Jurnal Pendidikan Kimia (JPKIM)
p-ISSN: 2085-3653| e-ISSN: 2549-3116
https://jurnal.unimed.ac.id/2012/index.php/jpk/index

10.24114/jpkim.v16i3.62816
J. Pendidik. Kim 2024, 16(3), 220– 224

Original Research Article

The potential of methanol extract nanoemulsion from gletang flower (Tridax procumbens) as an antibacterial agent against pathogenic bacteria

SRIWIJAYANTI
Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten 42411, Indonesia

AGRIN FEBRIAN PRADANA
Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten 42411, Indonesia

FAJAR RIZKI FAUJI
Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten 42411, Indonesia

IIN FARLINA
Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten 42411, Indonesia

BOIMA SITUMEANG - *https://orcid.org/0000-0002-5505-5928*
Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten 42411, Indonesia

Corresponding authors: Sriwijayanti (e-Mail: sriwijayanti@gmail.com)

Citation: Sriwijayanti, S., Pradana, A.F., Fauji, F.R., Farlina, I., & Situmeang, B. (2024). The potential of methanol extract nanoemulsion from gletang flower (Tridax procumbens) as an antibacterial agent against pathogenic bacteria. Jurnal Pendidikan Kimia (JPKIM), 16(3), 220 – 224. https://doi.org/10.24114/jpkim.v16i3.66529

ARTICLE INFO

Keywords:
E. coli;
Nanoemulsion;
S. aureus;
T. procumbens

History:
♦Received - 20 Aug 2024
♦Revised - 17 Dec 2024
♦Accepted - 18 Dec 2024

ABSTRACT

Gletang plant is a weed that grows wild and is widely distributed in various places such as rice fields, plantations, and roadsides. Phytochemical screening of Gletang flower extract using methanol revealed the presence of secondary metabolites such as alkaloids, flavonoids, steroids, phenols, terpenoids, and tannins. This study aims to formulate a nanoemulsion from the methanol extract of Gletang flowers. The extraction was performed by maceration with methanol 96%. The nanoemulsion was characterized by testing its pH, % transmittance, stability, viscosity and particle size using a particle size analyzer. The antibacterial activity was tested against *Staphylococcus aureus* and *Escherichia coli* using the Kirby-Bauer method. The results of pH characterization, % transmittance, stability and viscosity tests met the standards. The particle size analyzer showed that the particle size ranged between 300-1000 nm. The antibacterial activity tests indicated that all three formulations showed activity against the pathogenic bacteria *E. coli*, with formulation 3 showing the highest activity.

Introduction

One of the plants with development potential is Gletang (*Tridax procumbens*). Gletang is a wild-growing weed that is widespread in various locations such as rice fields, plantations, and roadsides (Debeturu et al., 2022; Ingole et al., 2022). This plant generally has a year-round life cycle and is not seasonal (Gubbiveeranna & Nagaraju 2016). In some rice fields in Cilegon City, the availability of this plant is abundant, but it has not been widely used by the community. However, the Gletang plant contains bioactive compounds that can be used as a source of raw materials for pharmaceuticals (Badruzzaman et al., 2020).

The part of the plant with the most potential for further research is the flower. Previous studies have shown that phytochemical screening of Gletang flower extract using methanol revealed the presence of secondary metabolites such as alkaloids, flavonoids, steroids, phenols, and terpenoids. These compounds have the potential to act as antibacterial agents, especially against the bacterium S. aureus. Widyawati et al. (2022), reported that Gletang flower extract has antibacterial activity against *S. mutans*.

The common treatment for these infections typically involves the administration of antibiotics. However, inappropriate use of antibiotics can lead to resistance. One approach to combat resistance is to use botanicals as the basis for therapy (Nandi et al., 2022). To maximize antibacterial activity, Gletang flower extract is formulated into a nanoparticle emulsion (Rai et al., 2023). The nanoparticle size increases the amount of dissolved active compounds, which is expected to inhibit the growth of pathogenic bacteria such as *S. aureus*, *E. coli* and *S. mutans* (Nair et al., 2023). Testing of nanoparticle emulsions in natural materials can be performed using instruments such as UV-visible spectrophotometer and particle size analyzer (PSA).

