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RESEARCH ARTICLE

Clove Extract and Grape Seed Oil Nanoemulsion for Oral Diseases Therapy: Antibacterial and Antioxidant Activities

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Abstract

The growth of micro-organisms that acquire resistance to most commercially available antibiotics is occurring rapidly. Consequently, a pressing necessity exists to identify and detect new antimicrobial substances. This study aimed to analyze the antioxidant and antibacterial activity of nanoemulsion clove extract and grape seed oil. This research was conducted in June 2023 using experimental methods at the Research Laboratory of the Universitas Islam Bandung Pharmaceutical Study Program by developing a nanoemulsion preparation containing clove extract (*Syzygium aromaticum* L.) and grape seed oil (*Vitis vinifera* L.). Antioxidant activity was tested using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Antibacterial activity was tested using the agar diffusion method by measuring the growth inhibitory diameter of *Staphylococcus aureus* and *Streptococcus mutans* bacteria and divided into four groups formulas based on the addition of clove extract with different concentrations in the nanoemulsion base (FA=0.25%, FB=0.5%, FC=0.75%, and FD=1%) to see the best results. The result shows nanoemulsion preparations have antioxidant properties in the DPPH test. The FA formula has the highest IC_{50}, namely 1,117.56 ppm. The antibacterial activity of *Staphylococcus aureus* and *Streptococcus mutans* has an inhibition zone, although it is still in the category of inhibiting bacterial growth, but does not kill growth. The nanoemulsion formulation, comprising clove extract and grape seed oil, has exhibited exceptional antioxidant properties and substantial antimicrobial efficacy against prevalent oral bacterial strains.

Keywords: Antibacterial, antioxidant, clove extract, grape seed oil, nanoemulsion

Introduction

Antibiotic-resistant microbes are a global issue. The utilization of medicinal plants as phytochemical sources is being developed to circumvent this issue. Many benefits come from active substances. One phytochemical that inhibits *Streptococcus mutans* has been found by studying many plant-derived substances and their active components for years.[1]

The gold standard for *Streptococcus mutans* treatment is chlorhexidine; however, adverse oral effects limit its usage. Worse, most antibacterials cause bacterial resistance. Current therapeutic research should focus on naturally available, safe medications, especially for oral disorders with few side effects, and can fully treat patients. Clove and grape oils have been examined for dental health. Several research studies show that this plant extract has antioxidant and antibacterial abilities.[2] Natural ingredients contain many biologically active chemical molecules.[3,4] Nanoemulsions are being developed as drugs. Nanoemulsions made from natural oils have been extensively studied for oral health issues.[5,6]

Clove (*Syzygium aromaticum*) is a plant native to the Maluku islands in Eastern Indonesia.[7] Clove oil contains essential oils such as β-caryophyllene, eugenol, and eugenol acetate.[8] Grape seed oil (GSO) is rich in bioactive components with health benefits. GSO possesses antioxidant, anti-inflammatory, and metabolic disease-fighting capabilities.[9] GSO contains several phenolic components, including flavonoids, carotenoids, phenolic acids, tannins, and stilbenes.[10] Jafri and Ahmad[11] found that clove oil and eugenol may fight dangerous bacteria biofilms alone or together. Microscopy confirmed

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the outstanding results of the combination treatment on preexisting *Staphylococcus aureus* and *Streptococcus mutans* biofilms. Grape seed extract was found to be antibacterial against *S. aureus* and *S. mutans* in another study.[12]

According to the explanation, phytodentistry does not currently use clove-grape seed oil nanoemulsion. This formulation is claimed to absorb antioxidants and antibacterials better. This study examines a nanoemulsion's antibacterial and antioxidant properties with clove extract and grape seed oil against bacterial-induced oral diseases.

