

1967-5706-1-PB.pdf

by

Submission date: 10-Sep-2022 04:30PM (UTC+0700)

Submission ID: 1896455419

File name: 1967-5706-1-PB.pdf (1.02M)

Word count: 4489

Character count: 24665

RESEARCH ARTICLE

Evaluations of Antibacterial Properties of *Zingiber purpureum* Essential Oil Against 13 Different Gram-positive and Gram-negative Bacteria

Nataniel Tandirogang¹, Silvia Anitasari^{1,2}, Enos Tangke Arung^{3,4,*},
Swandari Paramita⁴, Yung-Kang Shen⁵

¹Department of Medical Microbiology, Medical Program, Faculty of Medicine, Universitas Mulawarman, Jl. Krayan, Samarinda 75119, Indonesia

²Department of Dental Material and Devices, Dentistry Program, Faculty of Medicine, Universitas Mulawarman, Jl. Krayan, Samarinda 75119, Indonesia

³Laboratory of Forest Product Chemistry, Faculty of Forestry, Universitas Mulawarman, Jl. Penajam, Samarinda, 75119, Indonesia

⁴Research Center for Medicine and Cosmetics from Tropical Rainforest Resources (PUI-PT OKTAL), Universitas Mulawarman, Jl. Penajam, Samarinda 75119, Indonesia

⁵School of Dental Technology, College of Oral Medicine, Taipei Medical University, No. 250, Wuxing St, Taipei 110, Taiwan

*Corresponding author. E-mail: tangkearung@yahoo.com

Received date: Jun 9, 2022; Revised date: Jul 23, 2022; Accepted date: Jul 27, 2022

Abstract

BACKGROUND: Indonesia's tropical forest is home to around 80% of the world's medicinal plants. One of these is *Zingiber purpureum*, which have traditionally been used to treat joint discomfort, the common cold, and jaundice. The rhizomes of this plant have been suggested to possess antibacterial action in the treatment of infections. In this study, *Z. purpureum* was screened for antibacterial activity against 13 bacteria (Gram-positive and Gram-negative).

METHODS: *Z. purpureum* rhizomes were obtained and the distilled extracts were made to generate essential oil. The minimum inhibitory concentration (MIC) and Kirby Bauer disk diffusion methods were used to determine the antibacterial activity.

RESULTS: All bacteria activity were inhibited by the essential oil of *Z. purpureum* at concentrations ranging from 2.5 vol% to 10 vol%. However, several bacterias (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*) were inhibited at the lowest concentration (0.63 vol %), with the inhibition zones ranging from 6.7 mm to 8.0 mm. Meanwhile, the widest inhibition zone (13.3 mm) was reported on *E. cloacae* at 10 vol% concentration.

CONCLUSION: A 10 vol% *Z. purpureum* rhizome extract inhibits Gram-positive and Gram-negative bacteria, particularly those that are resistant to a variety of antibiotics.

KEYWORDS: *Zingiber purpureum*, antibacterial agents, susceptibility test, infection

Indones Biomed J. 2022; 14(3): 303-8

Introduction

Indonesia is a megadiverse country teeming with diverse plant species. Numerous plant species in Indonesia are utilized medicinally or in traditional herbs, for instance, *Zingiberaceae*, found in Indonesia and other countries. (1) Numerous *Zingerbeacea* species exist, one of which is *Zingiber purpureum* (Rose), with some other synonyms as *Zingiber cassumunar* (Roxb), *Zingiber cliffordiae*

(Andrews), *Zingiber luridum* (Salisb), and *Zingiber xantorrhizon* (Steud) which can be found throughout Southeast Asia, most notably in East Kalimantan, Indonesia. The Dayak ethnic group has long used this herb to relieve joint pain, the common cold, and jaundice. The extracts from stems, leaves, flowers, and rhizomes are used to cure conditions.(2,3)

