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FORMULATION AND ACTIVITY TESTS OF NANOEMULSION OF TURMERIC RHIZOME (CURCUMA LONGA L) EXTRACT FOR *METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS* (MRSA) BACTERIA

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ABSTRACT
Turmeric rhizome *(Curcuma longa* L) contains the main active compound curcumin, which has antibacterial activity which inhibits *Methicillin-Resistant Staphylococcus aureus* (MRSA). Developing a nanoemulsion formula for turmeric rhizome extract can overcome bacterial resistance by protecting the active substance from degradation and blocking the efflux pump in bacteria. This research aims to create a turmeric rhizome extract nanoemulsion formulation and determine the activity of against MRSA bacteria in vitro. Method: Turmeric rhizomes were extracted using the maceration method using 70% ethanol solvent. The technique of nanoemulsion preparation was by using the spontaneous emulsification method with varying extract concentrations of 1.25% (F1), 2.50% (F2), and 3.75% (F3). Evaluation of the physical characteristics of nanoemulsions (particle size, polydispersity index, zeta potential), viscosity, and pH. In vitro activity test formula using the diffusion method against MRSA bacteria. The turmeric rhizome extraction process produces a yield value of 14.3%. The best formula is F1 with a viscosity value of 134.6 ± 21.3 Cps, pH value of 6.34, particle size value of 33.4 ± 12.8 nm, polydispersity index of 0.407 ± 0.01, zeta potential -14.2±2.9 mV. Turmeric extract nanoemulsion can inhibit the growth of Methicillin-Resistant Staphylococcus aureus bacteria with an inhibition zone of 11.00 mm at F3. Conclusion: Curcumin can be formulated in a nanoemulsion system without providing significant changes in organoleptic tests, viscosity, pH, nanoemulsion type tests, particle size, polydispersity index, and zeta potential. Turmeric extract nanoemulsion has an antibacterial effect against MRSA in vitro.

Keywords: Nanoemulsion; MRSA; Turmeric Rhizome

1. INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a strain of Staphylococcus aureus that is genetically resistant to the antibiotic methicillin as well as various forms of other β-lactam antibiotics such as flucloxacillin, cephalosporins and carbapenems (Wildana et al., 2018). Vancomycin is the current treatment option for MRSA infections. However, the effectiveness of therapy is slower in vitro when compared to β-lactam antibiotics (Salsabil et al., 2021). The nose, throat, and perineum are the primary sites of MRSA colonization (Anggraini et al., 2021), with hands being the most prevalent site at 32.50%, followed by the nose at 17.50%. Notably, the Asia-Pacific region bears a substantial burden of MRSA, with transmission within the hospitals contributing significantly to its prevalence of 23.5%, ranging from 0.7%-10.4% (Budiman et al., 2020; Wong et al., 2018).

Turmeric (*Curcuma longa* L) contains the compound curcumin, which has pharmacological activity in the form of antibacterial. Curcumin has an antibacterial mechanism like other bacterial phenolic compounds, explicitly inhibiting metabolism by damaging the cytoplasmic membrane and denaturing cell proteins (Ramadhani et al., 2018). Curcumin at a concentration of 250 µg/mL was able to kill MRSA. Additionally, turmeric rhizome extract, at concentrations of 5%, 10%, 20%, and 40% can produce inhibitory zone diameters of 11 mm, 13.5 mm, 14.5 mm, and 15 mm against Staphylococcus aureus, respectively (Pangemanan et al., 2016; Teow & Ali, 2015). However, curcumin's susceptibility to crystallization inacidic solutions (pH < 7) and rapid degradation at elevated temperatures of 70 and 90ºC pose significant challenges (Kharat et al., 2017).