Methods

This experiment was conducted in June 2023 at the Research Laboratory of the Universitas Islam Bandung Pharmaceutical Study Program by developing a nanoemulsion preparation combining clove extract (*Syzygium aromaticum* L.) with grape seed oil (*Vitis vinifera* L.). Clove samples (*Syzygium aromaticum* L.) were taken from the West Java agricultural area via a company, Friends of Indonesian Spices. The clove samples were determined at the Herbarium Bandungense, School of Life Sciences and Technology, Institut Teknologi Bandung, with registration number 3470/IT1.CC11.2/TA.00/2023. Grape seed oil was obtained from PT Tamba Sanjiwani, Tabanan regency, Bali, using the TSb product brand.

Fresh cloves obtained in packaged form have gone through a washing and drying process in the sun using the natural air-dried method. The clove samples were then ground using a blender until they became simplicia powder.[13] The preparation was made into four different formulas based on adding clove extract with different concentrations on the nanoemulsion basis (FA=0.25%, FB=0.5%, FC=0.75%, and FD=1%).

The method used for the antioxidant test begins with preparing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution and determining the maximum wavelength. A total of 6 mg of DPPH powder was weighed and then dissolved in 100 ml of ethanol p.a. (60 ppm).[14] Preparation of blank solution was done as follows: a total of 2 ml of DPPH solution (60 ppm) was pipetted and put into a test tube, then added with ethanol p.a. to 2 ml and homogenized.[15] Preparation of test solutions for nanoemulsion preparations of grape seed oil and clove ethanol extract was done as follows: a nanoemulsion stock solution was made by weighing 500 mg of the nanoemulsion sample and then dissolving it in 50 ml of ethanol p.a. (10,000 ppm), a concentration of 400 was made; 600; 800; 1,000; and 1,200 ppm. 2 ml of each dilution concentration was taken into a test tube then 2 ml of 60 ppm DPPH solution was added.

The reaction tube was homogenized using a vortex and then incubated in the dark for 30 minutes. Preparation of clove ethanol extract test solution was done as follows: weighed 50 mg of clove extract, then dissolved it in 50 ml of ethanol p.a. (1,000 ppm), concentrations then made in 2, 4, 6, 8, and 10 ppm. 2 ml of each dilution concentration was taken into a test tube then 2 ml of 60 ppm DPPH solution was added. The reaction tube was homogenized using a vortex and then incubated in the dark for 30 minutes. Absorption measurements were done using a UV-Vis spectrophotometer; the blank solution, the test sample solution, and the comparison solution for vitamin C preparations were measured for absorption at a wavelength of 516 nm. Lastly, the calculation of % inhibition and IC_{50} was done as follows: The free radical inhibition activity of DPPH with the test solution of nanoemulsion preparation of grape seed oil and ethanol extract of guava leaves and DPPH with the comparison solution of vitamin C preparation was calculated using the formula:

$$\%\text{inhibisi} = \frac{\text{a DPPH} - \text{a sample}}{\text{a DPPH} \times 100}$$

The inhibitory concentration (IC_{50}) value is the antioxidant concentration (μg/ml), which can provide a radical scavenging percentage of 50% compared to the control via the line equation. The IC_{50} value is obtained from the line intersection between 50% resistance power and the concentration axis, then enter the equation y=a+bx. Where y=50 and the x value shows IC_{50}.[16]

The antibacterial activity of nanoemulsion preparations of grape seed oil and ethanol extract of guava leaves was tested using the agar diffusion method by measuring the growth inhibitory diameter against *Staphylococcus aureus* and *Streptococcus mutans* bacteria. Making tryptone soya agar (TSA) media was done as follows: a total of 20 grams of TSA, which will be used as a medium in the antibacterial test, is dissolved in 500 ml of distilled water, then heated while stirring with a hotplate stirrer. The media was

sterilized by autoclaving at a temperature of 121°C and a pressure of 1.5 atm for 15 minutes.[17] Equipment preparation and sterilization were done: the tools used were prepared, washed, cleaned, and dried. After that, the tools and media are wet sterilized using an autoclave.[18] Bacterial rejuvenation tests were done as follows: rejuvenation of the test bacteria is done by taking one cycle of the initial microbial culture and then planting it on TSB media. Next, it was incubated at 37°C for 24 hours.[19]