Previously published research on the rhizomes isolated a variety of phenylbutenoids, curcuminoids, and terpenoids with anti-inflammatory, analgesic, ovicidal,

insecticidal, and enzyme inhibitory properties. Additionally, the rhizomes of these plants had considerable antibacterial action.(4) Zingiberaceae family was found to be reactive with *Bacillus subtilis* (ATCC6633), *Enterococcus faecalis* (ATCC2921), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (TISTR1843), *Pseudomonas aeruginosa* (ATCC741), *Staphylococcus aureus* (ATCC25923), *Salmonella typhi*.(5)

Meanwhile, the oil plant's have been revealed as antimicrobial activity against *Escherichia coli*, *Propionibacterium acnes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Candida albicans*, *Cryptococcus neoformans*, *Epidermophyton occosum*, *Microsporium gypseum*.(6) The oil contained a minimum bactericidal concentration (MBCs) of 0.62-2.5%. However, the oil was tested for antibacterial activity as well as yeast and dermatophytes, and it was discovered that dermatophytes were the most responsive, followed by yeast and bacteria.(7,8)

Numerous herbs, spices, and plants have been reported to be potential sources of antibacterial, especially against multi drug resistant bacteria.(9) However, few have been studied concerning levels and range of activity. Hence, the aim of this study wants to screen the antibacterial activity of *Z. purpureum* against 13 kinds of bacteria, including Gram-positive and gram-negative, using minimum inhibitory concentration (MICs) and Kirby Bauer disk diffusion method because both are considered the gold standard for determining the antimicrobial susceptibility.

Methods

Collection and Identification of *Z. purpureum*

The rhizome of *Z. purpureum* was collected from Muara Badak village, Kutai Kertanegara, East Kalimantan, Indonesia, and then identification by Laboratory of Dendrology and Forest Ecology, Faculty of Forestry, Universitas Mulawarman, Indonesia (Identification Letter No. 123/UN17.4.08/LL/2022).

Preparation and Distillation of *Z. purpureum*

The fresh rhizomes of *Z. purpureum* were washed, removed from the outer skin, and then chopped. About 2 kg flesh of rhizome was put in the distillation apparatus. The distillation process was done in 6 hours under 100°C. The essential oil obtained from this process was 1% and then the oil was placed in a bottle and stored at room temperature until used for the experiment.(3)

Bacteria Strain Preparation

All process of antimicrobial susceptibility testing was done at Microbiology Laboratory of Faculty of Medicine, Universitas Mulawarman which referred to CLSI M-100-S25.(4,10) The testing process required bacteria to be isolated in pure culture and identified to the genus and species level. Various organisms were accessible from the laboratory of the Abdul Wahab Sjahranie Hospital, Samarinda, Indonesia, under the approval of Health Research Ethics Committee, Faculty of Medicine, Universitas Mulawarman (Ethical Clearance Letter No. 71/KEPK-FK/XII/2020) and from The American Type Culture Collection (ATCC). In this study, the *Z. purpureum* was used against both bacterial Gram-positive (Methicillin-resistant *S. aureus* ATCC 33591, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *Staphylococcus pyogenes* ATCC 19615, *Streptococcus mutans* ATCC 35668) and Gram-negative bacteria (*E. coli* 35218, *S. typhi* ATCC 14028, *Campylobacter jejuni* ATCC 33291, *P. aeruginosa* ATCC 15442, *Shigella sonnei* ATCC 25932, *E. cloacae* ATCC 13047, locally *E. coli*, *Porphyromonas gingivalis* ATCC 33277).

