeta Potential (mV) |
|---|---|---|---|---|
| 2.5% | 10 | 129.80 ± 0.23 | 0.38 ± 0.04 | -81.60 ± 0.20 |
| | 11 | 122.30 ± 0.42 | 0.38 ± 0.02 | -85.30 ± 0.82 |
| | 12 | 114.30 ± 0.35 | 0.51 ± 0.02 | -89.20 ± 0.12 |
| 5% | 10 | 248.20 ± 0.14 | 0.58 ± 0.03 | -84.00 ± 1.20 |
| | 11 | 187.60 ± 0.12 | 0.62 ± 0.03 | -93.47 ± 0.42 |
| | 12 | 137.40 ± 0.40 | 0.89 ± 0.01 | -94.70 ± 1.45 |
| 7% | 10 | 249.40 ± 0.26 | 0.39 ± 0.01 | -73.43 ± 1.98 |
| | 11 | 198.80 ± 0.52 | 0.54 ± 0.01 | -80.63 ± 0.55 |
| | 12 | 139.90 ± 0.40 | 0.84 ± 0.01 | -87.00 ± 0.78 |
| 10% | 10 | 301.83 ± 0.21 | 0.63 ± 0.03 | -76.07 ± 0.06 |
| | 11 | 276.30 ± 0.55 | 0.60 ± 0.05 | -83.33 ± 0.70 |
| | 12 | 267.50 ± 0.23 | 0.96 ± 0.02 | -90.73 ± 0.32 |
| 15% | 10 | 341.53 ± 0.21 | 0.27 ± 0.04 | -79.33 ± 0.14 |
| | 11 | 291.23 ± 0.21 | 0.51 ± 0.01 | -84.40 ± 0.20 |
| | 12 | 270.40 ± 0.38 | 0.59 ± 0.02 | -88.70 ± 0.20 |

It is seen that the formulation with HLB 12 produced a smaller droplet size compared with the formulation with HLB 10. HLB played an important role in the stabilization of nano cream which corresponded to droplet size. The higher the hydrophobicity of the surfactant, the higher the stability, and the smaller the droplet size. The formulation with HLB above 14 was able to produce an emulsion with a droplet size below 100 nm in the particular formulation. A similar study also reported

the mixture of surfactant with HLB 10 formed opaque nanoemulsion with bigger droplet size while HLB 12 formed stable transparent nanoemulsion with smaller droplet size. Thus it can be concluded that higher HLB can reduce the droplet size and ensure the EO is retained within the nanoemulsion [31].

The mixture of Span 80 and Tween 20 also proved to give a synergistic effect on nano cream stability. Span 80 as hydrophobic surfactant able to adsorb onto the continuous phase interface. Yet the excessive Span 80 led to flocculation which increased the droplet size as seen on formula F1. The increase of Tween 20, with the highest concentration in formula F3, can modulate interfacial properties as the result of the difference in adsorption capacities. Tween 20 possesses high surface activity and protects the emulsion against coalescence bridging to droplet size reduction [32,33].

The concentration CEO also affects the nano cream droplet size. As the CEO concentration increased with fixed surfactant concentration, the droplet size gradually increased. With the higher concentration of CEO, there was a decrease in specific surface area. High CEO concentration required higher surfactant concentration to maintain average droplet size. This was in agreement with a study by Kasprzak et al (2023) where the emulsion droplet size increased as the oil concentration increased. A large amount of oil creates a large oil interface and thus requires a larger surfactant proportion to stabilize the dispersion system [34]. The high oil concentration with low surfactant also increases polydispersity [35], this was in accordance with the result shown in Table 2.

