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Formulation and Characterization of Amomum Compactum Sol. Ex Maton Nano Emulsion and Its Antibacterial and Wound Healing Activity

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Abstract: Natural treatment is plant extract nanoemulsion. Forming nanoemulsions with a specific method may help skin wound healing. Fruit cardamom (Amomum compactum Sol. Ex Maton) was used as an antimicrobial, but its wound-healing activity was unstudied. This study compares nanoemulsion from cardamom seed ethanol extract to cardamom extract in suppressing bacterial growth, wound healing, and determining component makeup. Research methods with Lab experiments involve sampling, processing, extracting, and UV-VIS spectrophotometer testing secondary metabolite chemicals, creating nanoemulsions, and characterizing them with HPLC, FTIR, UV-VIS, and PSA. TPC and disc diffusion for antibacterial and wound healing characterization and SPSS analysis. The research results found high quantities of gallic acid, catechin, and epigallocatechin were found in 20% (v/v) cardamom extract nanoemulsion, with HPLC analysis showing levels of 4590.10 µg/mL, 2128.95µg/mL, and 755.42µg/mL, respectively. FTIR and UV-Vis tests indicate a single peak around the phenol compound's absorption area and describe its group by absorbing functional groups in the IR spectrum. Nanoemulsion suppressed *E. coli* and *S. aureus* better than cardamom extract. At 1% (v/v), nanoemulsion heals wounds faster than cardamom extract and gentamicin sulfate. The particle size of 53.13 ± 1.25 nm and particle size distribution of 0.56 ± 0.02 nm contribute to the stability and high activity of the nanoemulsion.

Keywords: Antibacterial; Cardamom; Nanoemulsion; Wound healing

Introduction

Preventing the spread of disease-causing bacteria during food production is an essential study area. Some examples of harmful microorganisms that can cause a wide range of illnesses and, in the worst circumstances, death include Staphylococcus aureus, Salmonella, and Campylobacteria (Ekumankama, 2022; El-Shenawy et al., 2016). Concerns about the possibility of foodborne illness are a constant source of anxiety for the food business, including producers, regulators, scientists, and consumers (Vieira et al., 2022). Plants can produce tens to hundreds of thousands of metabolites, each with unique biological features and functions. About one percent of all secondary metabolites in plants are volatile organic molecules produced in the plant's primary and secondary metabolic pathways (Derbassi et al., 2022).

Cardamom is utilized as a spice and traditional medicine. Cardamom treats dyspepsia, hiccups, vomiting, and alcohol detoxification (Duke et al., 2022). Additionally, cardamom is a traditional medication for mouth and throat infections, TB, and kidney and gall stones (Badnale et al., 2022; Karadağ et al., 2020). Previous research has shown that cardamom's antioxidant capability increases glutathione levels, which is directly proportional to the dose of essential oil from cardamom extract (Castillo et al., 2023; Raissa et al., 2020). Other studies suggest that cardamom leaf extract lowers blood sugar in diabetic rats. *Elletaria cardamom* fruit inhibited *Escherichia coli* and *Staphylococcus aureus*. Cardamom has 0.317-1.66 g of total phenolics and 11.33-

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14.63 g of total flavonoids per 100 g (Abdullah et al., 2021; Yassin et al., 2022).

Due to its hydrophobicity, volatility, and molecular reactivity, this natural substance still presents numerous challenges in use and application. Nanotechnology can solve these issues in several industries, including food (Kumar et al., 2020). Making nanoemulsions from natural substances may solve the issues. Nanoemulsions from natural component extracts boost biocompound physical stability and activity. Tiny nanoemulsion droplets destabilize the lipid bilayer by the cell membrane, increasing antibacterial action. This causes cell death and dysfunction. High-pressure and low-pressure approaches can make nanoemulsions (Hidajat et al., 2020). Research objectives to determine the secondary metabolite content of cardamom (Amomum compactum Sol. Ex Maton) and the effect of cardamom extract nanoemulsion as a potential antibacterial.

