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Antibacterial Properties of Turmeric Oil Nanoemulsion

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Abstract
Turmeric oil is an essential oil extracted from *Curcuma domestica* rhizome, containing active compounds like turmerone, atlantone, and zingiberone, with various pharmacological activities. This study aimed to evaluate the antibacterial activity of turmeric oil nanoemulsion formulations (F1, F2, F3) against *Staphylococcus aureus* and *Escherichia coli*. The nanoemulsions were characterized for organoleptic properties, pH, viscosity, stability, homogeneity, droplet size, and zeta potential. All formulas appeared as thick, yellow, aromatic liquids with good homogeneity, pH of 6.8–7.0, and showed no separation upon centrifugation. Droplet sizes were 23.2 nm (F1), 203.8 nm (F2), and 298.7 nm (F3), with zeta potentials of –18.5 mV, –13.4 mV, and –8.5 mV, respectively. Antibacterial tests using the diffusion method showed moderate activity with inhibition zones ranging from 5.0–9.8 mm. These findings indicate that turmeric oil nanoemulsions are physically stable and exhibit moderate antibacterial activity.

Keywords
Turmeric oil; Nanoemulsion; Antibacterial

INTRODUCTION

Turmeric, *Curcuma longa* or scintific synonym is *Curcuma domestica*, is a rhizomatous of *Zingiberaceae* family . The major constituents of the rhizomes are curcuminoid pigments and volatile oil, have been khown their pharmacological activity including antimicrobial properties [1]. Another significant substance that is produced from the curcuma rhizome in amounts of 3-6% is turmeric oil.[2].

The major components of turmeric oil are *zingiberene* (11%), *β-turmerone* (10%), *sesquiphellandrene* (10%), and *α-curcumene* (5%)[3,4]. Turmeric oil known has biological activity included antioxidant, anti-inflammatory, antidiabetic, cardiovascular, neuroprotective, nephroprotective, analgesic, anticancer, antinociceptive, antibacterial, antifungal, and antiparasitic [4].

Turmeric oil knows can be inhibited a pathogen for periodotitis like *Porphyromonas gingivalis*[5] *and Streptococcus mutans*[6], *restricting the development of bacteria Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa (Gram-negative) and Bacillus cereus, B. coagulans, B. subtilis, and Staphylococcus aureus (Gram-positive)*[7,8]. Turmeric oil successfully suppressed the development of Staphylococcus aureus (MIC of 38.8µl/mL), Staphylococcus epidermidis (MIC of 50.0µl/mL), Escherichia coli (MIC of 44.4µl/mL), and Pseudomonas aeruginosa [3]. Turmeric oil has antibacterial properties, particularly in combating antibiotic resistance, through cell wall degradation, cytoplasmic membrane disruption, DNA and RNA synthesis alterations, electron transport, and nutrient uptake.

Thus, nanotechnology may be an ally to strengthen the antibacterial properties of natural substances [9,10]. Their dispersion across biological membranes is increased and their physical-chemical properties are better conserved when they are nanoemulsified [11]. Using nanoemulsion as an antibacterial agent is a novel and exciting development. The nanoemulsion particles bind to lipid-containing organisms due to thermodynamic factors. This fusion is facilitated by the pathogen's anionic

charge and the cationic charge of the emulsion being attracted to each other electrostatically. When enough nanoparticles combine with the pathogens, some of the energy in the emulsion is liberated. The active element destabilizes the pathogen's lipid membrane, releasing energy that results in cell lysis and death. [12].
The present study analysed the formulation of nanemulsions turmeric oil, physical evaluation and antibacterial activities of nanoemulsion turmeric oil against any bacterial.