The delivery of nanoparticles, particularly for topical preparations, offers a promising approach to overcome bacterial resistance by protecting the active substance from degradation and blocking the efflux pump in bacteria. In addition, nanoparticles can release active substances with a long half-life, so using small doses can provide maximum therapeutic effects (Alabdali et al., 2022; Chamundeeswari et al., 2019). Nanoemulsions, in particular, enhance the stability and effectiveness of the active substances and protect them from environmental factors such as high temperature, pH, and oxygen (O'Sullivan et al., 2017). Moreover, nanoemulsions can prevent particle aggregation compared to conventional emulsions (Saifullah et al., 2016). The mechanism of nanoemulsion in delivering active substances is by combining nanoemulsion globules with the lipid bilayer structure on the bacterial cell membrane so that it can release the active substance, and this combination also damages the lipid membrane of the bacteria (Jiang et al., 2020).

The ratio of oil, surfactant, and water plays a pivotal role in shaping physicochemical properties, stability, and therapeutic efficacy of nanoemulsions, offering a fertile ground for innovation. The loading of curcumin into nanoemulsions was explored using a combination of medium chain triglyceride (MCT), Tween 80, and lecithin as surfactants. The precise ratio of medium chain triglyceride (MCT), Tween 80, lecithin, and water used (10:6:4:80, respectively) produces a particle size of 113.93 nm with a polydispersity index of 0.23 and a zeta potential of -36.23 mV. Furthermore, the synergistic effects of mixed surfactants, exemplified by combinations of Tween 80 and Span 80, were highlighted by Chong et al. (2018) underscoring the importance of leveraging diverse surfactant combinations for enhanced emulsion stability. Non-ionic surfactants, such as Tween 80, serve as a co-surfactant can prevent aggregation and increase pH stability. Surfactant concentration also influences physicochemical stability (particle size, polydispersity index (PDI), surface charge, and curcumin concentration) under the influence of ionic strength, pH, and thermal (Chuacharoen et al., 2019). Additionally, the utilization of virgin coconut oil (VCO) as the oil base presents a novel approach. Its unique composition, featuring oleic acid and medium-chain fatty acids, facilitates a bond with Tween 80, mitigating Ostwald ripening and yielding nanoemulsions with droplets smaller than 100 nm thus opening avenues for further exploration in nanoemulsion formulation design.

2. METHODS

2.1. Tools

Beker glass (Pyrex®), Magnetic stirrer (Thermo Scientific®), Particle Size Analyzer (Malvren®), ultra-turrax (IKA T25 digital®), sonicator, pH meter (Ohauss®) and Viscometer (Brrokfield®).

2.2. Material

Turmeric rhizome simplicia (PT Palapa Muda Perkasa), Methicillin-Resistant Staphylococcus aureus bacteria, tween 80, span 80, methylparaben, glycerin, VCO, Mueller Hinton agar (Oxoid®), Mannitol Salt Agar, paper disk (Macherey Nagel®), Nacl 0 solution .9% (w/v) (Otsu-Ns®), and vancomycin antibiotic disc.

2.3. Extraction Process

Turmeric rhizome was extracted using the maceration method. 600 g of turmeric rhizome simplicia powder was macerated using 6 L of 70% ethanol (1:10) solvent. The maceration was carried out for two days while stirring thrice a day for 2-3 minutes. The macerate was collected and subjected to evaporation and concentration using a rotary evaporator at 70 °C to obtain turmeric rhizome filtrate. Subsequently, to eliminate residual ethanol and water content, the filtrate underwent further treatment in a water bath. The yield of the thick extract was calculated.

2.4. Preparation of Turmeric Rhizome Extract Nanoemulsion

Turmeric extract nanoemulsion will be formulated using a water-in-oil (W/O) system and formulas as outlined in **Table 1**. The oil phase was prepared by dissolving VCO and Span 80, followed byhomogenization using a magnetic stirrer for 15 minutes. Meanwhile, the water phase was made by mixing Tween 80, thick turmeric extract, glycerin, methylparaben, and distilled water. This mixture is homogenized using a magnetic stirrer for 15 minutes. Next, nanoemulsion commences by slowly and homogenously adding the water phase into the oil phase while stirring continuously using a magnetic stirrer for 1 hour. The emulsion particle size was then reduced using an Ultra Turax Homogenizer at a speed of 14,000 rpm within 15 minutes until a stable nanoemulsion was obtained. Subsequently, the nanoemulsions undergo sonication for 30 minutes.

