Plumeriastuti *et al*. MKH (2021). 97-104
DOI: 10.20473/mkh.v32i2.2021.97-104

Antimicrobial effect of Methanol and Ethanol Extracts of Kembang Bulan (*Tithonia diversifolia*) Leaves against *Staphylococcus aureus*

Hani Plumeriastuti[1*], Muhammad Afif Habibi[2], Benjamin Christoffel Tehupuring[3], Mustofa Helmi Effendi[4], Agnes Theresia Soelih Estoepangestie[4], Suryanie Sarudji[5], Adiana Mutamsari Witaningrum[4]

[1]Division of Veterinary Pathology, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga
[2]Bachelor of Veterinary Medicine Study Program, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga
[3]Division of Veterinary Anatomy, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga
[4]Division of Veterinary Public Health, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga
[5]Division of Veterinary Microbiology, Department of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga

*E-mail: hani-p@fkh.unair.ac.id

ABSTRACT

The purpose of this study was to determine how *Staphylococcus aureus* responded to Kembang Bulan (*Tithonia diversifolia*) leaf extract's antibacterial properties. Eight *S. aureus* bacterial isolates from three Surabaya dairy farms were used in this study. *Tithonia diversifolia* leaf extract at concentrations of 10%, 5%, 2.5%, 1.25%, and 0% were employed in the study's samples. The antibacterial activity of *Tithonia diversifolia* leaf extract was assessed in this work using the disk diffusion method and an inhibition zone estimated in millimeters. Based on the findings of this study, it was determined that *Tithonia diversifolia* leaf extract at 5% and 10% concentrations had 100% antibacterial activity against *S. aureus* in eight samples. As with *S. aureus*, *Tithonia diversifolia* leaf extract at concentrations of 0%, 1.25%, and 2.5% did not exhibit any antibacterial action.

Keywords: Antimicrobial, *Tithonia diversifolia*, *Staphylococcus aureus*

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

INTRODUCTION

In the tropical nation of Indonesia, there are 28,000 different types of plants, with more than 7,000 of them purportedly having therapeutic properties (Pramono, 2002). While the remaining species still require scientific study, only 283 of them are utilized as traditional medicines (Khairullah et al., 2020). Indonesia's diverse flora serves a range of purposes, one of which is as a source of medicinal plants (Isnawati et al., 2019).

Medicinal plants are those that have been identified and proven to have substances that help prevent and treat illnesses, carry out specific biological functions, and protect against insects and fungi based on human observation (Mahomoodally, 2013). The local community is crucial as a source of information for the continued use of herbal medicines since it possesses traditional knowledge of therapeutic plants (Rahayu et al., 2020). Recent studies have focused on using anti-microbial screening to identify the medicinal uses of plants (Vaou et al., 2010; Manandhar et al., 2019; Romulo et al., 2018).

One of the medicinal plants used in traditional medicine in Indonesia belongs to the Asteraceae family and is called Kembang Bulan or *Tithonia diversifolia* (Rahman et al., 2021). This plant is effective in treating liver disease, leprosy, flatulence, and wounds or injuries that have bruised (Sari et al., 2016). The result from the phytochemical screening test of *Tithonia diversifolia* leaves contains flavonoids, glycosides, saponins, tannins, and triterpernoid or steroids (Otusanya and Ilori, 2012). The presence of flavonoid and tannin compounds is expected to inhibit bacterial growth and has antibacterial activity (Ogundare, 2007).

Staphylococcus aureus is a significant human pathogen because it can cause catastrophic life-threatening infections, moderate skin infections, and food poisoning (Hennekinne et al., 2012). Meanwhile, certain *S. aureus* bacterial strains are part of the typical human skin and respiratory tract flora (Otto, 2010). The effects of *S. aureus* species on animal health and their ability to spread from animals to humans and humans to animals make them harmful in veterinary medicine (Lozano et al., 2016). It has a significant negative effect on animal health and welfare and results in significant financial losses in the production of livestock because it can make cattle develop mastitis, which lowers milk supply (Karzis et al., 2014; Peton and Leloir., 2014).