Inoculum

It was important to standardize and find the optimum bacterial cell number employed in susceptibility testing to produce reliable and consistent results. A 0.5 McFarland standard was prepared to obtain final inoculum size. The recommended final inoculum size for broth dilution experiments was 1×10^8 colony-forming units (CFU)/mL.(10)

Evaluation of MICs

The extract of *Z. purpureum* on the 96-well microplates was incubated at 37°C for 24 hours. The MICs of plant extracts were defined as the lowest maximum dilution concentration at which no measurable bacterial growth occurred after 24 hours in microdilution wells. These experiments were repeated three times. This technique was used to determine the minimal inhibitory concentration.(11)

Kirby Bauer Disk Diffusion Method

Kirby Bauer Method with disk diffusion method was conducted as described as follows. Ten microliters of the extracts dissolved in ethanol were added to sterile filter paper discs (Whatman No.1). The discs were dried at 70°C overnight. The plates of Mueller-Hinton agar were applied with a 200 µL culture of bacteria. The discs contained extracts seeded on those plates. Five µg ciprofloxacin and 30 µg oxacillin were used as positive controls, while 0.4

µg DMSO was used as a negative control. The plates were then incubated at 37°C for 18-24 hours. The experiments performed in duplicate and the means of the diameters of the inhibition zones were calculated.(12)

Statistical Analysis

Antibacterial activity was described as the mean±standard error median (SEM) and statistical analysis was carried out by Kruskal Wallis test at a confidence level of 95 % (α level of 0.05) using SPSS ver. 23 (IBM Corporation, Armonk, NY, USA). The Mann Whitney multiple range test was also used for each subgroup.

Results

The potential of *Z. purpureum* rhizomes to inhibit Gram-positive and Gram-negative bacteria was revealed in Table 1, with extract concentrations ranging from 0.32 to 10 vol%. Result of Mann-Whitney test following Kruskal Wallis showed that *Z. purpureum* significantly inhibited both Gram-positive and Gram-negative bacteria with a $p=0.000$. Some of the lowest MIC range was reportedly found in Gram-negative bacterias (*E. cloacae*, *E. coli*, *C. jejuni*, and *P. aeruginosa*).

Table 1. MIC range of *Z. purpureum* against Gram-positive and Gram-negative bacteria.

Bacteria	MIC Range (vol %)
Gram-Positive	
<i>MRSA</i> ATCC 33591	2.08±0.42
<i>S. aureus</i> ATCC 25923	1.25±0.00
<i>S. pyogenes</i> ATCC 19615	5.00±0.00
<i>S. epidermidis</i> ATCC 14990	5.00±0.00
<i>S. mutans</i> ATCC 35668	4.17±0.83
Gram-Negative	
<i>E. coli</i> 35128	1.04±0.21
<i>C. jejuni</i> ATCC 33291	1.04±0.21
<i>Styphi</i> ATCC 14028	2.08±0.42
<i>P. aeruginosa</i> ATCC 15442	1.04±0.21
<i>S. sonnei</i> ATCC 25923	2.50±0.00
<i>E. cloacae</i> ATCC 13047	0.63±0.00
Locally <i>E. coli</i>	3.33±0.83
<i>P. gingivalis</i> ATCC 33277	2.50±0.00

Values represent in n=3 experiments. Data was expressed as mean±SEM.

The extract was shown to be able to inhibited bacteria with inhibition zones ranging from 13.3 mm to 6.7 mm (Table 2). Only *P. aeruginosa* inhibited bacteria at the lowest dose (0.32 vol%). Whila, at a 10 vol% concentration of extract *Z. purpureum* rhizomes, the highest zone inhibition was seen against *E. cloacae* (13.3±0.3 mm), followed by *P. aeruginosa* (13.0±0.6 mm), *S. aureus* (12.7±0.7 mm), and *C. jejuni* (12.7±0 mm). The minimum diameter of the zone of growth inhibition against *MRSA* (8±0 mm) and *S. pyogenes* (8±0 mm) was then determined. Additionally, only *E. coli*, *P. aeruginosa*, and *E. cloacae* preserved zone inhibition at a concentration of 0.63 vol%.

