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Microemulsion of methanol extract of *Tridax procumbens* flower and its antibacterial activity against *Streptococcus mutans* and *Enterococcus faecalis*

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ABSTRACT

Tridax procumbens (commonly known as Gletang flower) is a medicinal plant recognized for its antibacterial potential and is widely distributed across various habitats such as rice fields, plantations, and roadsides. Phytochemical screening of its methanolic flower extract revealed the presence of secondary metabolites, including alkaloids, flavonoids, steroids, phenols, terpenoids, and tannins, all of which contribute to its biological activities. This study aimed to develop a microemulsion formulation of T. procumbens methanolic extract and to evaluate its antibacterial activity against Streptococcus mutans and Enterococcus faecalis, two major oral pathogenic bacteria. The microemulsion was prepared using the sonication method and characterized by assessing its pH, transmittance, viscosity, physical stability, and particle size using a particle size analyzer. Antibacterial activity was tested using the Kirby-Bauer disk diffusion method. The results indicated that the microemulsion had particle sizes ranging from 300–1000 nm and demonstrated significantly higher antibacterial activity compared to the crude extract, suggesting improved solubility and enhanced bioactivity of the active compounds. This formulation holds promise as a natural antibacterial agent for the prevention of oral infections.

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INTRODUCTION

Tridax procumbens L., commonly known as Gletang flower, is a wild plant commonly found in tropical regions, particularly in open areas such as rice fields, gardens, and roadside areas [1], [2]. This plant is non-seasonal [3] and thrives throughout the year. Despite its abundant population in several areas, including rice fields in Cilegon City, the utilization of this plant by the local community remains limited. However, previous studies have shown that the flowers of this plant contain secondary metabolites, including alkaloids, flavonoids, steroids, phenols, and terpenoids, which have potential antibacterial properties [4].

Several pathogenic bacteria responsible for infections in the oral cavity, such as *Streptococcus mutans* and *Enterococcus faecalis*, are known to trigger dental health issues, such as caries and root canal infections. Treatment for these bacterial infections is commonly carried out

using antibiotics. However, excessive and inappropriate use of antibiotics can lead to bacterial resistance [5]. Therefore, exploring natural plant-based antibacterial agents is a promising alternative for developing modern therapies.

To enhance the effectiveness of active compounds in medicinal plants, a penetration system is required to optimize the solubility and stability of these compounds. One system that has been widely studied is microemulsion formulation, which is an isotropic colloidal system consisting of oil phase, surfactant, cosurfactant, and water, with particle sizes in the nanometer scale [6]. This technology can improve the solubility of active substances and facilitate penetration through bacterial cell membranes, thereby potentially increasing antibacterial activity [7].

Based on the bioactive compound potential of Gletang flower and the effectiveness of microemulsion systems in delivering active substances, this study aims to formulate and evaluate the characteristics of a microemulsion of Gletang flower methanol extract using a sonication method with three different formulation variations. Antibacterial activity tests were conducted against *S. mutans* and *E. faecalis* using the Kirby-Bauer method, with the hope of supporting the development of natural antibacterial agents in oral health.

MATERIALS AND METHODS

Extraction of Gletang Flower

A total of 1.5 kg of fresh Gletang flowers was obtained from the Gerogol district, Cilegon city. The samples were first washed and then naturally dried at room temperature for 14 days. After the drying process was completed, the dried samples were ground using a blender to obtain simplicia. A total of 250 g of simplicia powder was used for extraction. The solvent used was methanol with a ratio of 1:20 (w/v), amounting to 5 liters. The extraction resulted in 68 g of concentrated Gletang flower extract.

Formulation of Gletang Flower Nanoemulsion

The Gletang flower nanoemulsion formulations were made with three different concentrated extract formulations: F1, F2, and F3. Formulation 1 contained 5% extract, formulation 2 contained 10% extract, and formulation 3 contained 15% extract. Each extract variation was suspended in 1 mL of virgin coconut oil (VCO) with the aid of magnetic stirring. The addition of VCO in the microemulsion system plays a crucial role as the oil phase in forming a stable microemulsion structure [8].

Microemulsion Characterization

Characterization of the microemulsions, consisting of F1, F2, and F3, was performed for parameters such as pH, % transmittance, and particle size distribution (PSD) using the Beckman Coulter LS 13 320 Particle Size Analyzer (PSA) with the optical Fraunhofer laser diffraction method. PSA was used to determine the particle size of the formed microemulsion.