Zeta potential measurement was carried out as a parameter in determining the surface charge of colloidal particles and the stability level of nanoemulsion preparations from a system containing dispersed particles through the presence of repulsion between particles of the same charge when close together. Stable colloids

must have a zeta potential value of more than -30 mV and +30 mV [36]. An immense zeta potential value indicates high stability of the preparation, while a low potential zeta value will flocculate faster [37]. All formulas have a zeta potential of more than -30 mV means that the nano cream is stable. The zeta potential value is highly dependent on the composition of the constituents and the dispersion medium [28]. The negative surface charge value was caused by the negatively charged lipid. The ethylene oxide groups in Tween 20 which are capable of creating H bonds with $OH^-$ also took into account in contributing more negative charge [38]. This surface charge forms an electrical barrier on the droplet's surface which contributes to physical stability [39]. Zeta potential is also related to pH value, Hsu et al (2003) reported the negative charge zeta potential at pH values higher than 4, this is in agreement with the observation result shown in Table 4.

## CEO Nano Cream Stability Study

### Physical Appearance

The stability study was conducted for 4 weeks with parameters observed namely color, fragrance, pH, viscosity, and spreadability which is present in Table 3. The observation in four weeks showed that the nano cream had a white color and viscous semi-solid cream with a distinct cardamom fragrance. There was no separation observed in the formulation which showed excellent stability. It was in agreement with the previous discussion related to the particle size and zeta potential. Submicron droplet size and high zeta potential value potentially form a stable dispersion system [31]. The increase in CEO concentration caused the fragrance to become stronger and the viscosity became lower. Higher CEO concentration produced larger droplet size which led to less viscous nano cream [35].

**Table 3:** Stability Study of CEO Nano Cream

| Concentration | Formulation | Week 1 | | | Week 2 | | | Week 3 | | | Week 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | Spread (mm) | Viscosity (Cp) | pH | Spread (mm) | Viscosity (Cp) | pH | Spread (mm) | Viscosity (Cp) | pH | Spread (mm) | Viscosity (Cp) |
| 2.5% | F1 | 6,42 | 52 | 6900 | 6,35 | 52 | 6850 | 6,25 | 52 | 6780 | 6,22 | 53 | 6690 |
| | F2 | 6,38 | 53 | 6810 | 6,35 | 53 | 6790 | 6,23 | 54 | 6670 | 6,19 | 54 | 6510 |
| | F3 | 6,35 | 55 | 6600 | 6,31 | 55 | 6610 | 6,23 | 56 | 6580 | 6,17 | 56 | 6530 |
| 5% | F1 | 6,22 | 53 | 6730 | 6,19 | 53 | 6590 | 6,17 | 54 | 6420 | 6,07 | 54 | 6400 |
| | F2 | 6,19 | 54 | 6520 | 6,15 | 54 | 6500 | 6,11 | 55 | 6460 | 6,04 | 56 | 6420 |
| | F3 | 6,17 | 57 | 6300 | 6,15 | 58 | 6270 | 6,07 | 58 | 6250 | 6,04 | 58 | 6210 |
| 7% | F1 | 5,53 | 54 | 6150 | 5,34 | 55 | 6110 | 5,12 | 57 | 5980 | 5,07 | 58 | 5940 |
| | F2 | 5,45 | 56 | 5930 | 5,31 | 56 | 5890 | 5,27 | 57 | 5870 | 5,24 | 59 | 5750 |
| | F3 | 5,35 | 59 | 5820 | 5,23 | 59 | 5780 | 5,11 | 60 | 5750 | 5,04 | 61 | 5710 |
| 10% | F1 | 4,64 | 60 | 5840 | 4,57 | 61 | 5830 | 4,52 | 61 | 5810 | 4,49 | 63 | 5780 |
| | F2 | 4,57 | 62 | 5640 | 4,52 | 62 | 5530 | 4,46 | 64 | 5500 | 4,30 | 65 | 5480 |
| | F3 | 4,55 | 64 | 5450 | 4,47 | 64 | 5460 | 4,42 | 65 | 5390 | 4,30 | 67 | 5350 |
| 15% | F1 | 4,57 | 62 | 5620 | 4,52 | 64 | 5600 | 4,49 | 65 | 5580 | 4,47 | 66 | 5560 |
| | F2 | 4,55 | 64 | 5460 | 4,52 | 66 | 5440 | 4,47 | 67 | 5390 | 4,36 | 69 | 5370 |
| | F3 | 4,49 | 66 | 5310 | 4,42 | 67 | 5270 | 4,34 | 68 | 5240 | 4,31 | 69 | 5210 |