According to Table 2, bacteria were inhibited at the concentration ranging from 0.63 to 5 vol%. *E. cloacae* (0.63 vol%) had the lowest MIC on the *Z. purpureum* at 10 vol%, followed by *S. pyogenes* (5 vol%), *S. epidermidis* (5 vol%), and *S. mutans* (5 vol%). Other bacteria have a MIC of between 1.25 and 2.5 vol%. Only *C. perfringens* was unable to be inhibited by 10 vol% of *Z. purpureum*. We estimated that there was a significant different in the resistance of Gram-positive and Gram-negative bacteria to *Z. purpureum* rhizomes extract with $p<0.001$ (Table 3).

Discussion

The ancient and traditional use of plants as medicine, the emergence of multidrug-resistant pathogens, and the increasing use of essential oils make the study of their antibacterial activity timely and relevant. Some plants, that have long been used in traditional medicine for various illness, were already proven to have antibacterial properties in some research.(13-18) *Z. purpureum* or *Z. cassumunar*, which has traditionally been used to treat joint discomfort, the common cold, and jaundice, might have an antibacterial properties both for Gram-positive and Gram-negative bacterias.

Based on our results, we found that both Gram-positive and Gram-negative bacterias are affected and inhibition zone is reported from all bacterias. The lowest MIC scores are reported from four Gram-negative bacterias (*E. cloacae*, *E. coli*, *P. aeruginosa*, *C. jejuni*), indicating that from this study, *Z. purpureum* might has a stronger antibacterial activity against Gram-negative bacteria as compared to Gram-positive bacteria, especially in the lowest concentration (0.63 vol%). All these bacteria are always present in severe or nosocomial infections, and contribute to immunocompromised patients. However, further research and analysis is needed.

Table 2. Antibacterial zone of inhibition of *Z. purpureum* against Gram-positive and Gram-negative bacteria.

Bacteria	Zone of Inhibition in Diameter (mm)				
	0.63 vol% Concentration	1.25 vol% Concentration	2.5 vol% Concentration	5 vol% Concentration	10 vol% Concentration
Gram-Positive					
<i>MRSA</i> ATCC 33591	0.0±0.0	8.0±0.0	8.0±0.0	9.0±0.0	8.0±0.0
<i>S.aureus</i> ATCC 25923	0.0±0.0	10.7±0.3	11.0±0.0	12.3±0.3	12.7±0.7
<i>S.pyogenes</i> ATCC 19615	0.0±0.0	0.0±0.0	6.0±0.0	7.3±0.3	8.0±0.0
<i>S. epidermidis</i> ATCC 14990	0.0±0.0	0.0±0.0	6.0±0.0	8.0±0.0	8.3±0.3
<i>S. mutans</i> ATCC 35668	0.0±0.0	6.0±0.0	8.0±0.0	8.7±0.3	9.3±0.3
Gram-Negative					
<i>E. coli</i> ATCC 35128	8.0±0.0	8.0±0.0	9.0±0.0	10.0±0.7	11.0±0.6
<i>C. jejuni</i> ATCC 33291	0.0±0.0	9.3±0.3	10.3±0.3	12.0±0.0	12.7±0.3
<i>P. aeruginosa</i> ATCC 15442	7.0±0.0	9.7±0.7	12.0±0.0	12.3±0.9	13.0±0.6
<i>S. sonnei</i> ATCC 25923	0.0±0.0	0.0±0.0	8.0±0.6	8.3±0.3	10.0±0.0
<i>E. cloacae</i> ATCC 13047	6.7±0.3	8.3±0.3	11.3±0.3	13.0±0.6	13.3±0.3
Locally <i>E. coli</i>	0.0±0.0	0.0±0.0	6.7±0.3	8.0±0.0	9.3±0.3

Values represent in n=3 experiments. Data was expressed as mean±SEM.

