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Comparative Analysis of Essential Oil Profiles From Emprit Ginger Rhizome (*Zingiber officinale* var. amarum) Grown in Different Locations and Antibacterial Activity Againts *Staphylococcus aureus*

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Abstract

Emprit ginger (*Zingiber officinale* var. Amarum) is a native Indonesian medicinal plant used to treat various diseases. Apart from being an antioxidant, ginger emprit also has antibacterial potential. However, herbal materials used for medicinal purposes produce inconsistent effects due to the fluctuating chemical composition of the plants, usually caused by differences in growing locations that affect the content of active metabolites. This study aims to evaluate the antibacterial activity of essential oil of emprit ginger rhizome. Samples were obtained from 14 different growing locations namely Ponorogo, Magetan, Pacitan, Wonogiri, Karanganyar, Boyolali, Semarang, Magelang, Purworejo, Temanggung, Wonosobo, Banyumas, Bantul, and Kulonprogo. The essential oil profile of emprit ginger was obtained through Gas Chromathography Mass Spectometry (GCMS). Analyzed by multivariate calibration of *Principal Component Analysis* (PCA) and *Orthogonal Partial Least Squares* (OPLS) using SIMCA *software*. Antibacterial activity of essential oils was performed by microdilution method against *Staphylococcus aureus* bacteria. Analysis of antibacterial activity was determined by probit method to obtain the Minimum Inhibitory Concentration-50 (MIC_{50}). The results of the GCMS spectrum of essential oil showed that the main compound components in ginger emprit essential oil are *Citral, Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl, exo-(CAS), Z-Citral, Geranyl acetate, Camphene, 1,6 Octadiene, 7 Methyl-3-Metylene, 1,6-Cineole, Farnesene, Bornylene, Beta-Myrcene, Zingiberene, and Alpha-pinene*. The results of the *Staphylococcus aureus* antibacterial activity test on emprit ginger rhizome essential oil from Boyolali area show the highest MIC_{50} value of 0.2011% v/v.

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INTRODUCTION

Indonesia has plant species that are scattered in various regions, where the existing biodiversity can be utilized as medicinal raw materials. Indonesian people have long recognized and used traditional medicine to treat various diseases. One plant that is often used by the community is ginger (*Zingiber officinale* Roscoe), which is one of the spices in the temu-temuan tribe (*Zingiberaceae*).

Ginger is a medicinal plant and spice characterized by its pseudostem structure, scientifically known as *Zingiber officinale* var. Amarum. The variant known as emprit ginger is a rhizome plant that grows in low-lying to mountainous regions at altitudes ranging from sea level to 1500 meters.[1] Ginger originates from the Pacific Asia region, predominantly from India to China. As such, these two nations are often recognized as the first to utilize ginger, primarily as a beverage ingredient, cooking spice, and in traditional medicine. The largest global concentrations of ginger plants are in tropical regions, particularly across Asia and the Pacific Islands. Cultivation has recently extended to Jamaica, Brazil, Hawaii, Africa, India, China, Japan, the Philippines, Australia, New Zealand, Thailand, and Indonesia. In Indonesia, ginger is found throughout the country, grown in both monoculture and polyculture systems.[2] Based on the form, color, and size of the rhizomes, three types of ginger are recognized: large white ginger (also known as rhino ginger), small white ginger (or emprit ginger), and sunti ginger (or red ginger). This study uses ginger emprit because it is easy to obtain, affordable, and there has been no research on the content of ginger emprit compounds. Generally, all three types contain starch, essential oils, fiber, a small amount of protein, vitamins, minerals, and a proteolytic enzyme called zingibain[3] Beyond its use as a cooking ingredient, ginger has been empirically used as a component in various medicinal concoctions: such as remedies to boost immunity, combat inflammation, treat coughs, heal wounds, and counteract insect bite allergies.[4]

As a traditional medicinal plant, ginger is utilized to alleviate symptoms related to the throat and tongue, eliminate heart disturbances, and treat vomiting, ascites, cough, dyspnea, anorexia, fever, anemia, flatulence, colic, constipation, swelling, elephantiasis, and dysuria. Additionally, ginger has been used in treating diarrhea, cholera, dyspepsia, neurological diseases, diabetes, eye conditions, and ear inflammation.[5] The composition of ginger has been proven to have antibacterial effects. Research has identified terpenes as the key compounds responsible for these antibacterial properties. Against several microorganisms, terpenes act as bacteriostatic agents. These compounds can interact with the bacterial cell membrane, disrupting its permeability and subsequently impeding the transport of ions in and out of the cell. This disturbance in ion transport can disrupt the proton motive force, which in turn interferes with the energy production process within the cell.[6]

Secondary metabolites are chemical compounds produced by plants in small amounts and have no direct influence on plant growth and development.[7] Metabolite products produced by plants have specific properties and different levels for each species and plant part. In addition, differences in the content of metabolites produced can also be influenced by environmental geographical conditions such as altitude, temperature, rainfall, and soil type.[8] In this study, samples were taken from 14 different growing places in addition to geographical conditions; emprit ginger

rhizomes are cultivated directly by farmers not from wild plants so that the harvest age is uniform.