**pH Value**

The pH observation in 4 weeks showed that the pH value decreased as the CEO concentration increased. The highest pH value was observed in formulation with HLB 10 with the oil concentration of 2.5% which was 6.42 and 6.22 on week 1 and week 4 respectively. The lowest pH value was observed in formulation with HLB 12 with the oil concentration of 15% which was 4.49 and 4.3 on week 1 and week 4 respectively. The essential oil concentration affected the pH value of the nano cream. According to Nardyan (2021), the acid value of CEO was 2.9172 mgKOH/g which might affect the acidity properties of the nano cream [40]. Due to the acidic nature of the CEO, the high amount of oil can alter the pH value of the final formulation. Tween 20 and Span 80 as nonionic surfactants possess neutral pH [41]. Even though the HLB value was varied the concentration remained constant in all formulations which did not significantly affect the pH value.

The topical cream preparation with a low pH of 1-4 can irritate while pH 8-14 might cause scale on the skin. The suitable pH range for cream is 4.5-6.5 [42]. According to Indonesian National Standard (SNI) No 16-4399-1996 moisturizer cream is required to have a pH range of 4.5-7.5. In this study, the pH value ranged from 4.30-6.42 which fulfilled the SNI requirement, besides the formulation F3 (10%) and F3 (15%) which had slightly lower pH observed in week 4. However, the pH value can be adjusted to meet the requirement by adding lactic acid, sodium acetate, or sodium lactate [43].

**Viscosity**

The viscosity of nano cream for 4 weeks of observation as seen in Table 4 was in the range of 5210-6910 cP. Formulation with higher HLB and higher EO concentration produced lower viscosity. The composition of high HLB consisted of lower Span 80 and higher Tween 20. Tween 20 is the hydrophilic surfactant that most likely interacted with the water phase, while Span 80 is the hydrophobic surfactant that interacted with the oil phase [33]. The fixed concentration of surfactant on each formulation got a sufficient interaction with the water phase by Tween 20 through hydrogen bonding but the low concentration of Span 80 was unable to interact with all oil molecules. Interaction between oil, water, and a sufficient amount of surfactant produced a thick dispersion system. Accordingly, lesser interaction between the surfactant with oil phase produced a low viscosity value [44]. This lack of interaction bridged to larger droplet size and led to low viscosity as previously discussed.

The viscosity observation for 4 weeks showed a decreasing trend. The rising of environmental temperature can disrupt the durability of nano cream causing a viscosity decrease of the continue phase (water) and increasing the movement of the globule dispersed phase (oil) creating the gaps on each globul. In addition, oil in water

emulsion tends to decrease in viscosity because of the water absorption from the environment by the hygroscopic ingredients in the formulation [45]. However, based on SNI 16-4399-1996 about the standard quality of the cream, the good viscosity of cream preparation ranges from 2000-50000 cP [45]. Based on the result all formulations fulfilled the SNI standard of cream preparation.

**Spreadability**

Spreadability represents the area on which a cream formulation for skin application spreads during the application, which means the greater area indicates better spreadability. The result of spreadability was in the range of 5.1-6.9 cm as seen in Table 3. The highest spreadability value was formula F3 (15%) with values of 6.6 cm and 6.9 cm for week 1 and week 4, respectively. The lowest spreadability value was formula F1 (2.5%) with values of 5.2 cm and 5.3 cm for week 1 and week 4, respectively. Spreadability is inversely proportional to viscosity value. As the viscosity decreases, the creams are spread easily on the skin. It is in agreement with the previous discussion. The formulation with a high HLB value consisted of a lower amount of Span 80 which caused nanocream in formula F3 (15%) less viscous compared with nanocream F1 (2.5%). This low viscosity leads to a wide spreadability area. From the observation, the spreadability of the nanocream was in the range of 5.2-6.9 cm which meets the criteria of semisolid preparation with a general spreadability value of 4-7 cm [46].