METHODS

Materials and Tools

Materials: Turmeric oil were obtained from SESMU Indonesia, extracted by steam destilation form Curcuma domestica rhizome. Tween 80, Span 400, PEG 400, nipagin, and nipasol obtained from Sigma Aldrich, destilled water obtained from Bratachem.
Tools: The instruments used in this study include a pH meter, a Brookfield viscometer (Ametek), a particle size analyzer (Horiba Scientific SZ-100), a UV-Vis spectrophotometer (Shimadzu), a centrifuge, an analytical balance, a magnetic stirrer (Thermo), and a bath sonicator (Elma).

Nanoemulsion Preparation

Formulation of turmeric oil nanoemulsion devided in 3 concentration, can be seen in Table 1. Turmeric oil was diluted in VCO, span 80 and PEG 400 (oil phase) in glass beaker and stiring by 1000 rpm in 10 minutes. Tween 80 was diluted in destilled water (water phase) as an emulsifying agent. Water phase were slowly added to oil phase and stirring about 20 minutes in 500 rpm. The admixture were sonicated bath sonication about 30 minutes. The nanoemulsions were characterized included pH, viscosity, stability by centrifugation methode, particle size with Horiba Scinetific SZ-100, and the polydispersity index and zeta potential were measured. Three formulation were collected, and kept refrigated at 4°C.

Table 1. Turmeric oil nanoemulsion formulation

Materials	F1 (5%)	F2 (10%)	F3 (15%)
Turmeric oil	5	10	15
Tween 80	36	36	36
PEG 400	24	24	24
VCO	3	3	3
Span 80	1	1	1
Destilled water ad	100	100	100

Physical Evaluation

Organoleptic assay: Organoleptic tests were conducted by examining the color, odor, and clarity of the preparation through sensory observation. A clear appearance indicated that the preparation had small droplet sizes [13].
pH Determination: The pH meter was calibrated using solutions with pH values of 4.0 and 6.8. After calibration, the electrodes were rinsed with distilled water (aquadest), and then the pH meter was immersed in the preparation. The pH reading was allowed to stabilize on the meter's display. The pH range considered safe and tolerable for the skin in this preparation was between 4.2 and 7.0 [13].
Viscosity determination: Viscosity was measured using an LV-type Brookfield viscometer at room temperature. A 50 mL sample of the preparation was placed in a beaker and positioned in the available solvent trap. Spindle size 62 was used with a rotation speed of 50 rpm. The viscosity value was displayed on the Brookfield viscometer's readout.
Particle size analyzer, Polidispesity Indeks (PI), and Potential Zeta: The globule size, PI, and zeta potetial was determined using a Particle Size Analyzer (Horiba Scientific SZ-100).

Antibacterial Assay

Antibaterial assay of nanoemulsion turmeric oil against *E. coli, S. Aureus, S. Pyogenes, P. aeruginosa* by we diffusion method. 50 µL each formulation were added to the wells in solid medium Nutrient Agar (NA) with bacterial inoculum. Negative controls were also added to the wells. All plated were incubated at 37°C for 24 hours. The clear zone formed is measured using a caliper.

RESULTS AND DISCUSSION

The results show pH of turmeric oil nano emulsion between 6,8-7,0. Viscosity of turmeric oil nanoemulsion between 23-26,8 cps, there are no precipitation after centrifugation test. Physical evaluation can be seen in Tabel 2. The result of antibacterial assay against *E. coli, S. Aureus, S. Pyogenes, P. aeruginosa* can be seen in Table 3.

The preparation obtained based on organoleptic evaluation results all of formulation were thick liquid with yellow color with turmeric aromatic (Fig. 1). Yellow color is produced from the color of turmeric oil. Results of pH measurements of nanoemulsion turmeric oil between 6.8-7.0, where the pH is a normal pH for human body. Based on the results of viscosity measurements, the turmeric oil nanoemulsion viscosity value was 23–29 Cps. It is known that the requirements for viscosity values for nanoemulsion preparations range from 10-2000 Cps. This shows that the turmeric oil nenoemulsion preparation meets the viscosity requirements. Previous study has shown that a substance will be more stable the higher its viscosity because comparatively thick materials tend to make particle mobility more difficult[14]. Stability analysis was performed after the nanoemulsion centrifugation about 3800 rpm in 30 minutes. Result show there are no precipitation after the centrifugation, the nanoemulsion was stable.

