p-ISSN : 0215-8930; e-ISSN : 2775-975X

Media Kedokteran Hewan
(Veterinary Medicine Journal)

Figure source: Yesica et al. 2021. Media Kedokteran Hewan, 31 (1): 11-22

MKH (Vet. Med. J) Vol. 32, No. 2, May 2021, Pp. 52-96

p-ISSN 0215-8930; e-ISSN 2775-975X

Media Kedokteran Hewan (Veterinary Medicine Journal)

Volume 32, No.2, May 2021

Media Kedokteran Hewan (p-ISSN: 0215-8930) (e-ISSN: 2775-975X) (established 1985) publishes all aspects of veterinary science and its related subjects. Media Kedokteran Hewan publishes periodically three times a year (January, May, and September). Media Kedokteran Hewan publishes original articles, review articles, and case studies in Indonesian or English, with an emphasis on novel information of excellent scientific and/or clinical quality, relevant to domestic animal species and biotechnology of veterinary medicine from researchers, lecturers, students, and other practitioners around Indonesia and worldwide.

Editor in Chief
Yulianna Puspitasari, drh., M.VSc., Ph.D. Universitas Airlangga, Indonesia

Associate Editor
Dian Ayu Permatasari, drh., M.Vet. Universitas Airlangga, Indonesia
Dhandy Koesoemo Wardhana, drh., M.Vet. Universitas Airlangga, Indonesia

Assistant Editor
I Ketut Mudite Adnyane, drh., M.Si., Ph.D., PAVet. Institut Pertanian Bogor University, Indonesia

Administration Staff
Ardiono, Universitas Airlangga, Indonesia

Editorial Board Members

Prof. Dr. Pudji Srianto, drh. M.Kes. Universitas Airlangga, Indonesia
Prof. Dr. Suwarno, drh., M.Kes. Universitas Airlangga, Indonesia
Prof. Dr. Wiwik Misaco Yuniarti, drh., M.Kes. Universitas Airlangga, Indonesia
Prof. Dr. Lucia Tri Suwanti, drh., M.P. Universitas Airlangga, Indonesia
Dr. Tariq Jamil, Friedrich Loeffler Institute, Jena, Germany
Dr. Attaur Rahman, College of Animal Husbandry & Veterinary Sciences, Abdul Wali Khan University Mardan, Pakistan
Dr. Shekhar Chhetri, DVM, M.Sc. Royal University of Bhutan, Bhutan
Dr. Ugbo Emmanuel Nnabuike, Ebonyi State University, Nigeria
Dr. Abzal Abdramanov, M.Sc. Kazakh National Agrarian Research University, Kazakhstan

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

Dr. Jagdish Gudewar, B.VSc., M.VSc. College of Veterinary & Animal Sciences, Parbhani, India
Abdul Rehman, DVM., M.Phil., Ph.D. University of Veterinary and Animal Sciences, Lahore, Pakistan
Dr. Freshinta Jellia Wibisono, drh., M.Vet. Universitas Wijaya Kusuma, Surabaya, Indonesia
Roza Azizah Primatika, S.Si, M.Si. Universitas Gadjah Mada, Indonesia
Fidi Nuraini, drh.,M.Si. Universitas Brawijaya, Indonesia
Dr. Nanik Hidayatik, drh., Universitas Airlangga, Indonesia
Mohammad Thohawi Elziyad P, drh., M.Si. Universitas Airlangga, Indonesia
Ragil Angga Prastiya, drh., M.Si. Universitas Airlangga, Indonesia
Mirza Atikah Madarina Hisyam, drh., M.Si. Universitas Airlangga, Indonesia

Peer Reviewer:

Prof. Dr. Bambang Sektiari, drh., DEA. Universitas Airlangga, Indonesia
Prof. Dr. Imam Mustofa, drh, M.Kes. Universitas Airlangga, Indonesia
Prof. Dr. Fedik Abdul Rantam, drh. Universitas Airlangga, Indonesia
Prof. Dr. Ir. Sri Hidanah, MS. Universitas Airlangga, Indonesia
Prof. Dr. Widya Paramita Lokapirnasari, drh., MP. Universitas Airlangga, Indonesia
Prof. Dr. Tita Damayanti Lestari, drh, M.Sc. Universitas Airlangga, Indonesia
Prof. Ooi Hong-Kean, DVM.,Ph.D. Japan Science and Technology Agency, Japan
Sattar Mengal, DVM., M.Phil., Ph.D. Lasbela University of Agriculture, Water and Marine Sciences, Pakistan
Innocent Damudu Peter, DVM., M.VSc., Ph.D. University of Maiduguri, Nigeria
Mohd Farhan Hanif bin Reduan, DVM., Ph.D. Universiti Malaysia Kelantan, Malaysia
Nurrul Shaqinah Nasruddin, DVM, Ph.D. National University of Malaysia, Malaysia
Norfitriah Mohamed Sohaimi, DVM., Ph.D. Universiti Putra Malaysia, Malaysia
Dr. Mark Hiew Wen Han, Universiti Putra Malaysia, Malaysia
Taira Kensuke, DVM., Ph.D. Azabu University, Japan
Shin Yoon-Joo, Seoul National University, South Korea
Rey Oronan, University of the Philippines, Los Banos
Eddy Sukmawinata, DVM., M.Sc., Ph.D. Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Japan
Dr. Sri Rahmatul Laila, Institut Pertanian Bogor University, Indonesia
Dr. Chaerul Basri, Institut Pertanian Bogor University, Indonesia
Wredha Sandhi Ardha Prakoso,drh., M.Sc. Karantina Hewan, Badan Karantina Pertanian
Dilasdita Kartika Pradana, drh., M.Si. Balai Besar Veteriner Kelas I Denpasar, Bali, Indonesia
Dr. Sarasati Windria, drh. Universitas Padjajaran, Bandung, Indonesia
Shelly Kusumarini R, drh., M.Si. Universitas Brawijaya, Indonesia
Syaiful Rizal, drh., M.Si. Research Center for Biology - LIPI, Indonesia

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

Suryo Kuncorojakti, drh., M.Vet., Ph.D. Universitas Airlangga, Indonesia
Adiana Mutamsari Witaningrum, drh., M.Vet. Universitas Airlangga, Indonesia
Oky Setyo Widodo, drh., M.Si. Universitas Airlangga, Indonesia
Martia Rani Tacharina, drh., M.Si. Universitas Airlangga, Indonesia

Editorial Office : Faculty of Veterinary Medicine, Universitas Airlangga
Kampus C Universitas Airlangga Mulyorejo, Surabaya 60115
Telp. (031)5992785, 5993016 Fax. (031) 5993015
e-mail : medvetj@journal.unair.ac.id

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

Author Guidelines

Veterinary Medicine Journal is a journal that contains scientific articles on veterinary medicine and animal husbandry biotechnology published by Faculty of Veterinary Medicine Airlangga University 3 times per year on January, May and September. Veterinary Medicine Journal received manuscripts in the form of original research articles, review articles and case reports in Indonesian and English. Manuscripts received must be original, current and have never been published or are being planned to be published in other scientific journals.

Manuscripts must be submitted online through the Open Journal System (OJS) in Word format. The entire text is typed in Book Antiqua 11pt double spaced. The title is written with a Title case (bold, 14pt, align center). The full length of the manuscripts is a maximum of 12 pages of HVS paper. Italicize only for species names or terms that have not been standardized as Indonesian. Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum. Avoid unnecessary duplication of text.

The first page contains titles in Indonesian and English, followed by full names of all authors without titles and initials (bold, center), followed by the name and complete address of the respective institution (marked with numeric superscripts) and e-mail of corresponding authors (marked with *superscript).

The second page forward contain:
ABSTRACT in English and Indonesian, **INTRODUCTION**, **MATERIALS AND METHODS**, **RESULTS**, **DISCUSSION**, **CONCLUSIONS**, **APPROVAL OF ETHICAL COMMISSION**, **ACKNOWLEDGEMENTS** and **REFERENCES**.

Title should be concise but informative, as far as possible in no more than 12 words.