Method

This research method with lab experiments involves sampling, processing, extracting, and UV-VIS spectrophotometer testing of secondary metabolite chemicals. I am making nanoemulsions and characterizing them via HPLC, FTIR, UV-VIS, and PSA. Total plate count (TPC) and disc diffusion for antibacterial and wound healing characterization and SPSS data analysis.

The investigation was conducted at the NRE investigation Center, Medan, and a well-equipped and experienced facility. A variable is any measurable or calculable attribute, number, or quantity (Suwarno & Nugroho, 2023). Cardamom nanoemulsion formulation is the first variable in this study. The second variable is cardamom secondary metabolite compound analysis (Amomum compactum Sol. Ex Maton). The key variables are Independent, dependent, and controlled variables (Notoatmodjo, 2018). The study's independent variable was cardamom extract. The dependent variable is secondary metabolite compound analysis. Laboratory measurements of cardamom extract nanoemulsion production are the controlled variable.

This research utilized a macerator, ultrasonicator, test tube, spatula, 50 mesh sieve, Eppendorf micropipette (1-10 µl, 50-200 µl, 100-1000 µl), Borusil Tips 10, 200, 1000 µl, muslin cloth, Whatman filter paper No. 1, water bath, NEPTUNE Tips 10, 200, 1000 µl, pH meter (OHAUS Starter300 portable), Erlenmeyer, magnetic stirrer, hot plate (Thermo Fisher Scientific), and incubator (ESCO IFA-32). 15 ml falcon tube (SPL, 50015), 50 ml falcon tube (SPL, 50050), analytical balance (AXIS), 1.5 ml Eppendorf tube (SPL, 60015-1), ose needle, tweezers, HPLC, PSA, FTIR, UV-VIS, and TEM. Cardamom extract (Amomum compactum Sol. Ex Maton) was tested. E. coli and S. aureus. Triuoroacetic acid, KBr, NaCl, ethanol, Tween 80, NA, and distilled water are employed.

This project will use the maceration method to extract cardamom fruit without heating. HLPC and FTIR are used to describe the nanoemulsion after it is made. Measure ultraviolet/visible spectroscopy. Then, assess antibacterial activity with TPC and Disc Diffusion. Now, 25 white mice (male Wistar rats) were prepared for testing—treatment method. The mice were lab-adapted for seven days and fed conventional feed. Then, 25 white mice received wounds created with a 4 cm2 incision from the back skin of white mice at 1 mm depth. Data was examined in MS Excel 2019 and statistically calculated in IBM SPSS Statistics 21—Igor Pro.5 and Excel 2019 created graphs. The compound's chemical structure was drawn in ChemDraw Ultra 10.0.

Result and Discussion

High-Performance Liquid Chromatography (HPLC) Analysis

A reverse-phase HPLC approach was used using polar solvents such as water, methanol, ethanol, or acetonitrile with a C18 column. I was adding acetonitrile to the eluent accelerated compound separation (Kumar et al., 2022). HPLC separated nanoemulsion components from cardamom extract, but not optimally—peaks 2 and 3 almost overlapped but were still visible. Since the extract was crude, such overlap is usual. A purified extract would be different. Figure 1 shows the cardamom extract nanoemulsion analytical chromatogram.

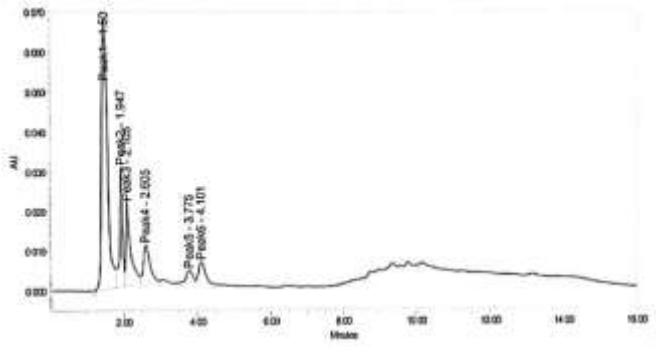

Figure 1. Chromatogram of the HPLC analysis of the cardamom extract nanoemulsion

In this investigation, six peaks indicated six substances. Peaks 2, 4, and 5 represent gallic acid (GA), epigallocatechin (EGC), and catechin. This study used standard curves for these three chemicals (Appendix). The chromatogram shows that Peak 1 is the chemical with the highest content in this ethanol extract of cardamom seeds, followed by Peak 2 (GA), Peak 3, Peak

4 (EGC), Peak 6, and Peak 5 (C). Figure 2 shows gallic acid, catechin, and epigallocatechin structures.