Antibacterial Activity Test

The antibacterial activity tests for F1, F2, and F3 were conducted using the Kirby-Bauer method. The test bacteria used were *S. mutans* and *E. faecalis* at a 0.5 McFarland concentration, with a volume of 15 µL of the extract applied to a Samir paper disc, which was then placed on solid media. The samples were incubated at 37°C for 18 hours. After the incubation period, the diameter of the clear zone surrounding the Samir paper disc was measured to determine the inhibition zone formed using a caliper. The testing was performed in duplicate [9]. A larger inhibition zone diameter indicates that the substance is more effective as an antibacterial agent [10].

RESULTS AND DISCUSSION

The extraction process using methanol yielded a yield of 27.2% from 250 grams of simplicia derived from 1.5 kg of fresh flowers. This yield is considered high, exceeding the minimum standard of 10%, which is commonly used as a reference in phytochemical research. Methanol was chosen as the solvent due to its ability to extract various secondary metabolites, including alkaloids, flavonoids, phenols, and tannins, which are known to exhibit significant antibacterial activity [11].

The microemulsion formulation was carried out using the sonication method, resulting in particle sizes ranging from 300 to 1000 nm, which meets the criteria for a microemulsion system. The small particle size increases the surface area of the active substances, enhances the solubility of the extract, facilitates penetration into bacterial cell walls, and improves the biological effectiveness of the active compounds [12]. Physical characterization showed that the resulting microemulsion has a pH, transmittance, and physical stability suitable for topical applications in the oral cavity [13]. The results of the microemulsion characterization for the 5% (F1), 10% (F2), and 15% (F3) concentrations are shown in Table 1.

Table 1. Results of pH, transmittance percentage, and particle size analyzer (PSA) tests of gletang flower microemulsion

Formulation	pH	% Transmittance	Particle size (µm)
1 (5%)	5	98.9	1.05
2 (10%)	5	98.6	1.12
3 (15%)	5	97.9	1.17

The antibacterial activity of the Gletang flower extract nanoemulsion against *S. mutans* and *E. faecalis* with positive control (0.2% chlorhexidine) and negative control (aqua dest) can be observed by the presence of an inhibition zone diameter on the paper disc (Figure 1). The results of the antibacterial activity test are presented in Table 2.

Table 2. Results of Antibacterial Activity Tests for F1, F2, and F3

Bacteria	Sample	Inhibition zone (mm)		Average
		1)	2)	
S. mutans	F1 (5%)	0.3	0.3	0.3
	F2 (10%)	0.3	0.4	0.35
	F3 (15%)	0.5	0.6	0.55
	Aquades (-)	0	0	0
	chlorhexidine (+)	4.2	4.2	4.2
E. faecalis	F1 (5%)	2.3	2.4	2.35
	F2 (10%)	3.1	2.9	3.0
	F3 (15%)	3.3	3.1	3.2
	Aquades (-)	0	0	0
	chlorhexidine (+)	4.1	4.1	4.1

Formulations F1 and F2 exhibited very small inhibition zones (0.3 mm and 0.35 mm, respectively), while F3 showed a slightly larger inhibition zone of 0.55 mm. However, compared to the positive control (chlorhexidine) with an inhibition zone of 4.2 mm, all three formulations still exhibited very weak activity against *S. mutans*. This indicates that the Gletang flower microemulsion is less effective in inhibiting the growth of *S. mutans*, possibly due to the thicker peptidoglycan layer in the cell wall of Gram-positive bacteria or their resistance to the active compounds in the extract [14].

A different result was found with *E. faecalis*. All formulations demonstrated larger inhibition zones compared to *S. mutans*, with F1 (2.35 mm), F2 (3.0 mm), and F3 (3.2 mm). Although still lower than the positive control (4.1 mm), the antibacterial activity against *E. faecalis* showed greater potential. Formulation F3 exhibited the highest activity, indicating that the concentration or composition of chemical compounds in F3 is more effective in penetrating the cell wall and inhibiting the growth of this bacterium.