In addition to serving as a key ingredient in the manufacture of traditional and modern medicines, the antioxidants and antibacterial secondary metabolites produced by *Zingiberaceae* plants can generally inhibit the growth of harmful human pathogens[8] These include *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and fungi such as *Neurospora, Rhizopus, Penicillium, Candida albicans*, and *Microsporum gypsiium*, all of which can cause mycotic diseases in humans and animals[10]. The microbial test subject used in this study was *Staphylococcus aureus*, a Gram positive, cocci-shaped bacterium that can exist individually, in pairs, or clusters.[10] This organism is found in the nasal cavity and skin and is a dangerous pathogen because it causes several diseases such as skin and respiratory infections. This bacterium can become a pathogen if it has the opportunity to enter the body, such as during the use of medical devices. It is most commonly associated with skin infection diseases. Infected body tissues will cause inflammation, necrosis, and abscess formation. [9] In addition to *Staphylococcus aureus*, this study also used the test microbe *Escherichia coli*, a Gram negative bacterium normally present in the gastrointestinal tract of humans and animals. This bacterium can also cause urinary tract infections and diarrhea.[11]

The fresh extract of ginger rhizome demonstrates the capacity to inhibit the growth of test microbes, as observed by the average diameter of the resultant microbe-free zones [12] This phenomenon is attributable to the antimicrobial compounds found in the fresh extract of ginger rhizome, which contain several essential oil components.[13] The ginger rhizome extract exhibited the largest inhibitory zone diameters against two test microbes, specifically, 15.83 mm for *Staphylococcus aureus* and 15.33 mm for *Escherichia coli*.[14] According to the inhibition capacity categorization, the ginger rhizome extract has a moderate inhibitory effect on the growth of *Staphylococcus aureus* and *Escherichia coli*.[15]

In addition to serving as a key ingredient in the manufacture of traditional and modern medicines, the antioxidants and antibacterial secondary metabolites produced by *Zingiberaceae* plants can generally inhibit the growth of harmful human pathogens [16]

MATERIAL AND METHOD

Material

The materials utilized in this study include emprit ginger rhizomes sourced from various cultivation lands in Ponorogo, Magetan, Pacitan, Wonogiri, Karanganyar, Boyolali, Semarang, Magelang, Purworejo, Temanggung, Wonosobo, Banyumas, Bantul, and Kulonprogo, Luria Bertani media, anhydrous Na_2SO_4 (Emsure, Germany), DMSO (Sigma Aldrich, USA), n-hexane (Emsure, Germany), agar (Himedia), distilled water, (Pyrex) 70% alcohol (Smartlab), *Staphylococcus aureus* bacteria (ATCC 25923), ampicillin (Sigma Aldrich) (as a comparative raw material), disposable 96 well microplates, disposable petri dishes, white tips, yellow tips, blue tips The instruments employed in this research include *Gas Chromatography Mass Spectrometer* (GCMS) (Shimadzu QP-2010S), micropipettes, distiller, analytical balance, autoclave, incubator, Laminar Air Flow (LAF) cabinet, (Airegard Work Station) vortex mixer, spectrophotometer UV (Optima SP-3000 nano), and microplate reader (Spark Tecan).

Method

Bacterial culture

Preparation of solid media as a place for bacterial culture was made using Luria Bertani media: agar with a ratio of 2.5 : 1 gram dissolved in 100 mL distilled water. Solid media was placed in Petri dishes for bacterial growth. Pure culture of Gram-positive *Staphylococcus aureus* bacteria (ATCC 25923) was taken 1 dose from glycerol stock and then inoculated into Luria Bertani agar media aseptically, and incubated for 1x24 hours at 37ºC in an incubator.[17]