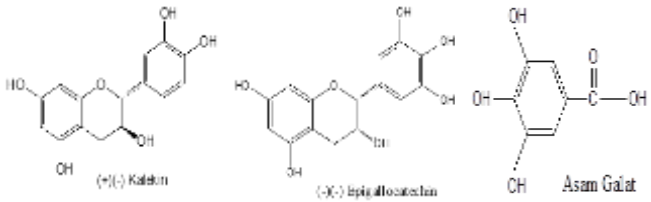

Figure 2. The chemical structures of Gallic Acid (GA), Epigallocatechin (EGC), and Catechin (C)

FTIR Characterization

FTIR examination of the nanoemulsion determined the isolated chemicals' functional groups. The FTIR spectrum records these functional group vibrations. Figure 3 shows the ethanol-extracted nanoemulsion components' FTIR spectra.

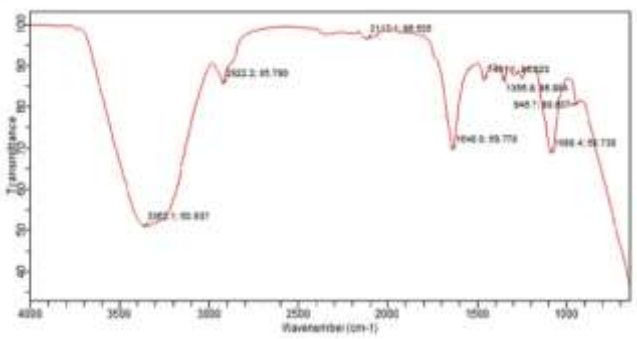

Figure 3. FTIR Spectrum of the nanoemulsion from ethanol extract of cardamom seeds

UV-Vis Analysis

UV-Vis spectrophotometry was used to analyze cardamom seed nanoemulsion and ethanol extract. This approach helps characterize extract chemical structures. UV-Vis spectrophotometry detects the wavelength and intensity of ultraviolet and visible light absorbed by the sample, cardamom seed nanoemulsion, and ethanol extract (Keivani Nahr et al., 2020). Figure 4 shows cardamom nanoemulsion and ethanol seed extract UV-Vis spectra.

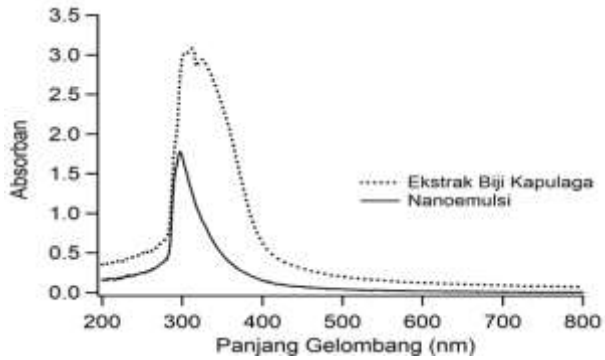

Figure 4. UV-Vis Spectra of nanoemulsion and ethanol extract of cardamom seeds

Characterization of Particle Size and Particle Size Distribution of Cardamom Seed Extract Nanoemulsion

A Particle Size Analyzer (PSA) was used to analyze the nanoemulsion from cardamom seed ethanol extract's particle size and dispersion. To ensure stability, the nanoemulsion was produced in water using an ultrasonic homogenizer (Ghazy et al., 2021). Figure 5 displays cardamom extract nanoemulsion particle size analysis results.