Z. purpureum contains phenylbutenoids, curcuminoids, sesquiterpenoids, benzaldehydes, and quinones, as well as essential oils containing monoterpenoids, neocassumunarin A, neocassumunarin B, 6-gingerol, and 12-gingerol.(19,20) Due to its phytochemical composition, *Z. purpureum* rhizomes extract possesses antibacterial, antifungal, antiviral, and antioxidant properties. Antibacterial activity was demonstrated for the phytochemical compounds against *P. aeruginosa*, *S. aureus*, *A. baumannii*, *E. coli*, *B. subtilis*, and *S. typhi*. Additionally, 6- and 12-gingerol showed antibacterial action against periodontal microorganisms. (21-23)

According to previous studies, *S. aureus*, *S. epidermidis*, and *S. mutans* were more reactive to *Z. purpureum* oil than other bacteria, owing to the fact that *Z. purpureum* oil contained 32 vol% terpinene-4-ol as the primary active compound and demonstrated activity

against a broad range of Gram-positive bacteria.(24,25) Gram-negative organisms are marginally less sensitive to oil-related contamination than Gram-positive bacteria due to the presence of hydrophilic lipopolysaccharides in their outer membrane that functions as a barrier to the hydrophobic compounds found in essential oils.(21-23) That result is contrary to our study which implies that the hydrophobic components of essential oils enable them to enter the periplasm of Gram-negative bacteria via porin proteins in the outer membrane. Bacteria which reported to have the lowest MIC scores are Gram-negative bacteria except *S. aureus* (Table 1). This might be due to the structure of the *S. aureus* cell wall, which allows hydrophobic molecules to flow through easily.(26-28)

Gram-negative bacteria have a more complex cell wall. It possesses a 2-3 nm thick peptidoglycan layer, which is thinner than the cell wall of Gram-positive bacteria and accounts for around 20% of the cell's dry weight. Outside of the thin peptidoglycan layer is an outer membrane. (27,29) Braun's lipoprotein forms a strong bond between the peptidoglycan and the outer membrane; this protein is covalently attached to the peptidoglycan and entrenched in the outer membrane. One of the characteristics that distinguish Gram-negative bacteria from Gram-positive bacteria is the existence of an outer membrane. It is made up of two layers of phospholipids connected by lipopolysaccharides (LPS) to the inner membrane. (30,31) The peptidoglycan layer is surrounded by an outer membrane composed of various proteins and LPS. This

Table 3. Difference of Gram-positive and Gram-negative resistance against *Z. purpureum*.

Concentration	p-value
0.63 vol%	0.000
1.25 vol%	0.001
2.5 vol%	0.001
5 vol%	0.001
10 vol%	0.001

Significant if $p < 0.001$.

LPS is composed of two polysaccharides: lipid A, the core polysaccharide, and the O-side chain, which provides the quid that enables Gram-negative bacteria to be more resistant to essential oils penetrating the cell wall and acting on both the cell wall and the cytoplasm.(32,33)

As a result, we propose that there was a change in the surface of Gram-negative bacteria's cell wall and that the mechanisms were dependent on chemical components found in the plant's essential oil. Furthermore, Table 2 indicated that the minimum inhibitory concentration of *E. cloacae* was 0.63 vol%. It was established that *E. cloacae* is more susceptible to *Z. purpureum* than other species, and a positive control of 5 µg ciprofloxacin was used. This finding may provide a prospective option for the treatment of antibiotic-resistant Gram-negative bacteria, such as those that produce extended-spectrum beta-lactamase (ESBL). Some studies found that *E. cloacae* is a common nosocomial organism that is resistant to carbapenem, meropenem, imipenem, ertapenem, penicillin, aztreonam, and first-, second-, and third-generation cephalosporins; the fact that our study discovered that 10% *Z. purpureum* could inhibit *E. cloacae* could be a promising future for future treatment of *E. cloacae* infection.(34-36)