Based on the description of the content of bioactive compounds and its potential, the researcher is interested in conducting a study on the formulation and characterization of a nanoemulsion of *T. procumbens* extract using a sonicator with three different formulations. The antibacterial activity will be tested using the Kirby-Bauer method.

Materials and Methods

Materials

The materials used include Gletang flowers, methanol (pro analysis), Virgin Coconut Oil (VCO), Tween 80, Polyethylene Glycol (PEG) 400, distilled water, bacteria, NaCl, Nutrient Agar (NA), and Nutrient Broth (NB). The equipment used includes an analytical balance, rotary evaporator, stirrer, hot plate, sonicator bath, UV-Vis Spectrophotometer, centrifuge, Ostwald viscometer, universal pH indicator, inoculation loop, and Particle Size Analyzer (PSA).

Sample extraction

The Gletang flowers used as samples in this study were collected from Cilegon City, Banten Province. The samples were cleaned from impurities using running water and then air dried. After drying, the samples were ground using a blender and then subjected to maceration extraction with 96% methanol for three days. The extraction results were filtered using Whatman 40 filter paper. The filtrate was then concentrated using a rotary evaporator at 50°C to obtain a thick Gletang flower extract. Phytochemical screening was performed on the thick Gletang flower extract to confirm the presence of secondary metabolites.

Nanoemulsion formulation of Gletang flower

The formulations of Gletang flower extract (5%, 10%, and 15%) were prepared by mixing them with 3 mL of Virgin Coconut Oil (VCO), 8.5 mL of Tween 80, and 4 mL of PEG 400 using a magnetic stirrer. Then 50 mL of distilled water was gradually added to the mixture while continuing to stir with the magnetic stirrer. After mixing, the solution was placed in a bath-type sonicator for 1 hour (Eqbal et al., 2021). The characterization of the Gletang flower nanoemulsion included testing the physical stability, viscosity, percent transmittance, pH, and particle size.

Antibacterial activity test

The antibacterial activity test refers to the research by Widyawati et al. (2022) and Situmeang et al. (2022) with some modifications. The antibacterial activity test was conducted against S. aureus and E. coli bacteria. A total of 15 µL of samples from formulations 1, 2, and 3, positive control (streptomycin), and negative control were applied to paper disks and then placed on a solid medium containing the bacteria. The plates were then incubated at 37°C for 24 hours. After 24 hours, the diameter of the clear zone around the disk was observed and measured using a caliper.

Results and Discussion

Extraction of Gletang flower

Fresh Gletang flower samples totaling 1.5 kg were collected from Cilegon. The samples were washed and then dried at room temperature for 14 days. Once dry, the samples were ground in a blender to produce a powder known as simplicia. The purpose of grinding is to increase the surface area of the sample so that the solvent can penetrate the cell walls of the sample during extraction. A total of 250 grams of simplicia was used. Methanol was used as the solvent at a ratio of 1:20 (w/v) with 5 L of methanol. The resulting concentrated methanol extract was 68 g with a yield of 27.2%. A yield greater than 10% is considered high. Methanol is a universal solvent capable of extracting secondary metabolites ranging from non-polar to polar compounds (Agidew, 2022).

Phytochemical screening of methanol extract

The phytochemical screening aims to identify the secondary metabolite compounds in the methanol extract of Gletang flowers. The results of the phytochemical screening of the methanol extract of Gletang flowers are shown in the Table 1.