Previous research using the extract from *Tithonia diversifolia* leaves, proved its antibacterial activity against *S. aureus* bacteria when used using the well diffusion method, therefore, using the disk diffusion method, the author of this study intends to investigate the antibacterial activity of *Tithonia diversifolia* leaf extract against other bacteria that harm cattle and cause

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

financial losses in the production of livestock, such as *S. aureus* (Ningsih et al., 2016).

Through the use of a disk diffusion technique with *Tithonia diversifolia* extract, this study seeks to identify the antibacterial activity of Kembang Bulan (*Tithonia diversifolia*) against *S. aureus* as a result of the existence of an antibacterial ingredient in the plants.

MATERIALS AND METHODS

Different concentrations of infusion are made using the *Tithonia diversifolia* leaf extract that was purchased from PT. Herbacore. This study used several concentrations of *Tithonia diversifolia* extract, including 10%, 5%, 2.5%, 1.25%, and 0%. For 10% of *Tithonia diversifolia* infusion use 1 gr extract in 9 ml methanol and ethanol as solvent, for 5% *Tithonia diversifolia* infusion use 0.5 gr extract in 9.5 ml methanol and ethanol as solvent, 2.5% *Tithonia diversifolia* infusion use 0.25 gr extract in 9.75 ml methanol and ethanol as solvent, 1.25% *Tithonia diversifolia* infusion use 0.125 gr extract in 9.875 ml methanol and ethanol as solvents, for 0% use only ethanol and methanol. Give each injection 24 hours. Each infusion should have a blank disk in it. Let it stand for 10 minutes. Take the infused disk and incubate it till it is dry after 10 minutes (Lemos et al., 2010).

Eight pure isolates of *S. aureus* were used in this study. They were found in isolated milk from instances of subclinical mastitis in the Surabaya dairy farms Kaliwaron, Jemursari, and Bendul Merisi.

Prepare a Petri disk measuring 90 x 15 mm that already has Mueller-Hinton media on it. Then, prepare a bacterial suspension that has been standardized with McFarland Standard no. 1 by dissolving bacterial colonies in physiological NaCl 0.9% solvent (PZ). Finally, drop 0.2 ml of the suspension on the Mueller-Hinton medium's surface in the Petri disk. Flatten with a bent crooked glass and let stand for 15 minutes (Abba et al., 2020).

Attaching a blank disc with various concentrations of *Tithonia diversifolia* extract to the surface of Mueller-Hinton agar media and letting it stand for 15 minutes is how the antibacterial test for the extract is carried out. *Tithonia diversifolia* extracts of 10%, 5%, 2.5%, 1.25, and 0% were employed in this study, along with a 30 µg disk of tetracycline, which was incubated for 24 hours at 30°C to 35°C. Observe the clear zones around the disk by millimeters using a caliper (Ramandinianto et al., 2020).

The inhibition zone is determined in this study using calipers in millimeters and compared to the norm in Tables 1 and 2.

RESULTS AND DISCUSSION

Table 3 shows the inhibition zone as determined by the antibacterial activity extract of *Tithonia diversifolia* leaf using

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

Table 1. Diameter of inhibition zone (CLSI,2013)

Antibacterial	Resistant	Intermediate	Sensitive
Tetracycline 30 µg	≤ 14 mm	15 mm–18 mm	≥ 19 mm

Table 2. The standard diameter of inhibition zone *Tithonia diversifolia* extract

Antibacterial	Resistant	Intermediate	Sensitive
Tithonia difersivolia ext	-	-	≥ 8 mm

Table 3. Measurement of inhibition zone of *Staphylococcus aureus* sensitivity to antibacterial from *Tithonia diversifolia* leaf extract with methanol as a solvent and tetracycline antibiotic as the positive control