Table 1. Nanoemulsion formula of rhizome tumeric extract

Ingridient	Concentration (%b/v)		
	F1	F2	F3
Rhizome Tumeric Extract	1.25 %	2.50 %	3.75 %
VCO *(Virgin Coconut oil)*	45%	45 %	45 %
Tween 80	1.5 %	2%	2.5 %
Span 80	10%	14.5 %	12.5 %
Glycerine	8%	8%	8%
Methylparaben	0.1 %	0.1 %	0.1 %
Aquadest	20 mL	20 mL	20 mL

2.5. Analysis of particle size, polydispersity index, and zeta potential

The nanoemulsion preparation that has been made was dispersed with aquabidest with a ratio of 1:1.000. Place 2 mL in the cuvette and measured it on the PSA tool to get the particle size, polydispersity index, and zeta potential results.

2.6. Physical Evaluation of Preparations

2.6.1. Organoleptic Test

Organoleptic testing was done visually by observing the preparation's shape, smell, and color.

2.6.2. Test pH

pH measurements were carried out using an electrode pH meter on a digital pH meter dipped in nanoemulsion until the pH meter showed a number at a temperature of 25°C ± 2.

2.6.3. Emulsion Type Test

This test was conducted by dropping the nanoemulsion preparation on a glass slide, then add one drop of methylene blue and observe under a microscope with 40x magnification.

2.6.4. Viscosity Test

A 20 ml sample was placed in a vial. A size 64 spindle with a rotation speed of 100 rpm is selected, then the tool was run. The viscosity value can be determined by observing the analysis results displayed on the Brookfield viscometer screen.

2.7. In Vitro Antibacterial Activity Test

2.7.1. Production of Mueller Hinton agar (MHA) media

Dissolve 19 grams of MHA in 500 ml of sterile distilled water, and heat the mixture on a hot plate until completely dissolved or homogeneous. Sterilize the media in an autoclave at 121 °C for 15 minutes. Pour the solution into a sterile petri dish; allow it to solidify, then invert and store the dish in the refrigerator (Sari et al., 2022).

2.7.2. Preparation of Mannitol Salt Agar (MSA) Agar Tilt Media

Dissolve 10.8 grams of MSA in 100 ml of sterile distilled water and heat it on a hot plate until completely dissolved or homogeneous. Sterilize the media in an autoclave at 121 °C for 15 minutes. Pour the solution into a sterile petri dish; after it hardens, then invert and store the dishin the refrigerator (Pasaribu, 2024).

2.7.3. Bacterial Rejuvenation

Aseptically streak one needle tube of MRSA bacteria onto the surface of the MSA medium in a zig-zag pattern. Incubate the mixture for 24 hours at 37 °C (Octaviani et al., 2022).

2.7.4. Making Bacterial Suspensions

MRSA bacteria rejuvenated with a sterile tube needle were then suspended in a tube containing 5 ml of 0.9% (w/v) NaCl until a turbidity that is the same as the standard turbidity solution of 0.5 Mc was obtained. Farland equals 1.5 x 108 (Colony Forming Unit) CFU/ml. Incubate the suspensions for 24 hours at 37 °C (Kurniawan et al., 2021).

2.7.5. Activity Test of Turmeric Extract Nanoemulsion Preparation

Antibacterial activity test utilizes the disc diffusion method On MHA plates previously inoculated with MRSA bacteria in 5 ml sterile NaCl were equated with MC Farland 108 CFU/ml/Mc Farland no.0.5. Lown's culture is made in MHA media by dipping a cotton swab in NaCl containing MRSA, then culture for 5 minutes to dry. Disc paper was dipped into each nanoemulsion preparation. The disc paper was drained until it did not drip, then left for 30 minutes to absorb the nanoemulsion into the disc. The paper discs were placed on MHA media inoculated with MRSA and then incubated at 37 °C for 18-24 hours. The inhibition zone or clear zone that forms was observed around the paper disc and then measured using a ruler and caliper to observe the diameter of the inhibition zone (Kurniawan et al., 2021).