Table 1. Phytochemcial screening of methanol extract of gletang flower

Phytochemical constituent	Test reagents	Result
Alkaloids	Dragendoff's reagent	+
	Wangner's reagent	+
Flavonoids	Lead acetate test	+
Phenolic	AlC_3	+
Steroids	Libermann test	-
Saponins	Froth formation test	-
Terpenoids	Salkowski test	+

Based on the results of phytochemical screening, the methanol extract of Gletang flowers contains alkaloids, flavonoids, phenolics, and terpenoids. According to the research conducted by Situmeang et al. (2022), flavonoid and phenolic compounds have the ability to inhibit the growth of *E. coli* and *S. aureus* bacteria. According to a study by Fazil et al. (2017), alkaloid compounds have antibacterial activity against *S. mutans* bacteria. Based on the phytochemical screening results, the methanol extract of Gletang flowers is likely to have antibacterial activity.

Formulation of gletang flower

Gletang flower nanoemulsions were prepared with extract concentrations of 5%, 10%, and 15%. These extract variations were suspended in 1 mL of virgin coconut oil (VCO) using a magnetic stirrer. The addition of VCO in the nanoemulsion

preparation process is expected to result in stable nanoemulsions. This is because the medium chain fatty acids in VCO are very stable when produced or stored at both low and high temperatures (Jaiswal et al., 2016).

Tween 80 was added at a concentration of 10 mL and mixed with a magnetic stirrer. Tween 80 acts as a surfactant. It is also a nonionic surfactant that does not irritate the skin, has low toxicity, and is soluble in water (Shakeel et al., 2008). PEG 400 was added as a co-surfactant at 1 mL and mixed with a magnetic stirrer until a homogeneous mixture was obtained. The co-surfactant helps to dissolve the solute in the dispersion medium by increasing the flexibility of the layer around the droplet area and reducing the surface free energy, thereby improving the stability (Sneha & Kumar, 2022). The nanoemulsion formulations of the methanol extract of Gletang flowers were prepared in three formulations. The formulations used are listed in Table 2.

Table 2. Formulation of gletang flower

Ingredient	Formula 1	Formula 2	Formula 3
Ekstrak	5%	10%	15%
VCO	1 mL	1 mL	1 mL
Solvent	10 mL	10 mL	10 mL
PEG	1 mL	1 mL	1 mL
Tween 80	10 mL	10 mL	10 mL
Aquadest	28 mL	28 mL	28 mL

The preparation of Gletang flower extract Nanoemulsions involves mechanical energy such as magnetic stirrers and ultrasonicators. The sonication method uses ultrasonic waves where an ultrasonic electric generator produces an electric signal that is then converted into physical vibrations or ultrasonic waves. This results in a very strong effect that causes the molecules in the nanoemulsion to break apart (Li et al., 2021). The samples are reduced to nanoparticle size using a bath sonicator. A bath sonicator applies ultrasonic wave energy to the liquid containing the particles, breaking large particles into smaller ones to achieve nanoparticle size (Qian et al., 2012).

pH, % Transmitan, pH, Viscocity and stability of nanoemultions

The transmittance percentage test aims to determine the clarity of Gletang flower extract Nanoemulsion. The pH test is conducted to measure the acidity of the nanoemulsion. The viscosity test is used to determine the resistance of a fluid to flow or deformation. The results of the transmittance percentage, pH, viscosity, and stability tests are shown in Table 3. According to Sneha & Kumar (2022), a transmittance percentage of 90-100% is classified as a visual indicator of high quality nanoemulsion formulations, indicating clarity and transparency. As the transmittance percentage increases, the droplet size being measured decreases, allowing more light to pass through the sample. When light can pass through the sample, the droplet size is very small, approaching the nanometer scale (Sneha & Kumar, 2022).