No.	Samples	Diameter of inhibition zone in mm					
		0%	1.25%	2.5%	5%	10%	Tetracycline 30 µg
1	1	6(R)	6(R)	6(R)	9.21(S)	11.21(S)	21.3(S)
2	9	6(R)	6(R)	6(R)	13.77(S)	15.6(S)	23.6(S)
3	15	6(R)	6(R)	6(R)	11.9(S)	13.7(S)	21.34(S)
4	17	6(R)	6(R)	6(R)	9.1(S)	10.2(S)	21.28(S)
5	18	6(R)	6(R)	6(R)	11.53(S)	15.1(S)	25.5(S)
6	23	6(R)	6(R)	6(R)	12.98(S)	19.8(S)	29.45(S)
7	26	6(R)	6(R)	6(R)	13.1(S)	16.7(S)	26.8(S)
8	28	6(R)	6(R)	6(R)	13.3(S)	20.2(S)	30.5(S)

Table 4. Measurement of inhibition zone of *S. aureus* sensitivity to antibacterial from *Tithonia diversifolia* leaf extract with ethanol as a solvent and tetracycline antibiotic as the positive control

No.	Samples	Diameter of inhibition zone in mm					
		0%	1.25%	2.5%	5%	10%	Tetracycline 30 µg
1	1	6(R)	6(R)	6(R)	8.21(S)	10.01(S)	20.12(S)
2	9	6(R)	6(R)	6(R)	11.17(S)	13.61(S)	22.56(S)
3	15	6(R)	6(R)	6(R)	10.12(S)	12.34(S)	20.44(S)
4	17	6(R)	6(R)	6(R)	8.7(S)	9.12(S)	21.56(S)
5	18	6(R)	6(R)	6(R)	9.23(S)	11.24(S)	22.67(S)
6	23	6(R)	6(R)	6(R)	10.64(S)	14.37(S)	26.15(S)
7	26	6(R)	6(R)	6(R)	11.38(S)	15.84(S)	25.35(S)
8	28	6(R)	6(R)	6(R)	12.1(S)	17.43(S)	28.41(S)

the disk diffusion method. Tables 3 and 4 show that extracts of the leaves of *Tithonia diversifolia* are 0%, 1.25%, and 2.55% resistant to S. aureus, while 5% and 10% are sensitive to S. aureus. All of the samples are responsive to the

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

antibiotic tetracycline. Through the inhibition zone that formed around the disk, the sensitivity is visible (Hombach et al., 2013).

Using the disk diffusion method to determine the antibacterial activity of *Tithonia diversifolia* leaf extract in tables 5 and 6, eight (100%) isolates were found to be sensitive to *Tithonia diversifolia* leaf extract at 5% and 10% when using ethanol and methanol as solvents, while eight (100%) isolates were found to be resistant at 0%, 1.25%, and 2.5% when using ethanol and methanol as solvents. Eight (100%) isolates were also found to be sensitive to tetracycline at 30 µg.

Table 5. Percentage (%) of antibacterial activity of *Tithonia diverfolia* leaf extract with methanol as a solvent and tetracycline antibiotic as the positive control

Sensitivity Categories	0%	1.25%	2.5%	5%	10%	Tetracycline 30 µg
Resistant	0 (0%)	0 (0%)	0 (0%)	8 (100%)	8 (100%)	8 (100%)
Intermediate	0 (0%)	0 (0%)	0 (0%)	8 (100%)	8 (100%)	8 (100%)
Sensitive	0 (0%)	0 (0%)	0 (0%)	8 (100%)	8 (100%)	8 (100%)

Table 6. Percentage (%) of antibacterial activity of Tithonia diverfolia leaf extract with ethanol as solvent and tetracycline antibiotic as control positive

Sensitivity Categories	0%	1.25%	2.5%	5%	10%	Tetracycline 30 µg
Resistant	0 (0%)	0 (0%)	0 (0%)	8 (100%)	8 (100%)	8 (100%)
Intermediate	0 (0%)	0 (0%)	0 (0%)	8 (100%)	8 (100%)	8 (100%)
Sensitive	0 (0%)	0 (0%)	0 (0%)	8 (100%)	8 (100%)	8 (100%)

The leaf extract from *Tithonia diversifolia* contains sesquiterpenes, tannin, flavonoids, and terpenes that have an antibacterial effect by blocking the autolysin enzyme, which is necessary to create openings for a continuous network of mucopeptides and adding new building blocks during growth, necessary to separate the two freshly created bacteria from one another and for modifying the cell wall to create the septum that separates one bacterium into two cells (Dewole, 2013). As a result, bacterial growth will be inhibited by the loss or suppression of autolytic enzyme activity (Ahmed et al., 2020).