The percent transmittance results for the three nanoemulsion formulations (F1, F2, and F3) indicate that all the formulations have excellent clarity. F1 and F2 both show a transmittance of 98.3%, while F3 is slightly lower at 97.2%. These high transmittance values suggest that the droplet sizes in all three formulations are very small, in the nanometer range. When the droplets are that small, the solution becomes more transparent, allowing more light to pass through. The fact that F1 and F2 have identical transmittance values suggests that these formulations are quite similar in terms of their clarity and stability. The slightly lower value for F3 might point to some minor differences in droplet size or the composition of the formulation, but it's still within an acceptable range for nanoemulsions [13].

Overall, the results show that all three formulations are clear and stable, with good dispersions of small droplets. This is a good sign that the formulations have the right properties for use in pharmaceutical or cosmetic products, where clarity and stability are key.

The measurement of particle size of nanoemulsion showed that ranging from 23.2-298 nm (Fig 1). The smallest particle size was obtained at F1 (23.2 nm). The smaller particle size and the value of the Polydispersity Index, also known as the particle size distribution (PDI), indicate how the mix of surfactants affects the stability of nanoemulsions. The distribution of particle size is displayed by the polydispersity index value. The narrower the particle size dispersion indicated by a lower polydispersity index value, the more uniform the particle diameter size [15]. The stability of the nanoemulsion is also influenced by co-surfactant in addition to surfactant combination. A short chain alcohol known as a co-surfactant can assist in lowering the interface voltage between the water phase and the oil phase. [14]

The addition of other chemicals, pressure, solution concentration, molecular weight and size, bond strength between molecules, and temperature rise all have an impact on a liquid's viscosity [14]. The refractive index of the nanoemulsion preparate is measured in relation to the particle size-dependent changes in nanoemulsion clarity. Light that is smaller than the light's wavelength and is emulsion-sized will be able to flow through the product without refracting [14]. Particle size changes are caused by interactions between the particles,

which leads to aggregation. Aggregation is caused by a rise in surface free energy [16].
The antibacterial activities of turmeric oil nanoemulsion against positive and negative gram by wells diffusion methode. The results showed different of growth inhibition depending on concentration of turmeric oil. It was found the turmeric oil nanoemulsion exhibited maximum zone of inhibition against E. Coli in F3 (21.6 mm). The minimum zone of inhibition was shown against *P. Aeruginosa* in F1 (7.7 mm). The antibacterial activities of turmeric oil naomeulsion showed different of growth inhibition depending on concentration of turmeric oil and strain of bacterial [17].
Turmeric oil restrained the growth of Gram-positive and gram-negatives bacteria [18][19].. The components of turmeric oil has also indicated the antibacterial such as *zingiberene*, *sesquiphellandrene*, *β-turmerone*, *α-curcumen* α-phellandrene, camphor, eucalyptol, terpinolene, and α-pinene [18][19][20].

CONCLUSION

The turmeric oil nanoemulsion formulations demonstrated good physical characteristics, including appropriate pH (6.8–7.0), acceptable viscosity (23–29 Cps), uniform particle size distribution (23.2–298 nm), and physical stability after centrifugation. The presence of surfactants and co-surfactants played a key role in maintaining stability and uniformity of the emulsions. The formulations also showed clarity consistent with their particle sizes. Antibacterial testing revealed that the nanoemulsions had varying inhibitory effects against both Gram-positive and Gram-negative bacteria, depending on the concentration and bacterial strain. The highest antibacterial activity was observed against *E. coli* in F3, while the lowest was against *P. aeruginosa* in F1. These results suggest that turmeric oil nanoemulsions are stable and possess antibacterial potential influenced by formulation composition.

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