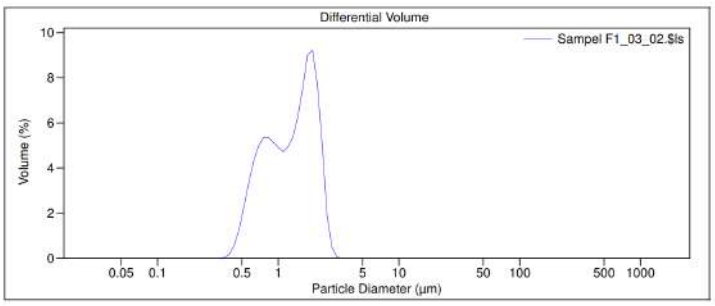
Table 3. pH, % tranmitan, viscocity and stability of nanoemultions

Formula	pH	% Transmitan	Viskositas (cP)	Stability
1 (5%)	5	98.9	1.71	Clear
2 (10%)	5	98.6	1.71	Clear
3 (15%)	5	97.9	1.85	Clear

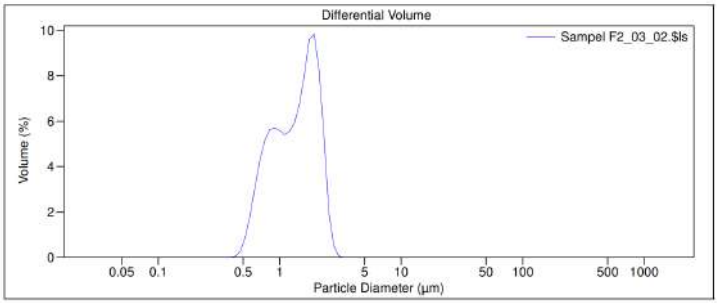
The physical stability test of Gletang flower extract nanoemulsion using centrifugation was conducted to determine the stability of the nanoemulsion formulation (Pandey et al., 2020). Based on the results, the physical stability test showed that the Gletang flower extract nanoemulsion did not show any separation or sediment formation, indicating good stability. According to Table 3, the viscosity measurements for formulations 1, 2, and 3 show that the Gletang flower extract nanoemulsions have a higher viscosity than water, which has a viscosity of 0.89 cP. The viscosity values of the Gletang flower extract nanoemulsions are still within the typical range for nanoemulsions, which is between 1-100 cP (Sneha & Kumar, 2022). Table 3 also shows the pH test results, where formulations F1, F2, and F3 of the Gletang flower extract nanoemulsion all have a pH of 5. This pH is within the standard range and is considered safe for use.

Particle Size Analyzer (PSA)

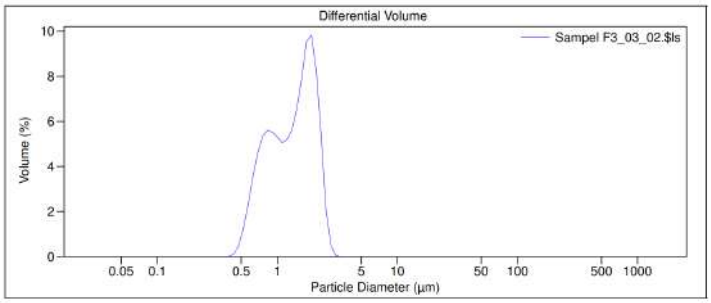
The result of particle size of nanoemultion sampel shown in Fig-1. The particle size distribution of the nanoemulsion, as determined by particle size analyzer measurements, exhibited a median particle size of 900 nm with a relatively narrow distribution (PDI = 0.2). This indicates that the emulsification process was successful in producing particles with a high degree of homogeneity. Particles with sizes below 1000 nm have been shown to demonstrate enhanced stability and higher bioavailability potential.



(a)



(b)



(c)

Fig-1. Particle Size Analyzer of formulations 1 (a), formulation 2 (b) and formulation 3 (c) of gletang flower nanoemultion

Antibacterial activity test result

The antibacterial activity of the Gletang flower extract nanoemulsion against *S. aureus* and *E. coli* bacteria can be observed by measuring the diameter of the inhibition zone on the paper disc (Widyawati et al., 2022). The fundamental principle of this methodology is that the Gletang flower extract nanoemulsion will diffuse into the inoculated medium, thereby inhibiting the growth of *S. aureus* and *E. coli*. The result of antibacterial activity test shown in Table 4 and Fig-2.