2.8. Analysis of Results

Data analysis from research on nanoemulsion preparations of turmeric rhizome extract was carried out descriptively, where the data obtained were described and compared to the specification requirements that had been determined. The data obtained will be subjected to comparative analysis using the ANOVA analysis method to determine significant differences between the nanoemulsion preparation formulas. If the results obtained (p Value) are <0.05, it shows that there is a significant difference.

3. RESULTS AND DISCUSSION

3.1. Extraction Process

The development of the nanoemulsion formula for turmeric rhizome ethanol extract starts from extraction. One way to see the effectiveness of extraction is the % yield value. **Table 2** indicates a favorable yield, surpassing the threshold of 11% (Depkes RI, 2017), suggesting a robust extraction process. The greater the yield value of the extract produced, the more efficient the treatment determined and the better the active compound components extracted (Dewatisari et al., 2018).

Table 2. The yield value of turmeric rhizome ethanol extract

Dry Simplicia Weight (Grams)	Condensed Extract Weight (Grams)	% yield
600	86.18	14.3

3.2. Physical Characteristics of Turmeric Rhizome Extract Nanoemulsion

The organoleptic assessment of nanoemulsion formulation is presented in **Table 3**, complemented by visual representation in **Figure 1**. The development of a nanoemulsion formula based on the characteristics of turmeric extract contains non-polar polyphenolic compounds, which are relatively insoluble in water but higher solubility in oil (Subagia et al., 2019). The stability of the nanoemulsion system is based on the interaction of surfactant, cosurfactant, water phase, and oil phase. To ensure biocompatibility and safety, non-ionic surfactants are preferred over ionic ones due to their lower toxicity. There needs to be more than surfactants to reduce the interfacial tension between oil and water. Therefore, cosurfactants are needed to help reduce the interfacial tension. Non-ionic surfactants such as tween and span are widely used for drug delivery systems because of their low toxicity and ability to form stable emulsions on nanocarriers (Gupta et al., 2010; Hua et al., 2018).

VCO is one of the ingredients in the nanoemulsion formulation because it can prevent Ostwald ripening and produce preparations with a droplet size of <100 nm. VCO is an oil that contains 43-53% lauric acid, which is a medium-chain saturated fatty acid (MCFA). A more stable nanoemulsion can be produced using VCO by forming smaller droplet sizes than other MCT oils (Hartini, 2016). The potential for nanoemulsion formation can be done in two ways: spontaneous and non-spontaneous. Spontaneous formation occurs during the mixing process of water and oil phases aided by a magnetic stirrer. Conversely, non-spontaneousl formation necessitates external energy input, typically achieved through a sonicator.

Table 3. Organoleptic observation results

Visualization	F1	F2	F3
Color	Yellow	Yellow	Yellow
Odor	Turmeric	Turmeric	Turmeric

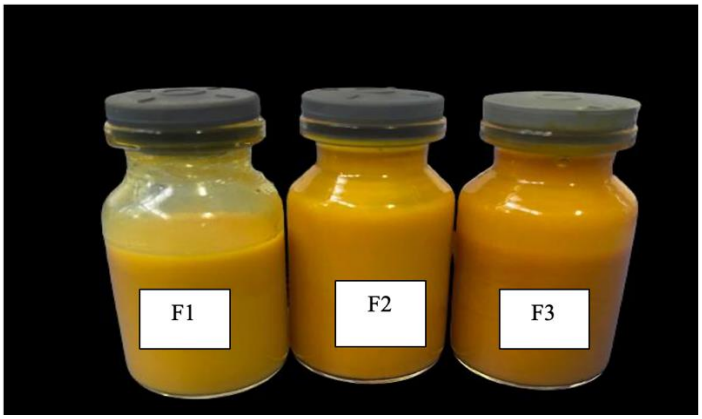

Figure 1. Visualization of Turmeric Rhizome Extract Nanoemulsion Preparations: F1: Nanoemulsion Formula Contains Turmeric Extract 1.25 %; F2: Nanoemulsion Formula Contains Turmeric Extract 2.50 %; F3: Nanoemulsion Formula Contains Turmeric Extract 3.75 %