Preparation of bacterial suspension was done as follows: making a bacterial suspension is done by measuring the results of bacteria that have been incubated using a UV-Vis spectrophotometer at a wavelength of 625 with an absorbance range of 0.08–0.12.[20] Inhibitory power test of nanoemulsion preparations was done as following; the test bacteria were inoculated as much as 100 µl into 20 ml of agar media in a petri dish then aseptically, the inoculated media was made with a hole of 8 mm. Comparative preparation of 1% antibiotic solution as a positive control, test samples of grape seed oil nanoemulsion and clove flower ethanol extract, grape seed oil nanoemulsion base were dropped 45 µl each in the wells that had been made in the test medium. After that, the media was incubated at 37°C for 18–24 hours. Then, measure the diameter of the obstacle area around the hole using a caliper.[20]

This study was approved by the Health Research Ethics Committee of Universitas Islam Bandung (110/KEPK-Unisba/XI/2023).

Results

The preparation formulation begins with preliminary grape seed oil nanoemulsion optimization, and then the percent transmittance test is evaluated. The optimum formula is F6, with the highest transmittance percentage, 99.65%±0.17. The preliminary research formula can be seen in Table 1.

After obtaining the optimum preliminary formula, optimization was done by adding clove extract, namely 0.25%, 0.5%, 0.75%, and 1% (Table 2).

Antioxidant activity tests were carried out on nanoemulsion preparations of formulas A, B, C, and D and also on ethanol extract of clove flowers to determine the inhibitory power and IC_{50} value of nanoemulsion preparations and ethanol extract of clove flowers against DPPH radical compounds. The IC_{50} value and antioxidant activity test curve results can be seen in Table 3. In testing antioxidant nanoemulsion preparations, the FA formula had the highest IC_{50}, 1,117.56 ppm, followed by the FB formula, 949.14 ppm, FC 874.58 ppm, and FD 811.28 ppm. Based on the test results, adding the concentration of clove flower ethanol extract affects the antioxidant strength of the preparation. The more extract added, the lower the IC_{50} value, meaning the higher the antioxidant strength.

The free radical scavenging activity of DPPH is based on the ability of the test material to reduce or capture DPPH radicals, which can be

Table 1 Optimization Nanoemulsion Formulas

Material	Formulas (%w/w)					
	F1	F2	F3	F4	F5	F6
Grape seed oil	4	4	4	4	4	4
Tween 80	20	22	24	26	28	30
PEG 400	20	22	24	26	28	30
Aquadest	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Table 2 Nanoemulsion Formula of Clove Extract and Grape Seed Oil

Material	Formulas (%w/w)			
	FA	FB	FC	FD
Clove extract	0.25	0.5	0.75	1
Grape seed oil	4	4	4	4
Etanol	10	10	10	10
Tween 80	30	30	30	30
PEG 400	30	30	30	30
Aquadest	ad 100	ad 100	ad 100	ad 100

Table 3 Antioxidant Test Results

Samples	Unit	Value IC_{50} (ppm)	Description
Sample FA	Ppm	1,172.56	DPPH
Sample FB	Ppm	949.14	DPPH
Sample FC	Ppm	874.58	DPPH
Sample FD	Ppm	811.28	DPPH
Clove extract	Ppm	8.18	DPPH

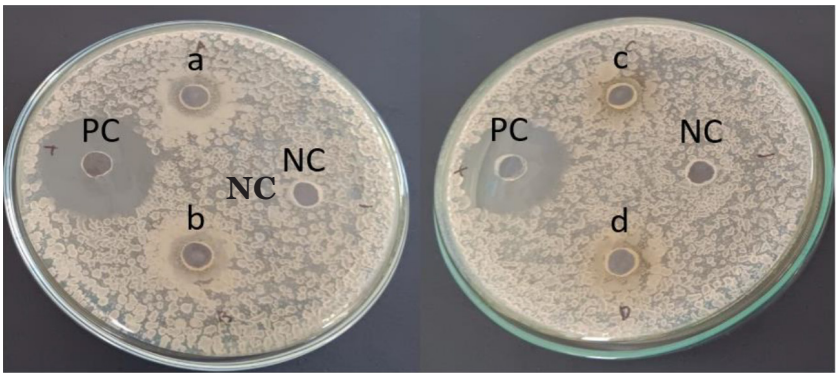

Figure 1 Inhibition Zones *Staphylococcus aureus*
Note: PC: positive control, NC: negative control, a: formula A, b: formula B, c: formula C, d: formula D