Sesquiterpenes are a class of secondary metabolites found in *Tithonia diversifolia* leaf extract that function as an antibacterial agent by rupturing the phospholipid membrane of *S. aureus* (Merciline and Dominic, 2020).

In this study, tetracycline 30 µg was utilized as a positive control to demonstrate that the bacteria are

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

susceptible to antibiotics. Tetracycline is a broad-spectrum antibiotic that can stop both Gram-positive and Gram-negative bacteria from growing (Grossman, 2016). Tetracycline serves as a comparison in this study between *Tithonia diversifolia* leaf extract and tetracycline, which possesses antibacterial action against *S. aureus* (Ogunfolaka et al., 2010). In this study, all samples are tetracycline sensitive. Tetracycline works by preventing bacteria from synthesizing proteins (Barrenechea et al., 2021).

CONCLUSIONS

In this research, tetracycline has a function as the comparison of *Tithonia diversifolia* leaf extract that can have the same effect as tetracycline that has antibacterial activity against S. aureus.

REFERENCES

Abba, B.N., A. Romane, and A.T. Ilagouma. 2020. Antibacterial Activity of Endostemon tereticaulis (Poir.) M. Ashby Essential Oil and Ethanolic Extract Against Resistant Pathogenic Bacteria. Nat. Prod. Com; 15(9): 1-9.

Ahmed, B., F. Ameen, A. Rizvi, K. Ali, H. Sonbol, A. Zaidi, M.S. Khan, and J. Musarrat. 2020. Destruction of Cell Topography, Morphology, Membrane, Inhibition of Respiration, Biofilm Formation, and Bioactive Molecule Production by Nanoparticles of Ag, ZnO, CuO, TiO2, and Al2O3 toward Beneficial Soil Bacteria. ACS Omega; 5(14): 7861-7876.

Barrenechea, V., M. Vargas-Reyes, M. Quiliano, and P. Milón. 2021. A Complementary Mechanism of Bacterial mRNA Translation Inhibition by Tetracyclines. Front. Microbiol.; 12: 682682.

Dewole, J. 2013. Phytochemical and Antimicrobial Studies of Extracts from the Leaves of *Tithonia diversifolia* for Pharmaceutical Importance. IOSR J. Pharm. Biol. Sci; 6(4): 21-25.

Grossman, T.H. 2016. Tetracycline Antibiotics and Resistance. Cold Spring Harbor Persp. Med; 6(4): a025387.

Hennekinne, J.A., M.L. De Buyser, and S. Dragacci. 2012. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. FEMS Microbiol. Rev; 36(4): 815-836.

Hombach, M., R. Zbinden, and E.C. Böttger. 2013. Standardisation of disk diffusion results for antibiotic susceptibility testing using the sirscan automated zone reader. BMC Microbiiol; 13: 225.

Isnawati, A., R. Gitawati, M. Raini, S. Alegantina, and V. Setiawaty. 2019. Indonesia basic health survey: self-medication profile for diarrhea with traditional medicine. African Health Sci; 19(3): 2365-2371.

Karzis, J., I.M. Petzer, V. Naidoo, and E.F. Donkin. 2021. The spread and antimicrobial resistance of *Staphylococcus aureus* in South

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

African dairy herds - A review. Onderstepoort J. Vet. Res; 88(1): e1-e10.

Khairullah, A.R., T.I. Solikhah, A.N.M. Ansori, A. Fadholly, S.C. Ramandinianto, R. Ansharieta, A. Widodo, K.H.P. Riwu, N. Putri, A. Proboningrat, M.K.J. Kusala, B.W. Rendragraha, A.R.S Putra, and A. Anshori. 2020. A Review of an Important Medicinal Plant: Alpinia galanga (L.) Willd. Sys. Rev. Pharm; 11(10): 387-395.