Figure 2 illustrates the entire nanoemulsion formula, depicting a water-in-oil type emulsion, as evidenced by theblue coloration of water phase. The utilization of methylene blue, awater-soluble dye, aids confirming the emulsion type. After testing, the presence of blue granules throughout the entire nanoemulsion formula corrobates its classification as a sater-in-oil-emulsion. The choice of methylene blue as the dye stems from its intrinsic water solubility, facilitating its dipersion within water phase (Anief, 2019; Hartini, 2016).

3.3. pH and Viscosity Test

Table 4 shows the pH and viscosity values. The pH test determines the degree of acidity or alkalinity of the resulting emulsion. pH measurement is essential in determining the suitability

of topical preparations because the preparations will be applied directly to the skin. Topical preparation must align with the skin's pH to mitigate the risk of skin irritation and esure comfort during its application. Skin's pH divided into some of layer. The pH gradient of the stratum corneum (SC) is characterized by a steady decline, with values around pH 6.8–7.2 near the stratum granulosum (SG), which is the topmost layer of living keratinocytes located just below the SC. This gradient decreases towards the surface of the SC, where the pH ranges between 4.5 and 5.5 (Fukuda et al., 2024). After carrying out a pH test, each formulation has been confirmed to fall within the desired skin pH range, underscoring their safety and compatibility for topical use. Furthermore, the inclusion of excipients in the nanoemulsion preparations is carefully calibrated to maintain a pH that harmonizes the skin's natural pH. This strategic formulation approach ensures that when combined, the excipients contribute to a final pH that is conducive to safe and effective topical application.

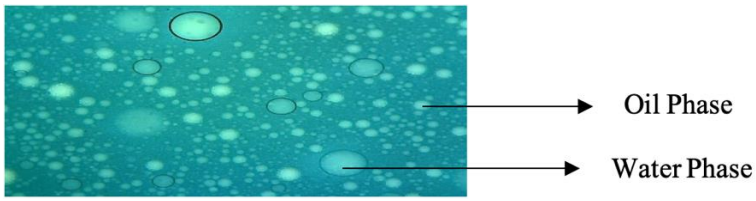

Figure 2. Emulsion type microscope observation (40x magnification)

Table 4. pH and viscosity

Formula	pH	Viscosity (cPs)	*P Value
F1	6.34±0.3	134.6 ± 21.3	
F2	6.36±0.03	154 ± 14.9	>0.05
F3	6.41±0.04	184.6 ± 11.4	

The viscosity test aims to determine the viscosity of the turmeric rhizome extract nanoemulsion. Numerous factors, such as changes in temperature, pH, manufacturing conditions, and the quality and concentration of raw materials, influence the viscosity value. A higher viscosity indicates improved product stability; however, they may also render the preparation challenging to apply and remove from its container. Among the formulations tested (F1, F2, and F3), F3 exhibits superior viscosity value compared to F1 and F2 (**Table 4**). The greater the extract concentration causes an increase in the viscosity of the nanoemulsion preparation. The increase in viscosity is directly proportional to the rise in surfactant because Span 80 surfactant has a dominant effect on increasing density due to the lipophilic chemical nature of Span 80, which tends to bind to the oil phase. It causes the nanoemulsion to be thicker and has a higher viscosity due to the water phase being wrapped in the oil. Nejadmansouri et al. (2016) show that Span 80 affects the viscosity of fish oil nanoemulsions because it interacts more with the oil phase, reducing the fluidity of the droplets and increasing the overall viscosity of the system.