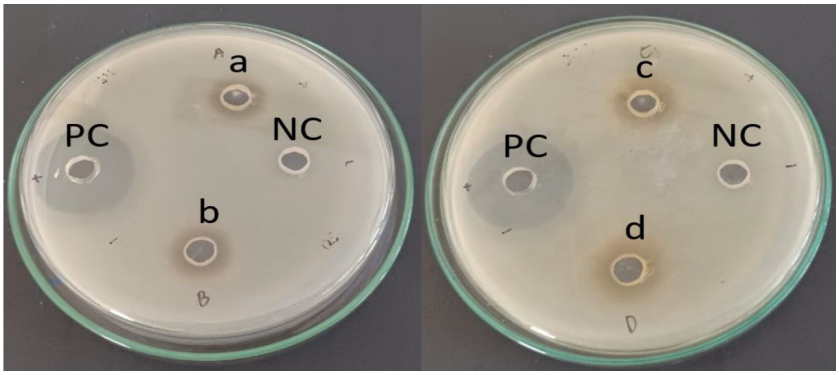

Figure 2 Inhibition Zones of *Streptococcus mutans*
Note: PC: positive control, NC: negative control, a: formula A, b: formula B, c: formula C, d: formula D

seen from the purple color change of the DPPH solution after being mixed with the test sample to yellow. Thus, the ethanol extract of clove flowers and the nanoemulsion preparation have antioxidant properties in the DPPH test.

The IC_{50} value was obtained based on the linear regression equation derived by plotting the percent reduction of DPPH as a parameter of antioxidant activity against the concentration of the test solution (ppm). The abscissa represented the concentration of the test solution along the X-axis, while the ordinate represented the percent reduction value along the Y-axis. Determination of the potential DPPH free radical scavenging activity of ethanol extract of clove flowers and nanoemulsion preparations is expressed by the IC_{50} parameter, namely the concentration of the test compound, which causes free radical scavenging of 50%.

The antibacterial activity test of the nanoemulsion preparation was carried out against *Staphylococcus aureus* bacteria. The diffusion method uses wells.

The test was carried out using an 8 mm diameter perforator by adding up the horizontal and vertical diameters of the inhibition zones (Figure 1 and Figure 2). Antibacterial activity testing was carried out on nanoemulsion base formula (F6), FA formula with an extract concentration of 0.25%, FB formula with an extract concentration of 0.5%, FC formula with an extract concentration of 0.75%, and FD formula with an extract concentration of 1%. The results of testing the antibacterial activity of microemulsion preparations can be seen in Table 4.

Based on Tables 4 and Table 5, the results of the antibacterial tests that have been carried out, it was found that each nanoemulsion formulation of FA, FB, FC, and FD has a fragile antibacterial activity for *Staphylococcus aureus* because the inhibition zone is almost invisible, however

Table 4 Results of Antibacterial Testing for *Staphylococcus aureus*

Groups	Inhibition Diameter *S. aureus* (mm)	Categories based on Inhibition Zones
FA	4.03	Very weak
FB	4.17	Very weak
FC	4.6	Very weak
FD	4.3	Very weak
Base	4	Very weak
Positive control	21.9	Very strong
Negative control	–	It has no antibacterial activity

Note: very weak: <5 mm, weak: 5–10 mm, strong: 10–20 mm, very strong: >20 mm

visually there is the potential to reduce growth in the area around the *Staphylococcus aureus* bacterial media just doesn't kill the bacteria. Meanwhile, in the results of the antibacterial activity of *Streptococcus mutans*, the inhibition zone was more visible. However, it was still inhibiting bacterial growth, not to the point of killing growth.