Antibacterial activity by microdilution method

Antibacterial activity test on essential oil of emprit ginger rhizome was conducted by microdilution method. Bacterial culture on Luria Bertani agar media was suspended in 10 ml of Luria Bertani liquid media aseptically, then incubated in an orbital incubator for approximately 2.5 hours with the aim of obtaining the growth phase of bacteria in the log phase until an optical density (OD) value of 0.25-0.30 was obtained using a spectrophotometer at a wavelength of 600 nm.[18] The bacterial suspension obtained was then used for antibacterial activity testing, no more than 30 minutes after measuring the absorbance of the bacterial suspension.[19]

One essential oil sample was made into three series of levels in the antibacterial activity test. A total of 40 µL of 100% DMSO was placed in a microtube, added 40 µL of essential oil, then added with sterile distilled water up to 1 mL and homogenized. The stock solution had an essential oil concentration of 4% v/v. A total of 800 µL, 700 µL, 600 µL, 500 µL, and 400 µL of the stock solution were placed into five microtubes, then sterile distilled water was added to 1 mL of each, so that the concentrations were 3.2%; 2.8%; 2.6%; 2.0% and 1.6% v/v, respectively. The essential oil concentration series solution was used in the microdilution method antibacterial activity test. A total of 25 µL of essential oil concentration series was placed in the wells of 96 wells microplate added with 150 µL Luria Bertani liquid media and 25 µL bacterial suspension, so that the final concentration of essential oil was 0.4%; 0.35%; 0.3%; 0.25% and 0.2% v/v. The antibacterial activity test was conducted on 96 wells microplate. The wells were divided into treatment sample wells with different concentrations, positive control wells, negative control wells, and blank wells.[20] Each of the treatments, positive control, negative control, and blank wells, was repeated three times to verify the accuracy of the results. Each side of the lid of the 96 wells microplate was glued with parafilm and incubated at 37ºC for 16-18 hours, then the optical density in each well was viewed with a wavelength of 600 nm. [21] The absorbance was read using a microplate reader at a wavelength of 600 nm, then the % inhibition of each concentration was obtained. The percent inhibition was analyzed using probit; from the % inhibition and probit analysis, the MIC_{50} result were obtained.

RESULTS AND DISCUSSION

The Essential Oil Yield

The steam distillation yield of essential oil from the rhizomes of emprit ginger sourced from different regions was as follows: Ponorogo, 0.175% v/b; Magetan, 0.242% v/b; Pacitan, 0.200% v/b; Wonogiri, 0.300% v/b; Karanganyar, 0.213% v/b; Boyolali, 0.183% v/b; Magelang, 0.173% v/b; Semarang, 0.253% v/b; Purworejo, 0.133% v/b; Temanggung, 0.167% v/b; Wonosobo, 0.286% v/b; Banyumas, 0.270% v/b; Bantul, 0.187% v/b; and Kulonprogo,

Table 1. Compounds with Highest Percentage Areas.(Basic data, 2022)

Compounds	Regions	Formula	Highest areas (%)
Camphene	Semarang	$C_{10}H1_6$	10.315
1,8-Cineole	Temanggung	$C_{10}H_{18}0$	8.16
Linalool	Pacitan	$C_{10}H_{18}0$	2.845
3-Cyclohexene-1-methanol, ,alpha,,,alpha,,4-trimethyl-, (S)- (CAS) p-Menth-1-en-8-ol, (S)-(-)-	Semarang	$C_{10}H_{18}0$	1.89
Z-Citral	Wonogiri	$C_{10}H_{16}0$	21.38
Citral	Pacitan	$C_{10}H_{16}0$	26.03
Geranyl acetate	Purworejo	$C_{12}H_{20}O_2$	7.715
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS) ar-Curcumene	Boyolali	$C_{15}H_{22}$	4.31
Zingiberene (CAS)	Karanganyar	$C1_5H_{24}$	5.59
Farnesene	Ponorogo	$C_{15}H_{24}$	4.205
Beta,-Sesquiphellandrene (CAS)	Magelang	$C_{15}H_{24}$	4.19
3a(1H)-Azulenol, 2,3,4,5,8,8a-hexahydro-6,8a-dimethyl-3-(1-methylethyl)-, [3R-(3,alpha,,3a,alpha,,8a,alpha,)]- (CAS)	Semarang	$C_{15}H_{26}0$	2.28

0.036% v/b. The essential oil content of the emprit ginger rhizome was not less than 0.80% v/b.[22] The distillation yield of essential oil from the emprit ginger rhizomes across these 14 locations was lower than the standard set by FHI. In this study, the rhizomes intended for distillation were dried in an open-air environment for a significantly longer duration than the time taken for harvesting. This process allowed for the evaporation of the essential oil. The difference in essential oil yields could be attributed to the post-harvest treatment of the rhizomes, such as the drying process and slicing, which could reduce essential oil yield.