## CEO Nano Cream Antibacterial Activity

The antibacterial activity of CEO and CEO nano cream is shown in Figure 1. The result shows that the higher the concentration of CEO, the greater the diameter of the inhibition against bacteria. The nanocream formulation F1 (2.5%) had the lowest

inhibition zone diameter 6 mm and 6.4 mm for *E. coli* and *S. aureus*, respectively. While nanocream F3 (15%) had the highest inhibition zone 10.2 mm and 11.4 mm for *E. coli* and *S. aureus*, respectively. Several studies also reported the antibacterial activity of CEO against both Gram-positive and Gram-negative bacteria [18,19,47]. CEO has stronger antibacterial power against *S. aureus* than against *E. coli* bacteria as seen in Figure 2. Gram-positive are found to be more sensitive to essential oils than Gram-negative bacteria. This is indicated by the relatively impermeable cell walls in which the outer membrane is rich in lipopolysaccharide in Gram-negative bacteria [48].

The biological activity of CEO is linked to their chemical composition such as ester α-terpinyl acetate and monoterpene 1,8-cineole, α-terpineol, linalool, and α-pinene [1]. The monoterpene hydrocarbons and oxygenated monoterpenes in the essential oil of different plants possess major antimicrobial, antifungal, and antiviral activities [49]. Some investigations have proven that these constituents present antimicrobial effects through cell membrane disruption, restricting oxygen intake, and hanging up of virulence factors [50]. CEO has a hydrophobic nature, which enables the oil to partition the cell membrane and bacteria mitochondria. The antibacterial mechanism of essential oil is disrupting bacterial cell walls, damaging the cytoplasm membrane and protein membrane, thus leading to cell damage, cytoplasm coagulation, and depletion of the proton-motive force [51].