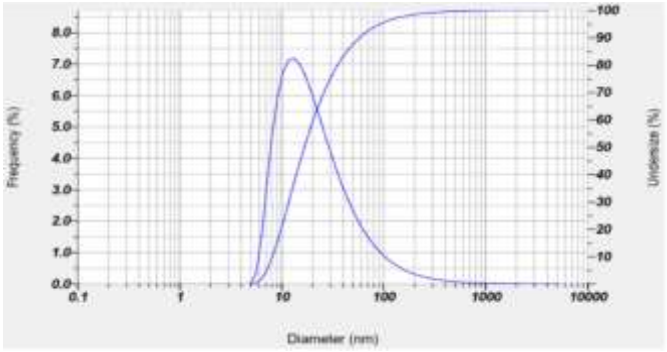

Figure 5. Results of the particle size analysis of the cardamom seed extract nanoemulsion using PSA

In Figure 5, the peak at 12.4 nm indicates that the nanoemulsion sample's most prevalent particle population has a diameter of 12.4 nm and a center of gravity of 31.3 nm. To ensure measurement accuracy, this procedure was repeated three times. Table 1 shows the Z-Average and PDI (Polydispersity Index) results.

Table 1. The values of Z-Average and PDI (Polydispersity Index)

Repetition	Z-Average (nm)	PDI
1	52.2	0.567
2	52.3	0.541
3	54.9	0.589

Antibacterial Activity of Cardamom Seed Extract Nanoemulsion and Cardamom Seed Extract against Escherichia coli

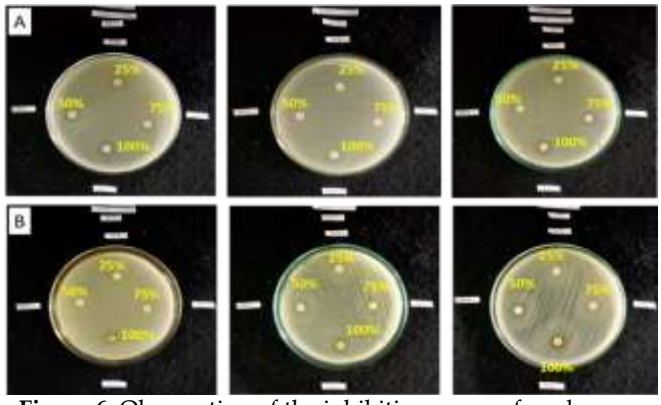

Figure 6. Observation of the inhibition zones of cardamom seed extract nanoemulsion (A) and cardamom seed extract (B) Against E. Coli with three repetitions

The antibacterial activity of cardamom seed extract nanoemulsion and cardamom seed extract at concentrations of 25%, 50%, 75%, and 100% was tested. Each petri dish was placed with a series of concentration variations of the sample, with three repetitions. The results of observations after the Petri dishes were incubated for 24 hours can be seen in Figure 6.

Antibacterial Activity of Cardamom Seed Extract Nanoemulsion and Cardamom Seed Extract against Staphylococcus aureus

The potential of cardamom seed extract nanoemulsion and extract to prevent S. aureus growth was also examined. Four 24-hour incubations were performed on 25%, 50%, 75%, and 100% samples. Figure 7 shows the inhibition zone area and antibacterial index observations and computations.

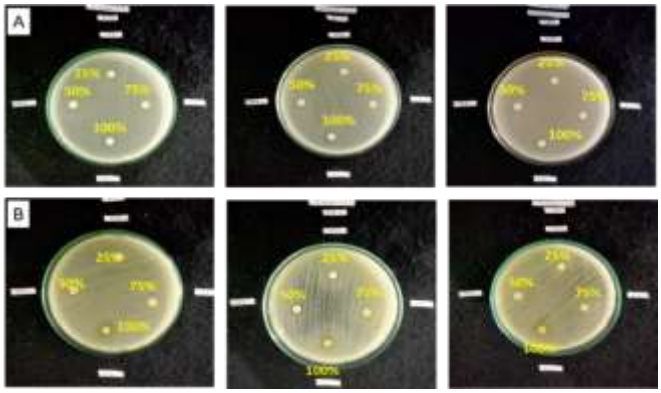

Figure 7. Observation of the inhibition zones of cardamom seed extract nanoemulsion (A) and Cardamom seed extract (B) Against S. Aureus with three repetitions