Essential oils work by disrupting the cell wall and cytoplasmic membrane, resulting in the lysis and leaking of intracellular chemicals. Increased disruption of the cell membrane disrupts a variety of essential processes, including energy conversion, nutrition processing, structural macromolecule synthesis, and growth regulator secretion. (37) The essential oil of *Z. purpureum* has been observed to promote cellular component leakage and ion loss across the membrane. As a result, Gram-negative bacteria have a larger inhibitory zone than Gram-positive bacteria, with the exception of *S. aureus* (Table 1). Three bacterias which being suppressed by 10 vol% *Z. purpureum* rhizomes the most were *E. coli* (ATCC 35128), *P. aeruginosa* (ATCC 15442), *E. cloacae* (ATCC 13047). These bacterias are opportunistic human pathogens that are usually associated with infections of the lower respiratory tract and urinary tract acquired in hospitals.(26) These microorganisms can colonize and grow on the epithelial lining of the urinary tract in response to acidic conditions and the presence of adhesin or fimbriae and exhibit cytotoxic activity, implying the production of bacterial toxins by the cell host. However, that the essential oil of *Z. purpureum* (Rosca) may be capable of reversing this process. We hypothesize that the plant could neutralize the urinary organ's acidity and so prevent these bacteria from adhering, thereby flushing them from the body.(38-40)

Conclusion

The essential oil of *Z. purpureum* rhizome has shown the ability to inhibit Gram-positive and Gram-negative bacteria, particularly those resistant to a variety of antibiotics, including beta-lactams, carbapenem, and first-, second-, and third-generation cephalosporins. Based on these results, this oil can be used for application in humans with safety being a priority concern, therefore further experiments need to be done.

Acknowledgments

The Lembaga Pengelola Dana Pendidikan (LPDP) funded a part of this study through the RISPRO program (Grant No. 6/F1/P-KCOVID-19.2B3/IX/2020). We'd also like to express our gratitude to Universitas Mulawarman for supporting research laboratories in conducting this study, and School of Dental Technology, College of Oral Medicine, Taipei Medical University for the collaboration.

Authors Contribution

NT and ETA were involved in conceiving and planning the research, NT and SP performed the data acquisition/ collection, NT and SA calculated the experimental data and performed the analysis, SA and SP drafted the manuscript and designed the table and figures, NT and YK aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

References

- O'Bryan CA, Pendleton SJ, Crandall PG, Ricke SC. Potential of plant essential oils and their components in animal agriculture- in vitro studies on antibacterial mode of action. *Front Vet Sci.* 2015; 2: 35. doi: 10.3389/fvets.2015.00035.
- Lim TK. *Edible Medicinal and Non-medicinal Plants* Vol. 10. Netherlands: Springer; 2016.
- Paramita S, Aminyoto M, Ismail S, Arung ET. Anti-hypercholesterolemic effect of *Zingiber montanum* extract. *F1000Res.* 2019; 7: 1798. doi: 10.12688/f1000research.16417.2.
- Anitasari S, Ismail S, Wiratama BS, Budi HS. Antibacterial and phytochemical analysis of two plants menispermaceae family. *Syst Rev Pharm.* 2020; 11(5): 150-6.
- Panphut W, Budsabun T, Sangsuriya P. In vitro antimicrobial activity of Piper retrofractum fruit extracts against microbial pathogens causing infections in humans and animals. *Int J Microbiol.* 2020;