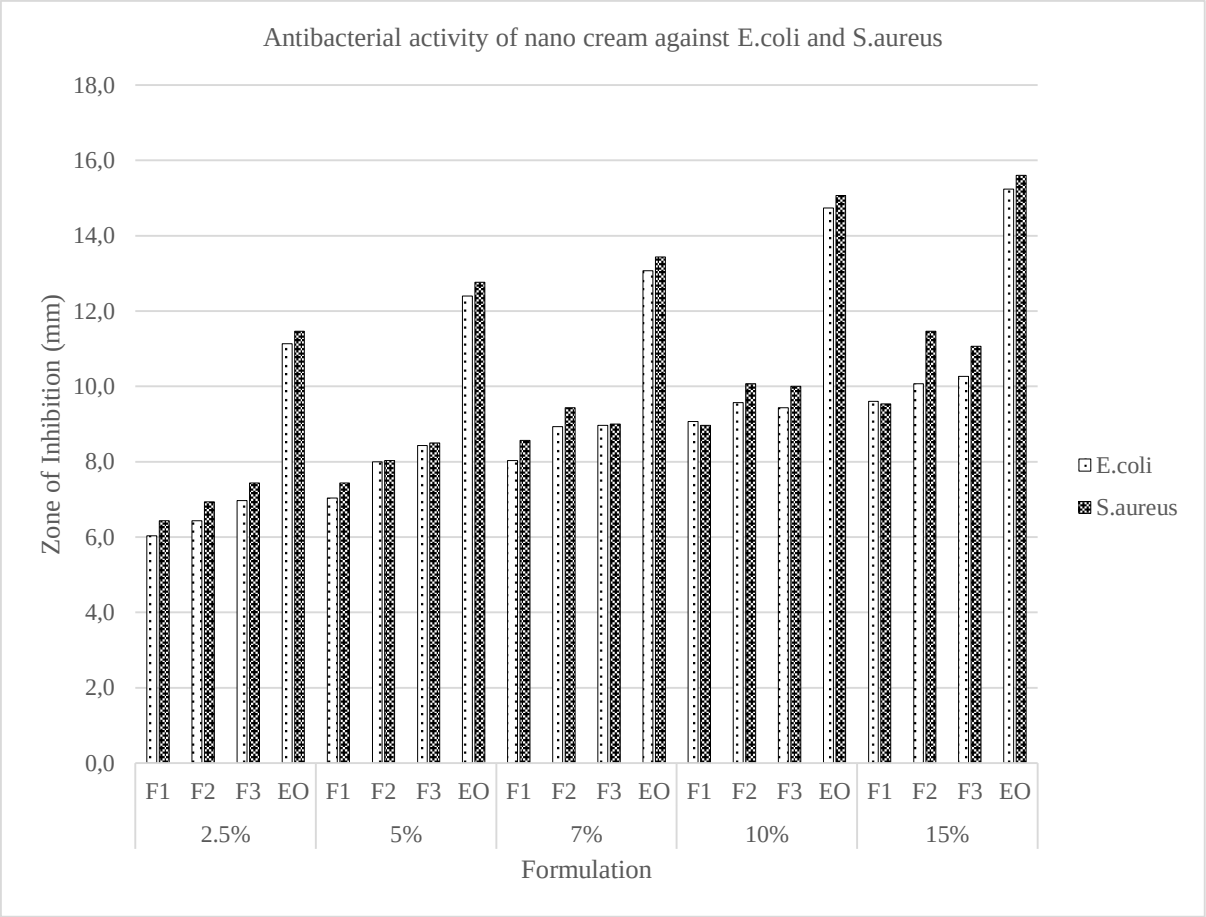


**Figure 1:** Antibacterial activity of nano cream against E.coli and S.aureus

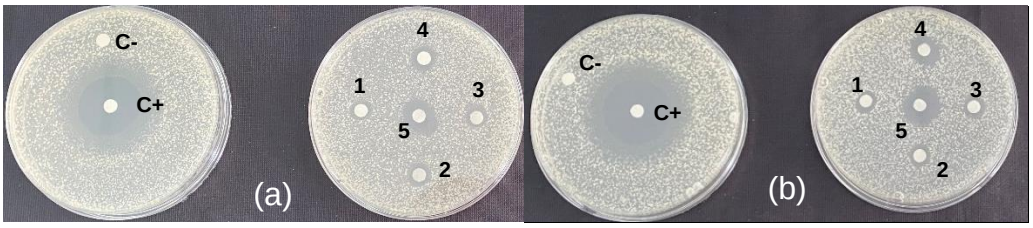


**Figure 2:** Antibacterial Activity Against (a) *E. coli* and (b) *S. aureus* of DMSO as Control Negative (C-), 1,8 Cineol as Control Positive (C+) and CEO with Concentration (1) 2.5%, (2) 5%, (3) 7%, (4) 10%, and (5) 15%