Table 4. The result of antibacterial activity test of formulation nanoemultion gletang flower

Formula	Zona hambat (mm)	
	E. coli	*S. aureus*
1	0	0
2	0.25	0
3	0.5	0
kontrol (+)	8.4	8.0
kontrol (-)	0	0

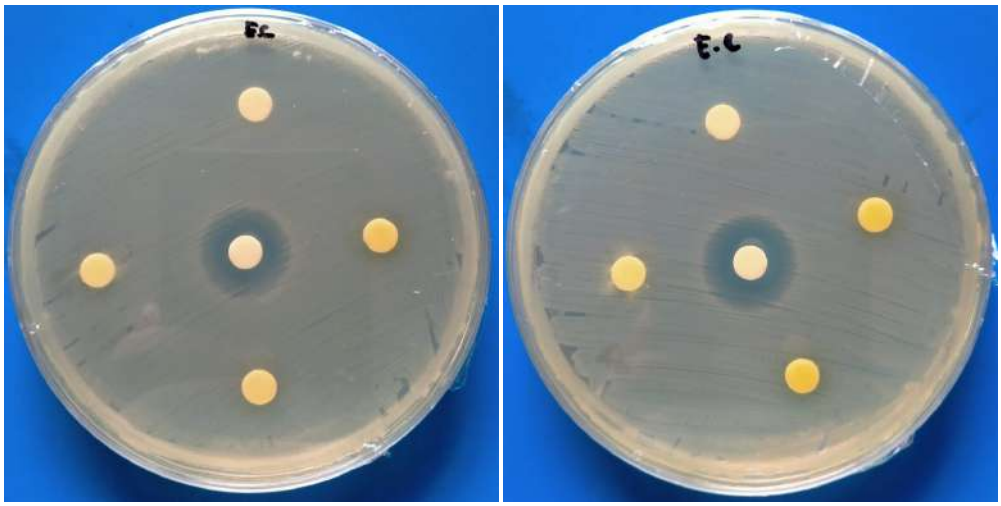


Fig-2. Inhibision zone of gletang nanoemultion against E. coli bacteria

The results of the antibacterial activity test of the Gletang flower extract nanoemulsion demonstrate that the nanoemulsion exhibits antibacterial activity against *E. coli*, as evidenced by the presence of a clear zone around the paper disc. Nevertheless, no activity was observed against S. aureus. The inhibitory effect of the sample preparations on the growth of *E. coli* in all formulations is classified as weak.

Conclusion

The characterization results for pH, percent transmittance, stability, and viscosity were found to meet the established standards. The results of the particle size analyzer test indicate that the particle size ranges from 300 to 1,000 nanometers. The results of the antibacterial activity tests indicate that all three formulations exhibit only minimal inhibitory activity against the pathogenic bacterium *E. coli*.

Conflict of Interests

The author declares that there is no conflict of interest in this research and manuscript.

Acknowledgment

The authors would like to thank the Indonesian government, through the Ministry of Education, Culture, Research, and Technology, for providing research funding through the 2024 Beginner Lecturer Scheme for Sriwijayanti.

References

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bull Natl Res Cent*, 46(87). doi:10.1186/s42269-022-00770-8

Badruzzaman, H., Haryati, E., & Susanto, H. (2020). Uji efektivitas suspensi ekstrak daun songgolangit (Tridax procumbens L.) terhadap penurunan kadar asam urat pada tikus putih jantan. *Jurnal Farmasi dan Sains*, 1(1), 72-79.

Debeturu, S., Tulandi, S., Paat, V., & Tiwow, G. (2022). Uji aktivitas analgesik ekstrak etanol daun songgolangit (Tridax procumbens L.) terhadap tikus putih (Rattus norvegicus). *Biofarmasetikal Tropis*, 5(1), 66-72. doi:10.55724/jbiofartrop.v5i1.371

Eqbal, A., Ansari, V. A., Hafeez, A., Ahsan, F., Imran, M., & Tanweer, S. (2021). Recent applications of nanoemulsion based drug delivery system: A review. *Research Journal of Pharmacy and Technology*, 14(5), 2852-2858. doi:10.52711/0974-360X.2021.00502

Gubbiveeranna, V., & Nagaraju, S. (2016). Ethnomedicinal, phytochemical constituents and pharmacological activities of Tridax procumbens: a review. *Int J Pharm Pharm Sci*, 8(2), 1-7.