- 2020: 5638961. doi: 10.1155/2020/5638961.
6. Pithayanukul P, Tubprasert J, Wuthi-Udomlert M. In vitro antimicrobial activity of Zingiber cassumunar (Plai) oil and a 5% Plai oil gel. *Phytother Res.* 2007; 21(2): 164-9.
 7. Patterson JE, McElmeel L, Wiederhold NP. In vitro activity of essential oils against gram-positive and gram-negative clinical isolates, including carbapenem-resistant enterobacteriaceae. *Open Forum Infect.* 2019; 6(12): ofz502. doi: 10.1093/ofid/ofz502.
 8. Bučková M, Puškárová A, Kalászová V, Kisová Z, Pangallo D. Essential oils against multidrug resistant gram-negative bacteria. *Biologia.* 2018; 73: 803-808.
 9. Semeniuc CA, Pop CR, Rotar AM. Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *J Food Drug Anal.* 2017; 25(2): 403-8.
 10. Clinical and Laboratory Standard Institute. M100-S25 Performance Standards for Antimicrobial Susceptibility Testing: Twenty-fifth Informational Supplement. Wayne: Clinical and Laboratory Standard Institute; 2015.
 11. Shaheen A, Ismat F, Iqbal M, Haque A. Characterization of putative multidrug resistance transporters of the major facilitator-superfamily expressed in *Salmonella typhi*. *J Infect Chemother.* 2015; 21(5): 357-362.
 12. Ifesan BOT, Ibrahim D, Voravuthikunchai SP. Antimicrobial activity of crude ethanolic extract from *Eleutherine Americana*. *J Food Agric Environ.* 2010; 8 (3/4 Part 2): 1233-6.
 13. Tandirogang N, Paramita S, Yasir Y, Yuniati Y, Aminyoto M, Fitriany E. Aktivitas antimikroba ekstrak Daun Karamunting (*Melastoma malabathricum* L.) terhadap bakteri penyebab diare. *Jurnal Sains dan Kesehatan.* 2017; 1(7): 345-51.
 14. Parawansah P, Nurtamin T, Mulyawati SA, Nuralifah N, Misneni WOA. Immunomodulatory effect of *Momordica charantia* L. fruit ethanol extract on phagocytic activity and capacity of mice peritoneal macrophages. *Indones Biomed J.* 2018; 10(2): 144-7.
 15. Yana HY, Hidayati L, Wijayanti N, Nuringtyas TR. Immunomodulatory activity of agarwood *Aquilaria malaccensis* Lamk. leaf extracts on *Staphylococcus aureus*-infected macrophages in vitro. *Indones Biomed J.* 2022; 14(2): 156-63.
 16. Callixte C, Damascene DJ, Ma'aruf A, Dachlan YP, Sensusuati AD, Daniel N, et al. Phytoconstituent analysis and antibacterial potential of epicarp extracts from mature fruits of *Persea americana* Mill. *Mol Cell Biomed Sci.* 2020; 4(2): 94-9.
 17. Wahyuni D, Waluyo J, Prihatin J, Kusumawardani FI, Kurniawan A. Pheretima javanica K. ethanol extract Granules' effects on eosinophil level, immunoglobulin E level, and organs histopathology in *Rattus norvegicus* B. *Indones Biomed J.* 2021; 13(2): 208-15.
 18. Sudiono J, Hardina M. The effect of *Myrmecodia pendans* ethanol extract on inflamed pulp: study on sprague dawley rats. *Mol Cell Biomed Sci.* 2019; 3(2): 115-21.
 19. Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Front Microbiology.* 2011; 3: 12. doi: 10.3389/fmicb.2012.00012.
 20. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Front Microbiol.* 2018; 9: 1639. doi: 10.3389/fmicb.2018.01639.
 21. Li ZH, Cai M, Liu YS, Sun PL, Luo SL. Antibacterial activity and mechanisms of essential oil from *Citrus medica* L. var. *sarcodactylis*. *Molecules.* 2019; 24(8): 1577. doi: 10.3390/molecules24081577.
 22. Gadisa E, Usman H. Evaluation of antibacterial activity of essential oils and their combination against multidrug-resistant bacteria isolated from skin ulcer. *Int J Microbiol.* 2021; 2021: 6680668. doi: 10.1155/2021/6680668.
 23. Han AR, Kim H, Piao D, Jung CH, Seo EK. Phytochemicals and bioactivities of *Zingiber cassumunar* roxb. *Molecules.* 2021; 26(8): 2377. doi: 10.3390/molecules26082377.