Abstract written in Indonesian and English, do not exceed 200 words, containing elements of background, material and methods, results and conclusions.

Keywords maximum of 5 (five) words or phrases, written after the abstract in each languages, alphabetically ordered. As far as possible avoid using keywords from the title.

Introduction should be brief, containing elements of background, problems, objectives and reference sources that support.

Research materials (materials and equipment) do not need to be mentioned separately, but rather integrated in the method used, complete with the brand and catalog number if applicable. Do not include common supplies, such as test tubes, pipette tips, beakers, etc. or standard laboratory equipment.

Method must be concise but sufficiently detailed (with reference or modification) so research can be repeated by other researchers.

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

Results are displayed in a concise but clear narrative with/without tables or figures.

Tables are made without vertical lines (use only lines at the top and bottom of the table as well as for separating heading from the main table), with table title placed before the table, numbered in Arabic numerals (**Table X**), and have to be referred in the text. The description of the table is placed after the table; it must be concise but clear enough so that the table separately can be understood without referring to the text. The table along with the title and description are placed after the References.

Figures presented are only those that support the findings of the study, and not restatement of data from tables in the form of figures. When resulted data in the form of figure is more informative, interesting or significant, presentation of data in table form is not required. Figure title is placed after the figure, numbered in Arabic numerals (**Figure Y**), and have to be referred in the text. The description of the figure is placed under the title of the figure; it must be concise but clear enough so that the figure separately can be understood without referring to the text. Image (in JPEG format) is sent in separate file. The title and description of the figure are placed after the References.

Discussion contains explanation of what are found related to **the importance of your study** and how it may be able to answer the research question, comparison of findings (internally, between research data, and externally, compared with findings from other studies) and cause-effect analysis.

Conclusion does not only repeat the results of the study, but summarize the findings into a narrative that impacts on the development of science and/or practitioners in the field of veterinary reproduction.

Approval of Ethical Commission have to be stated (number and institution) if the manuscript is constructed based on a research using live animals.

Acknowledgements are delivered to the research funders (state the name, number and recipient name of the grant, if applicable), and to those who have helped carry out the research.

References are sorted alphabetically based on the author's last name. The *titles* of *journals* should be *abbreviated* according to the style *used* by each journal. Sixty (60%) scientific journal/article and (40%) text book.

Roitt, I., J. Brostoff, and D. Male. 2005. Immunology. *6th* Ed. Black Well Scientific Pub. Oxford.

Staropoli, I., J.M. Clement, M.P. Frenkiel, M. Hofiiung and V. Deuble. 2006. Dengue-1 virus envelope glycoprotein gene expressed in recombinant baculovirus elicits virus neutralization antibody in mice and protects them from virus challenge. Am.J. Trop. Med. Hygi; 45: 159-167.

Review article

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

Veterinary Medicine Journal received review articles on topics included in the scope of the journal which are of current interest. Reviews are invited by the editor. The review must be a comprehensive analysis and perspective on the state of the field and where it is heading. Reviews will be subject to the same peer review process as is applied for original papers. The manuscript is arranged in the same way as the original article with an unstructured abstract (maximum of 200 words). The number of references is limited to 50-70, with 75% of them have to be from publications in the past 10 years. The total length should not exceed 12 pages (HVS paper, double spaced 11pt Book Antiqua).

Case reports:

Veterinary Medicine Journal receives quality and interesting case reports with topics included in the scope of this journal. All reports are peer reviewed as is applied for original articles. All instructions are the same as for the original articles.

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

P-ISSN 0215-8930; E-ISSN 2775-975X

Media Kedokteran Hewan
(Veterinary Medicine Journal)

Vol. 32 No. 2 May 2021

LIST OF CONTENT

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

Media Kedokteran Hewan by Unair is licensed under a Creative Commons Attribution-ShareAlike 4.0 International License

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*

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
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.

©2021. Plumeriastuti *et al*. Open access under CC BY–SA license, doi: 10.20473/mkh.v32i2.2021.97-104
Received: 25-04-2021, Accepted: 07-05-2021, Published online: 24-05-2021
Available at https://e-journal.unair.ac.id/MKH/index