3.4. Characteristics of Turmeric Rhizome Extract Nanoemulsion

Table 5 presents characteristics of turmeric rhizome extract nanoemulsion. The particle size in the nanoemulsion preparation will increase skin penetration (Kaur & Ajitha, 2019). An emulsion is categorized as a nanoemulsion if the particles have a size range of 10-1000 nm (Prakash et al., 2018). Based on **Table 5**, the droplet size of turmeric extract nanoemulsion is included in the nanoparticle category. Particle size influences absorption and release rates. The smaller the particle size formed, the greater the surface area, so the drug will be easily absorbed. However, variations in the extract concentration affect the need for the oil phase to coat the extract droplets. Higher concentrations of extract lead to increased droplet sizes as the oil layer expands to accommodate the extract. Moreover, surfactant concentration also influences physicochemical stability (particle size, polydispersity index (PDI) and surface charge) (Chuacharoen et al., 2019).

Table 5. Characteristics of turmeric rhizome extract nanoemulsion

Formula	Size of Particle (nm)	Polydispersity Index	Zeta Potential (mV)	**P Value*
F1	334.4 ± 12.8	0.407 ± 0.01	-14.4 ± 2.9	
F2	440.4 ± 6.8	0.573 ± 0.01	-7.2 ± 2	>0.05
F3	632.6 ± 24.5	0.305 ± 0.01	-19.1 ± 0.2	

The polydispersity index is a measurement of particles' dispersity quality, indicating whether the particles are monodispersed or polydispersed. A polydispersity index value of less than 0.5 is an ideal condition for nanoparticle formulation because it prevents aggregation (Kaur & Ajitha, 2019). **Table 5** shows nanoemulsion preparations F1 and F3 produce values < 0.5, but F2 has a polydispersity index value > 0.5. It can increase the occurrence of aggregation due to collisions between particles (Danaei et al., 2018).

The zeta potential value will determine the potential for flocculation to occur. It is done to predict the storage stability of the preparation. A high zeta potential will increase the prevention of flocculation because the electro is stable; preferably, a low zeta potential value tends to thicken and be unstable. The zeta potential value of a stable preparation is < -30 Mv and > +30 Mv (Prakash et al., 2018). The zeta potential value of the preparation of the turmeric extract nanoemulsion shows the stability of a system containing dispersed globules through repulsive forces between particles with the same charge when they are close together (**Table 5**).

3.5. Antibacterial Activity Test

The results of the in vitro test of turmeric rhizome extract nanoemulsion against MRSA bacteria suggest that the high concentration of turmeric extract will affect the antibacterial activity produced by the nanoemulsion preparation (**Table 6**).

Table 6. Antibacterial activity test

Group	Mean ±SD
F1	8,17±0,53
F2	9,64±0,81
F3	11,00±0,36

The antibacterial activity of turmeric extract nanoemulsion preparations was tested against MRSA bacteria using the disc diffusion method (Kirby-Bauer). This method is considered more efficient in work and has a lower risk of failure than other test methods. The sensitivity of MRSA bacteria to the turmeric extract nanoemulsion was assessed by measuring the size of the clear zone or inhibition zone formed. The test parameter observed was the clear zone, namely the clear area around the paper disk, as an indication of inhibited growth of microorganisms' excretion of antimicrobial substances by their competitors. The mechanism by which curcumin inhibits MRSA's growth is through binding curcumin to peptidoglycan found in the cell wall membrane. The results of this interaction cause the integrity of the MRSA cell wall to be damaged (Mun et al., 2013). As depicted in **Table 6**, the results highlight that nanoemulsion with a concentration of 3.75% (F3) is the most effective in inhibiting MRSA bacteria and has relatively strong antibacterial power.

4. CONCLUSION

Turmeric rhizome extract nanoemulsion emerges as a promising delivery system to combat MRSA resistance, particularly evident in F3 formulation. This formulation has good particle characteristics and a substantial inhibition zone. This condition is partly influenced by the comparison of the concentrations of span 80 and tween 80 which not only affects the characteristics of the nanoemulsion but also the effectiveness of the formula.

5. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

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