The inhibition zone results for the positive control of clindamycin, FA, FB, FC, and FD formulas were 13.93 mm, 4.03 mm, 4.17 mm, 4.27 mm, and 4.63 mm, respectively. The concentration of the extract in the preparation influences the results of the inhibition zone. In the negative control in the form of a nanoemulsion base without adding extracts, no clear zone was formed around the disc. The positive control group was included in the solid bacterial inhibition zone category. This weak inhibitory power is caused by the low concentration of clove flower ethanol extract, which has yet to be tested for its minimum concentration of antimicrobial substances.

Discussion

In this study, testing antioxidant nanoemulsion preparations, the FA formula had the highest IC_{50}, 1,117.56 ppm, followed by the FB formula, 949.14 ppm, FC 874.58 ppm, and FD 811.28 ppm. Based on the test results, adding the concentration of clove flower ethanol extract affects the antioxidant strength of the preparation. The more extract added, the lower the IC_{50} value, meaning the higher the antioxidant strength. However, a sample with an IC_{50} value of more than 200 ppm must be more substantial than a clove flower ethanol extract sample.

This study, by the statement of Partayasa et al.,[21] states that the antioxidants contained in

Table 5 Results of Antibacterial Testing for *Streptococcus mutans*

Groups	Initial Inhibition Diameter *S. mutans* (mm)	Perforator Diameter (mm)	Inhibition Diameter *S. mutans* (mm)	Categories based on Inhibition Zones
FA	12.03	8	5.03	Weak
FB	12.17	8	5.17	Weak
FC	12.27	8	5.6	Weak
FD	12.63	8	5.3	Weak
Base	There are no inhibition zones	–	–	There are no inhibition zones
Positive control (clindamycin for *S. aureus*; gentamycin for *S. mutans*)	21.93	8	13.93	Strong

Note: very weak: <5 mm, weak: 5–10 mm, strong: 10–20 mm, very strong: >20 mm

the sample decrease because the antioxidants are easily oxidized by the external environment, thereby reducing their activity in reducing DPPH free radicals.In testing the antioxidants of clove extract, the IC_{50} value was 8.18 ppm, which means it has antioxidant power in the strong category.

Based on the IC_{50} strength table, according to Leksono et al., a sample with an IC_{50} value of less than 50 ppm has antioxidant activity in the very strong category.[22]

The results of a good inhibition zone in the nanoemulsion dosage form are because the resulting nano-sized particles can penetrate bacterial cell membranes. The antibacterial effect of nanoparticles is attributed to their significant surface area, facilitating optimal interaction with microbes.[23,24] Apart from that, the bioactive compounds contained in clove flowers, such as flavonoids, phenols, and tannins, can also inhibit the activity of living bacteria.[25]

Referring to the antibacterial inhibitory power classification of Dewi et al.,[26] the positive control has very strong antibacterial effectiveness with an inhibitory diameter value of >20 mm, and the negative control has no antibacterial activity because there is no inhibition zone around the well. Factors that influence antibacterial activity include the bacteria being inhibited, the content of antibacterial compounds, the concentration of the extract, the diffusion ability of an extract, and differences in the structure of bacterial cell walls, which will influence the activity, penetration, and binding of antibacterial compounds.

According to research by Daulay,[27] in clove flower ethanol extract nanoemulsion, extract concentrations of 1.25% and 2.5% have a weak inhibitory category, and at 5%, it has a weak category. This study's highest extract concentration was 1%, with a transmittance percentage of 82,874. If the extract concentration is increased, the transmittance percentage will likely be smaller.

Further research needs to be carried out in vivo to clinically test the ability of nanoemulsion preparations containing clove extract and grape seed oil in their activity as antioxidants and antibacterials.

Conclusion

The nanoemulsion of clove extract and grape seed oil has exhibited exceptional antioxidant properties and substantial antimicrobial efficacy against prevalent oral bacterial strains.

Conflict of Interest

All of the authors have declared that they have no conflicts of interest.

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