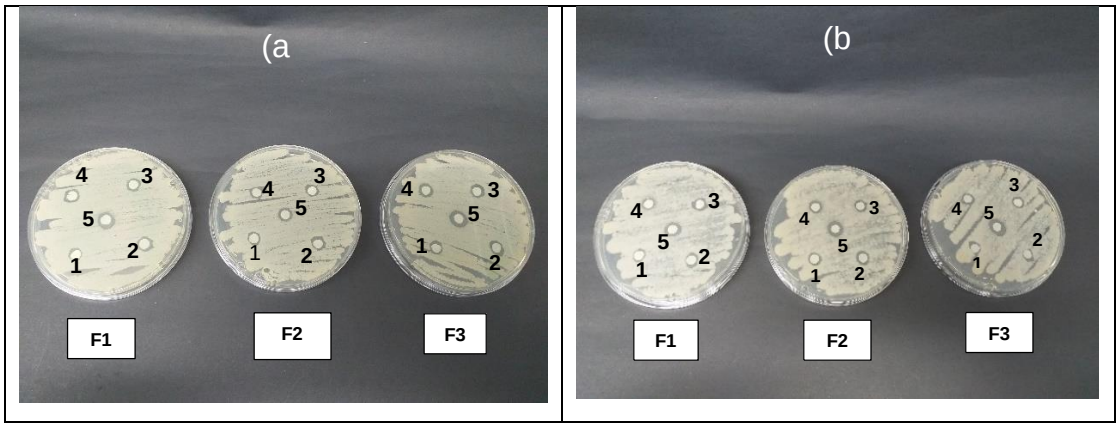


**Figure 3:** Antibacterial Activity of Cardamom Nanocream against (a) *E. coli* and (b) *S. aureus* with Cardamom Oil Concentration (1) 2.5%, (2) 5%, (3) 7%, (4) 10%, and (5) 15%

It is also seen that the CEO in nano cream formulation had lower antibacterial activity compared with free CEO. The nano cream formulation experienced several processes including homogenizing with high speed which might cause the volatile compound to degrade and evaporate due to heat which was generated during the process which might contribute to the activity of the CEO [52]. However, the loaded CEO in the nano cream system has sustained release that can prolong the action of the CEO on microorganisms. The activity might be lower compared to the free CEO, but the effect could last longer. The study by Feng, et al. (2022) reported that free essential oil gave higher antibacterial activity on certain microorganisms compared with the nanoemulsion form. Nonetheless, the essential oil loaded in a nanoemulsion system could maintain its inhibitory activity against bacteria within 12 hours [53].

The effect of variable HLB and CEO concentration against antibacterial activity was evaluated statistically. The statistical analysis shows that the inhibition zone data was normal-distributed ($p > 0.05$) for the difference in HLB and concentration for *S.aures* and otherwise for *E.coli*. The data of *S.aures* was identical for every HLB (Sig. = 0.22 > 0.05) and non-identical for concentration factor HLB (Sig. = 0.03 < 0.05),

which means there is an influence of concentration on antibacterial activity. For the HLB factor, ANOVA and post hoc test (Tukey's HSD and Duncan) showed that Sig, >0.05, which means HLB or formulations did not affect the antibacterial activity. Meanwhile, for the concentration factor, the Kruskal Wallis test showed that Sig. <0.05. For the data of *E.coli*, the Kruskal Wallis test also showed that Sig. <0.05 which means concentration influences the antibacterial activity for both bacteria.

# Conclusion

The preparation, characterization, and antibacterial activity of cardamom essential oil nano cream was successfully carried out. The nano cream containing cardamom essential oil was stable during the stability test for 4 weeks based on the parameters of physical appearance, pH, viscosity, and spreadability. The particle size of the nano cream ranged from 100-300 nm and zeta potential -80 – (-90) mV which also represents a stable nano cream. Cardamom essential oil showed favorable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Statistical analysis showed that the concentration of the essential oil was the significant factor that affected the antibacterial activity while the HLB value did not significantly play a role in enhancing the antibacterial activity. The high antibacterial activity should took into account to provide good efficacy. Thus, the optimum condition was F3 (15%) with inhibition zone 10.2 mm and 11.4 mm for *E. coli* and *S. aureus*, respectively. The average particle size was 270.40 ± 0.38 nm and zeta potential -88.70 ± 0.20 mV, wherein still meets the nanoemulsion size range that enable penetrated into stratum corneum. Eventually, cardamom essential nanocream can be considered as a potential antibacterial topical preparation.