Wound Healing

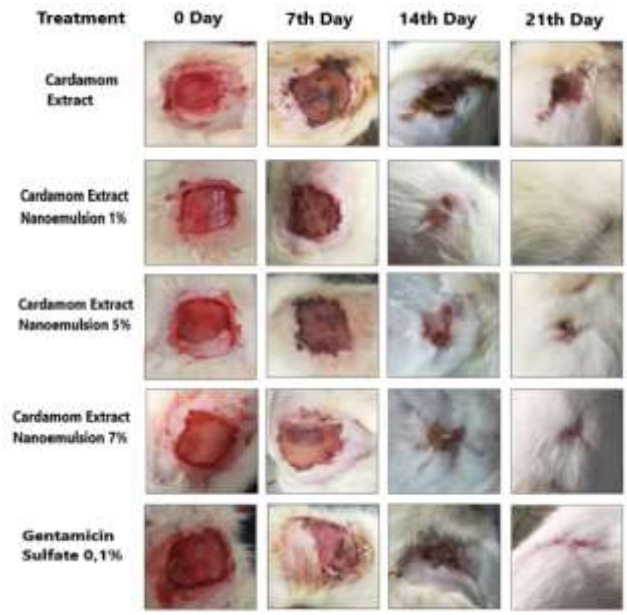

Figure 8. Observation of wounds on wistar rats on days 0, 7, 14, and 21

Five groups were tested for cardamom seed extract and nanoemulsion's ability to heal rat wounds: one with incision wounds treated with extract, one with 1% (v/v), one with 5%, one with 7%, and a positive control group treated with gentamicin sulfate. Treatments were repeated five times over 21 days. The observations are shown in Figure 8.

Discussion

Cardamom seed extract nanoemulsion's bioactive components, including gallic acid, catechin, and epigallocatechin, make it promising for wound healing and antimicrobial applications. This work developed a stable cardamom seed extract nanoemulsion and tested its antibacterial and wound-healing properties. The gallic acid content in 20% (v/v) nanoemulsion of ethanol extract from cardamom seeds is 4,590.1 µg/mL or 4.59 mg/mL, as determined by the chromatogram. This is higher than the content of cardamom seed ethanol extract phenolic component (Ghazy et al., 2021).

Gallic acid is recognized for its antioxidant properties due to its three hydroxyl (-OH) groups and carboxylic acid (-COOH) group. It has anti-diabetic, anti-tumor, anti-bacterial, and anti-inflammatory properties (Bai et al., 2021). The 20% (v/v) nanoemulsion sample of cardamom extract contains 755.43 µg/mL or 0.755 mg/mL of catechin, the second discovered component. Catechin, which has anti-diabetic, anti-cancer, antioxidant, and cytoprotective characteristics, is essential despite its low level (Abdallah et al., 2019). Epigallocatechin is the latest confirmed compound. EGC is a flavonoid like catechin. EGC is green tea's second most abundant flavonol (Ambigaipalan et al., 2020).

Due to its hydrogen bond (-OH) stabilization of free radicals, EGC has significant radical-scavenging activity (Qiu et al., 2022). HPLC examination shows that the cardamom seed ethanol extract contains a high concentration of these chemicals, proving the cold extraction method's efficacy. These chemicals have high content, suggesting food and medicinal applications (Ghazy et al., 2021).

According to FTIR, the cardamom seed ethanol extract contains phenolic chemicals (Raissa et al., 2020; Srivastav et al., 2021). The firm, broad FTIR peaks at 3362.1 cm-1 indicate carboxylic acid and phenolic group O-H bonding. In addition, FTIR analysis shows C=C stretching vibrations at 1461.1 cm-1, indicating phenyl rings in the compounds. Nanoemulsion and cardamom extract had a single UV-Vis peak at 297 and 312 nm, respectively. Typical absorption peaks of phenolic compounds are 200-400 nm (Sanches et al., 2022). Differences in extract solvents may explain the minor absorption peak shift (Xu et al., 2020).