 24. Tripathi M, Chawla P, Upadhyay R, Trivedi S. Essential oils from family zingiberaceae for antimicrobial activity - a review. *Int J Pharma Bio Sci.* 2013; 4(4): 149-62.
 25. Burt S. Essential oils: Their antibacterial properties and potential applications in foods - a review. *Int J Food Microbiol.* 2004; 94(3): 223-53.
 26. Pius O, Oluwadunsin O, Benjamin O. Antibacterial activity of ginger (*Zingiber officinale*) against isolated bacteria from respiratory tract infections. *J Biol Agric Healthcare.* 2015; 5(19): 131-8.
 27. Wahidah BF, Hayati N, Khusna UN, Rahmani TPD, Khasanah R, Kamal I, et al. The ethnobotany of zingiberaceae as the traditional medicine ingredients utilized by colo muria mountain villagers, Central Java. *J Phys Conf Ser.* 2021; 1796: 012113. doi: 10.1088/1742-6596/1796/1/012113.
 28. Batubara I, Wahyuni WT, Susanta M. Antibacterial activity of zingiberaceae leaves essential oils against *Streptococcus mutans* and teeth-biofilm degradation. *Int J Pharma Bio Sci.* 2016; 7(4): 111-6.
 29. Latifian E, Otur C, Abanoz-Secgin B, Arslanoglu SF, Kurt-Kizildogan A. Evaluation of antimicrobial activity in extracts of different parts of three tagetes species," *Turkish J Field Crops.* 2021; 26: 116-22.
 30. Raja SA, Ashraf M, Anjum A, Javeed A. Antibacterial activity of essential oils extracted from medicinal plants against multi-drug resistant *Staphylococcus aureus*. *J Anim Plant Sci.* 2016; 26 (2): 415-23.
 31. Yusoff MM, Ibrahim H, Hamid NA. Chemical characterization and antimicrobial activity of rhizome essential oils of very closely allied zingiberaceae species endemic to Borneo: *Alpinia ligulata* K. SCHUM. and *Alpinia nieuwenhuizii* VAL. *Chem Biodiver.* 2011; 8(5): 916-923.
 32. Widyowati R, Agil M. Chemical constituents and bioactivities of several Indonesian plants typically used in jamu. *Chem Parm Bull.* 2018; 66(5): 506-518.
 33. Annavajhala MK, Gomez-Simmonds A, Uhlemann AC. Multidrug-resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. *Front Microbiol.* 2019; 10: 44. doi: 10.3389/fmicb.2019.00044.
 34. Mezzatesta ML, Gona F, Stefani S. *Enterobacter cloacae* complex: Clinical impact and emerging antibiotic resistance," *Future Microbiol.* 2012; 7(7): 887-902.
 35. Davin-Regli A, Pagès JM. *Enterobacter aerogenes* and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. *Front Microbiol.* 2015; 6: 392. doi: 10.3389/fmicb.2015.00392.
 36. Soetjipto H. Antibacterial properties of essential oil in some Indonesian herbs. In: El-Shemy, HA, editor. *Potential of Essential Oils.* London: IntechOpen; 2018.
 37. Habsah M, Amran M, Mackeen MM, Lajis NH, Kikuzaki H, Nakatani N, Rahman AA, Ghafar, Ali AM. Screening of Zingiberaceae extracts for antimicrobial and antioxidant activities. *J Ethnopharmacol.* 2000; 72(3): 403-10.
 38. Masuda T, Jitoe A. Phenybutenoid monomers from the rhizomes of *Zingiber cassumunar*. *Phytochemistry.* 1996; 39(2): 459-461.
 39. Han AR, Kim H, Piao D, Jung CH, Seo EK. Phytochemicals and Bioactivities of *Zingiber cassumunar* Roxb. *Molecules.* 2021; 26(8): 2377. doi: 10.3390/molecules26082377.
 40. Sanatombi R, Sanatombi K. Biotechnology of *Zingiber montanum* (Koenig) Link ex A. Dietr.: a review. *J Appl Res Med Aromat Plants.* 2017; 4: 1-4. doi: 10.3390/separations6020031.

ORIGINALITY REPORT

20%
SIMILARITY INDEX

12%
INTERNET SOURCES

15%
PUBLICATIONS

2%
STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

1%
★ "Natural Oral Care in Dental Therapy", Wiley, 2020
Publication

Exclude quotes Off
Exclude bibliography On

Exclude matches Off