# Experimental

## Essential Oil Extraction

Cardamom (Amomum cardamomum) was obtained from Padang, West Sumatra, Indonesia. The essential oil was extracted using the ultrasonic-assisted hydrodistillation (UAE-HD) process. As a pre-treatment, 100 g of sample was immersed in 2L of water and irradiated with ultrasonic waves using a Qsonica Sonicator Q1375 with a size of probe 1” (25 mm) to a depth of about ± 3 cm, with 90% amplitude for 15 minutes. Then, the mixture was hydrodistilled for 6 hours using clevenger type equipment [40]. The oil extracted stored in the dark airtight containers at room temperature away from direct sunlight to preserve their stability and efficacy.

## Gas Chromatography Analysis

The components of cardamom essential oil compounds were analyzed using the gas chromatography–mass spectrometry (GC-MS) instrument. GC-MS analysis was performed using an Agilent 15977A Network GC System equipped with an Agilent 7890B Series autoinjector and an Agilent 5977A Mass Selective Detector. The carrier gas used was He at 40 mL. $min^{-1}$, DB 1 as the column (pressure 8.8085 psi), and Electron Impact (EI) as the ionizer. Samples were heated from 70 °C to 250 °C at a heating rate of 10 °C/min. The temperature of the detector and injector is 250 °C for the first time for 1 minute.

## Nano Cream Formulation

The nano cream formula was made according to Septiyanti (2017) with variations in HLB values referring to the calculation of surfactant HLB [55]. The HLB was calculated using the equation (1).

$$HLB\ blend = (\%\ Surfactant\ A \times HLB\ Surfactant\ A) + (\%Surfactant\ B\ \times HLB\ Surfactant\ B) \quad (1)$$

The nano cream formulation consists of oil and water phases which are specified in Table 4. The oil and water phases were stirred using a hotplate stirrer at a speed of 350 rpm, at a temperature of 70 °C for 30 minutes separately. Then, the oil phase was gradually added to the water phase while stirring using an ultra turrax homogenizer at a speed of 12000 rpm for 30 minutes.

**Table 4:** The Composition of Cordomum Essential Oil Nanocream

| HLB | Formula | Oil phase (%) | | | | | | Water phase (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Essential Oil | Span 80 | Cetyl alcohol | Stearic acid | Avocado oil | Glycerin | Tween 20 | Methyl paraben | Propylene glycol | Distilled water |
| 10 | F1 (control) | 0 | 5.4 | 5 | 3 | 2 | 4 | 4.6 | 0.25 | 4 | 71.75 |
| | F1 (2.5%) | 2.5 | 5.4 | 5 | 3 | 2 | 4 | 4.6 | 0.25 | 4 | 69.25 |
| | F1 (5%) | 5 | 5.4 | 5 | 3 | 2 | 4 | 4.6 | 0.25 | 4 | 66.75 |
| | F1 (7%) | 7 | 5.4 | 5 | 3 | 2 | 4 | 4.6 | 0.25 | 4 | 64.75 |
| | F1 (10%) | 10 | 5.4 | 5 | 3 | 2 | 4 | 4.6 | 0.25 | 4 | 61.75 |
| | F1 (15%) | 15 | 5.4 | 5 | 3 | 2 | 4 | 4.6 | 0.25 | 4 | 56.75 |
| 11 | F2 (control) | 0 | 4.6 | 5 | 3 | 2 | 4 | 5.4 | 0.25 | 4 | 71.75 |
| | F2 (2.5%) | 2.5 | 4.6 | 5 | 3 | 2 | 4 | 5.4 | 0.25 | 4 | 69.25 |
| | F2 (5%) | 5 | 4.6 | 5 | 3 | 2 | 4 | 5.4 | 0.25 | 4 | 66.75 |
| | F2 (7%) | 7 | 4.6 | 5 | 3 | 2 | 4 | 5.4 | 0.25 | 4 | 64.75 |
| | F2 (10%) | 10 | 4.6 | 5 | 3 | 2 | 4 | 5.4 | 0.25 | 4 | 61.75 |
| | F2 (15%) | 15 | 4.6 | 5 | 3 | 2 | 4 | 5.4 | 0.25 | 4 | 56.75 |
| 12 | F3 (control) | 0 | 3.8 | 5 | 3 | 2 | 4 | 6.2 | 0.25 | 4 | 71.75 |
| | F3 (2.5%) | 2.5 | 3.8 | 5 | 3 | 2 | 4 | 6.2 | 0.25 | 4 | 69.25 |
| | F3 (5%) | 5 | 3.8 | 5 | 3 | 2 | 4 | 6.2 | 0.25 | 4 | 66.75 |
| | F3 (7%) | 7 | 3.8 | 5 | 3 | 2 | 4 | 6.2 | 0.25 | 4 | 64.75 |
| | F3 (10%) | 10 | 3.8 | 5 | 3 | 2 | 4 | 6.2 | 0.25 | 4 | 61.75 |
| | F3 (15%) | 15 | 3.8 | 5 | 3 | 2 | 4 | 6.2 | 0.25 | 4 | 56.75 |