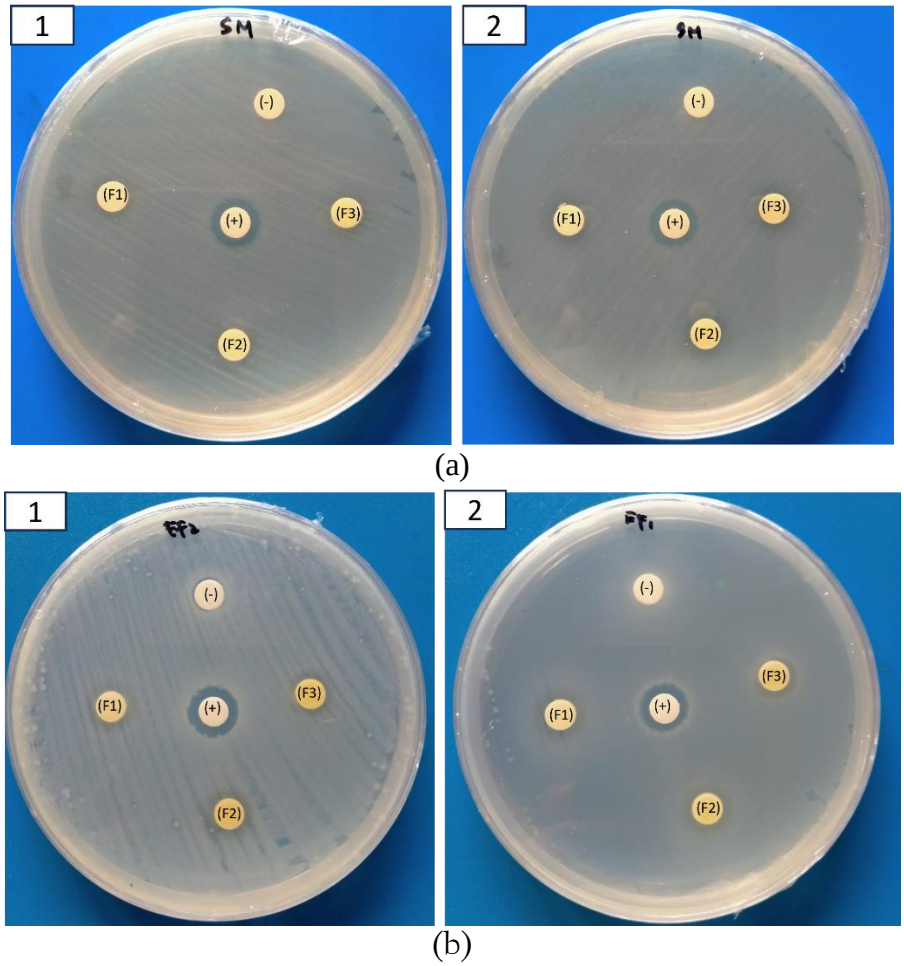

Figure 1. Inhibition zones of *S. mutans* (a) and *E. faecalis* (b) bacteria against Gletang flower microemulsion samples F1, F2, and F3.

The antibacterial activity test using the disc diffusion method showed that the microemulsion had larger inhibition zones compared to the extract without formulation, as conducted by Widyawati et al. (2022), indicating an increase in the bioavailability of the active compounds [15]. This finding is consistent with previous studies, which have shown that formulations in nano or microemulsion forms can enhance the stability and biological effectiveness of active compounds [16].

The microemulsion formulation based on Tridax procumbens methanol extract shows potential as an antibacterial agent against *S. mutans* and *E. faecalis*, two major bacteria responsible for oral infections. Furthermore, other studies have shown that methanol extracts from other parts of *T. procumbens*, such as stems, also exhibit antimicrobial activity against specific bacteria. This article supports the potential of *T. procumbens* as a source of natural antibacterial agents applicable for the prevention of oral infections. The use of a microemulsion formulation not only enhances antibacterial activity but also holds promise for the development of environmentally friendly and sustainable natural pharmaceutical products.

CONCLUSION

Physical characterization showed that the resulting microemulsion had suitable pH, transmittance, and physical stability for topical application in the oral cavity. Antibacterial effectiveness increased with the formulation: F3 › F2 › F1. This is likely due to the increased concentration of active extract or better microemulsion stability in formulation F3, thereby enhancing the diffusion of antibacterial compounds into the medium. The Gletang flower microemulsion shows antibacterial potential, particularly against *Enterococcus faecalis*.

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