Ingole, V. V., Mhaske, P. C., & Katade, S. R. (2022). Phytochemistry and Pharmacological Aspects of Tridax procumbens (L.): A Systematic and Comprehensive Review. *Phytomedicine Plus*, 2(1), 100199. doi:10.1016/j.phyplu.2021.100199

Jaiswal, M., Dudhe, R., & Sharma, P. K. (2015). Nanoemulsion: An advanced mode of drug delivery system. *Biotech*, 5(2), 123-127. doi:10.1007/s13205-014-0214-0

Li, G., Zhang, Z., Liu, H., & Hu, L. (2021). Nanoemulsion-based delivery approaches for nutraceuticals: Fabrication, Application, characterization, biological fate, potential toxicity and future trends. *Food & Function*, 12(5), 1933-1953. doi:10.1039/D0FO02686G

Nair, A., Greeny, A., Nandan, A., Sah, R. K., Jose, A., Dyawanapelly, S., Junnuthula, V., Athira K. V., & Sadanandan, P. (2023). Advanced drug delivery and therapeutic strategies for tuberculosis treatment. *Journal of Nanobiotechnology*, 21(414). doi:10.1186/s12951-023-02156-y

Nandi, T., Nayan, M. I. H., & Nisha, M. H. (2022). Phytochemical and Biological Investigation of the Leaves of Tridax procumbens. *Journal of Complementary and Alternative Medical Research*, 20(3), 34-40. doi:10.9734/jocamr/2022/v20i3418

Pandey, P., Gulati, N., Makhija, M., Purohit, D., & Dureja, H. (2020). Nanoemulsion: A Novel Drug Delivery Approach for Enhancement of Bioavailability. *Recent Patents on Nanotechnology*, 14(4), 276-293. doi:10.2174/1872210514666200604145755

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Nanoemulsion delivery systems: Influence of carrier oil on β-carotene bioaccessibility. *Food Chemistry*, 135(3), 1440-1447. doi: 10.1016/j.foodchem.2012.06.047

Rai, S. K., Mishra, N., & Yadav, K. S. (2023). Nanoemulsions: A potent carrier system for transdermal drug delivery. *Journal of Drug Delivery Science and Technology*, 79, 104049. doi:10.1016/j.jddst.2023.104049

Shakeel, F., Baboota, S., Ahuja, A., Ali, J., Aqil, M., & Shafiq, S. (2007). Nanoemulsions as vehicles for transdermal delivery of aceclofenac. *AAPS PharmSciTech*, 8(104), E1-E9. doi:10.1208/pt0804104

Situmeang, B., Ibrahim, A.M., Bialangi, N., Musa, W.J.A., & Silaban, S. (2019). Antibacterial activity and phytochemical screening of kesambi (Sapindaceae) against Eschericia coli and Staphylococcus aureus. *Jurnal Pendidikan Kimia*, 11 (1), 14-17. doi:10.24114/jpkim.v11i1.13078

Sneha, K., & Kumar, A. (2022). Nanoemulsions: Techniques for the preparation and the recent advances in their food applications. *Innovative Food Science & Emerging Technologies*, 76, 102914. doi:10.1016/j.ifset.2021.102914

Widyawati., Arma, U., Fadriyanti, O., Situmeang, B., and Silaban, S. (2022). Antibacterial activity of different parts of gletang (Tridax procumbens) from West Sumatera Indonesia. *Rasayan Journal of Chemistry*. 5 (4): 2382-2386. doi:10.31788/RJC.2022.1547084