The UV-Vis data show that emulsification does not change the chemical characteristics of the extract's

bioactive components. PDI data show that nanoemulsion particle sizes are relatively homogeneous. Stability results from monodispersity and particle size uniformity with a PDI below 1 (Baliyan et al., 2020). The nanoemulsion's Z-Average results show submicron-sized particles at 53.13 nm. The nanoemulsion is stable and ideal for food and pharmaceutical applications due to its small particle size, achieved by ultrasonication with Tween 80. Antimicrobial activity of nanoemulsion and cardamom extract against E. coli and S. aureus. Compared to Gram-positive bacteria, the nanoemulsion and extract more effectively inhibit Gram-negative bacteria, especially E. coli. Phenolic chemicals break bacterium cell walls, causing cell leakage and contributing to their antibacterial properties (Aldulaimi, 2018). Discrepancies in bacterial cell wall structure and components explain the discrepancies in action against different strains. Gram-negative bacteria are more permeable to polar substances like cardamom extract because porin proteins produce hydrophilic holes in their outer membranes. This explains the more potent Gram-negative bacteria inhibition (Bajaj et al., 2016).

The active components of gallic acid, catechin, and EGC in the 1% nanoemulsion therapy can expedite wound healing. These chemicals increase fibroblast viability, migration, and collagen formation, speeding wound healing. These chemicals' antioxidant characteristics limit ROS and oxidative stress, which aid wound healing (Barku, 2019; Juszczak et al., 2022). The study found that ethanol cardamom extracts better suppress harmful microorganisms like E. coli and S. aureus than methanol extracts. The cardamom seed ethanol extract and nanoemulsion show promising biological capabilities, including antioxidant and antibacterial activity and wound healing potential.

Conclusion

The formulation of cardamom seed extract nanoemulsion presents a promising approach for antibacterial applications and wound healing. The study reveals a high concentration of bioactive compounds, such as gallic acid, catechin, and epigallocatechin (EGC), in the nanoemulsion. These compounds are known for their antibacterial, antioxidant, and wound-healing properties. The developed nanoemulsion exhibits a stable and monodisperse particle size distribution with an average particle size of approximately 53.13 nm. The particle size is ideal for enhanced stability and efficient drug delivery. Antimicrobial tests conducted on Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) demonstrate the promising antibacterial activity of the nanoemulsion, especially against E. coli. The formulation is more effective against Gram-negative bacteria (E. coli) than Gram-positive bacteria (S. aureus). The observed antibacterial activity is attributed to the polar compounds present in the cardamom seed extract. In wound healing studies, the nanoemulsion with 1% concentration exhibits accelerated wound closure. This is likely due to the bioactive compounds in the nanoemulsion, including flavonoids, alkaloids, terpenoids, tannins, gallic acid, catechin, and EGC, which promote fibroblast migration and enhance antioxidant activity. These properties are pivotal in reducing oxidative stress, controlling reactive oxygen species (ROS), and speeding up wound healing. In summary, the nanoemulsion derived from cardamom seed extract demonstrates significant potential for use in antibacterial applications and wound healing. The high content of bioactive compounds, stability, and antimicrobial activity, particularly against E. coli, make it a promising candidate for further development in pharmaceutical and biomedical fields. The findings open the door to novel natural formulations for addressing bacterial infections and improving wound healing processes.

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Author Contributions
Ali Napiah Nasution conceptualized the research idea, designed of methodology, management and coordination responsibility; Rina Fitrisia and Susanthy Tio analyzed data, conducted a research and investigation process; Ermi Girsang and Maya Sari Mutia conducted literature review and provided critical feedback on the manuscript.

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Conflicts of Interest
The author declared no conflict of interest.

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