## Nano Cream Characterization

Physicochemical characterization studies were carried out to characterize and control the physical stability of the formulated nano cream. The physicochemical properties studied were particle size, zeta potential, pH values, spreadability, viscosity, and organoleptic. Determination of nano cream particle size and zeta potential was carried out using a particle size analyzer (PSA) (Horiba Nano Partica SZ-100). The

particle size was analyzed by diluting 1 mg of sample in 10 ml deionized water and put in the glass cuvette. The scattering angle is 90$^{o}$, with ND filter 10-30%, temperature sample holder 25$^{o}$C. The pH value was measured by immersing the pH electrode into the diluted nano cream using a pH meter (744 pH Meter Metrohm). The spreadability test was conducted by weighing and placing 0.5 grams of nano cream in the center of the petri dish. Then the petri dish loaded with 250 grams of glass on the nano cream surface and left for 1 minute. The diameter of the nano cream was measured by taking the average diameter length from several sides. The nano cream viscosity test was carried out based on SNI 16-4399-19 using a Brookfield Viscometer. The organoleptic test was conducted by observing the smell, color, and appearance of the nano cream.

## Antibacterial Study

The test bacteria were made in the form of a bacterial suspension of *E. Coli* (Gram negative) and *S. Aureus* (Gram positive). The bacterial suspension was measured at a wavelength of 625 nm and reached an absorbance of 0.08-0.1, equivalent to a microorganism concentration of $1.5 \times 10^{8}$ mL based on McFarland standards. Each suspension of *E. coli* and *S. aureus* bacteria was taken as much as 100 µL and was put into a sterile petri dish. Then 10-15 mL of Mueller Hinton Agar (MHA) medium was added and homogenized. A paper disk was affixed to the medium and dripped with 10 µL of cardamom oil with concentrations of 2,5%; 7%; 5%; 10%; and 15% diluted by DMSO, DMSO (negative control), and standard cineol (positive control) without further dilution and then incubated at 37 °C for 24 hours. The same procedure was done with nanocream sample. CEO nanocream was diluted by DMSO with concentration of 2,5%; 7%; 5%; 10%; and 15%, then 10 µL solution was dropped into the paper disc and then incubated at 37 °C for 24 hours. The clear zone was

measured using a ruler from one end of the clear zone to the other and subtracted from the diameter of the disc (5 mm).

### Statistical Analysis

All statistical tests and analyses were achieved using SPSS (IBM SPSS Version 23.0). Data were analyzed using Shapiro-Wilk and Levene's tests to evaluate normality and homogeneity of variance. Then, analysis of variance (ANOVA) was applied for the normal-distributed data and Kruskal-Wallis analysis otherwise. The post hoc Tukey's HSD and Duncan tests were conducted when the data differed significantly from one another according to the preliminary testing. A significance level of a = 0.05 was selected, and differences were considered significant at $p < 0.05$.

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