Lemos, J.A., J. Abranches, H. Koo, R.E. Marquis, and R.A. Burne. 2010. Protocols to study the physiology of oral biofilms. Methods Mol. Biol; 666: 87-102.

Lozano, C., H. Gharsa, B.K. Slama, M. Zarazaga, and C. Torres. 2016. *Staphylococcus aureus* in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African Continent. Microorganisms; 4(1): 12.

Mahomoodally, M.F. 2013. Traditional medicines in Africa: an appraisal of ten potent african medicinal plants. Evidence-Based Compl. Alt. Med; 617459.

Manandhar, S., S. Luitel, and R.K. Dahal. 2019. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. J. Trop. Med; 1895340.

Merciline, O. and M. Dominic. 2020. Phytochemical screening and antimicrobial activity of crude extract of Tithonia diversifolia. Open J. Biol. Sci; 5(1): 28-33.

Ningsih, W., Firmansyah, and S. Anggraini. 2016. Formulation and Test of Antibacterial Activity Hand Sanitizer Gel Ethanol Extract of Kembang Bulan. Sci. J. Pharm; 12(2).

Ogundare, A.O. 2007. Antimicrobial Effect of *Tithonia diversifolia* and Jatropha gossypifolia Leaf Extracts. Trends Appl. Sci. Res; 2(2): 145-150.

Ogunfolaka, O., O.S. Kolawole, and A. Olowe. 2010. In vitro Antimicrobial Activity of *Tithonia diversifolia* Leaf Extracts on Bacterial Isolates from Wound Infections from a Nigerian Hospital. Res. J. Med. Sci; 4(5): 305-308.

Otto, M. 2010. Staphylococcus colonization of the skin and antimicrobial peptides. Expert Rev. Dermatol; 5(2): 183-195.

Otusanya, O. and O. Ilori. 2012. Phytochemical Screening and the Phytotoxic Effects of Aqueous Extracts of *Tithonia diversifolia* (Hemsl) A. Gray. Int. J. Biol; 4(3): 97-101.

Peton and Leloir. 2014. *Staphylococcus aureus* in Veterinary Medicine. Infect. Genet. Evol; 602-615.

Pramono, E. 2002. Prospects and Potential for Agromedicine Commodity Development in Indonesia. Proceedings of the National Symposium II of APINMAP Medical and Aromatic Plants. Biol. Res. Cent; 31-37.

Rahayu, Y.Y.S., T. Araki, and D. Rosleine. 2020. Factors affecting the use of herbal medicines in the universal health coverage system in

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index

Indonesia. J. Ethnopharm; 260: 112974.

Rahman, N.F., Nursamsiar, Megawati, Handayani, and C.A.M. Suares. 2021. Total Phenolic and Flavonoid Contents and Antioxidant Activity of Kembang Bulan Leaves (*Tithonia diversifolia* (Hemsley) A. Gray). Indonesian J. Pharmaceut. Sci. Technol; 1(1): 57-65.

Ramandinianto, S.C., A.R. Khairullah, and M.H. Effendi. 2020. MecA gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from dairy farms in East Java, Indonesia. Biodiv; 21(8): 3562-3568.

Romulo, A., E.A.M. Zuhud, J. Rondevaldova, and L. Kokoska. 2018. Screening of in vitro antimicrobial activity of plants used in traditional Indonesian medicine. Pharmaceut. Biol; 56(1): 287-293.

Sari, Y.O., D. Dillasamola, S. Dharma, and M. Clara. 2016. The Influence of Ethanol Extract and Variety of Fractions from *Tithonia diversifolia* on Blood Glucose Level of Male White Mice. Schol. Res. Lib; 8(19): 446-450.

Vaou, N., E. Stavropoulou, C. Voidarou, C. Tsigalou, and E. Bezirtzoglou. 2021. Towards Advances in Medicinal Plant Antimicrobial Activity: A Review Study on Challenges and Future Perspectives. Microorganisms; 9(10): 2041.

Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index