Identification of Essential Oil Compound Groups from Ginger in 14 Regions via GCMS

The chromatogram profiles of the constituent compounds of the essential oil were analyzed in duplicate with *Gas Chromatography Mass Spectrometry* (GCMS). GCMS analysis showed that the 14 regions respectively had between 48 and 50 compounds. The peak area produced will correlate with the detected compounds; hence, the larger the peak area, the greater the number of secondary metabolites. The 14 essential oil growth locations in ginger rhizomes had similar numbers of compounds, but there were differences in compound concentration presentation in each sample. The essential oil components were chosen for identification based on peak criteria with an area greater than 0.5%. Emprit ginger rhizomes grown in Pacitan contained 12 monoterpene group essential oil components, with Citral having the highest area percentage of 26.03%. The details can be seen in Table 1.

Antibacterial Activity

Antibacterial activity was investigated by optimizing DMSO on *Staphylococcus aureus* bacteria. It was observed that DMSO at a final concentration under 0.8% v/v did not inhibit the growth of either bacteria. The antibacterial activity of essential oil from ginger rhizomes collected from 14 different growth regions was tested using DMSO at a final concentration of less than 0.5% v/v.

Additionally, *Staphylococcus aureus* bacteria contain teichoic acid, a water-soluble polymer, suggesting that the cell wall is polar. The non-polar nature of the ginger rhizome essential oil sample makes it more challenging to penetrate the polar cell wall of *Staphylococcus aureus* bacteria. The essential oil from ginger rhizomes contains oils derived from the terpenoid group. The mechanism of

terpenoids in antibacterial activity occurs at the transmembrane proteins located on the outer membrane of the bacterial cell wall. The essential oil can form strong polymer bonds until damage occurs to the transmembrane proteins, which serve as pathways for compound exchange. This damage results in a nutritional deficiency in bacterial cells that may inhibit bacterial growth, leading to eventual cell death.

Principal Component Analysis

The Principal Component Analysis (PCA) objective in the similarity analysis of essential oils from emprit ginger rhizomes from 14 different growth locations is to group correlated variables and replace them with a new group known as principal components. PCA is conducted to comprehend the relationship between the distribution of essential oil compound contents and the growth locations of the emprit ginger rhizome samples. The results of the PCA score scatter plot analysis are depicted in Figure 1.

The PCA results in a *score plot* curve can be utilized to estimate the data structure, serving as a basis for differentiating essential oils from emprit ginger rhizomes based on geographical disparities. The distance between samples indicates the similarity between the samples. Far and near distances among samples exhibit the extent of similarity among them. Essential oils from emprit ginger rhizomes from 14 growth locations lie in distinct quadrants. It suggests the presence of differences in the characteristics of essential oil compound contents of emprit ginger rhizomes from these 14 different growth locations. Essential oils from emprit ginger rhizomes with growth locations in Ambarawa (Semarang), Wonosobo, Temanggung, Karanganyar, Kulonprogo, and Magetan have similar percentage areas, possibly indicating similar compound contents.

However, areas with larger percentage differences exhibit differing compound contents. The areas of Kulonprogo and Magetan show the same trend. The processed results of the PCA of essential oils from emprit ginger rhizomes in the form of a *score plot* are presented in Figure 1.

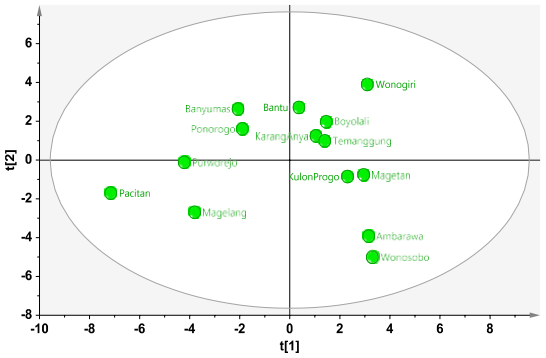

Figure 1. PCA *score plot.*

In thehe OPLS DA analysis, Figure 2 shows the results for compounds influencing the growth of emprit ginger across 14 locations are presented. Compounds that significantly impact the growth of emprit ginger across these 14 locations include *Citral, (2R)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol, Z-Citral, Geranyl acetat, Camphene, (6E)-octa-1,6-diene, 7-methyl-3-methylideneoct-6-enal, Farnesene, Bornylene, Beta-Myrcene, Zingiberene, Alpha-pinene*.

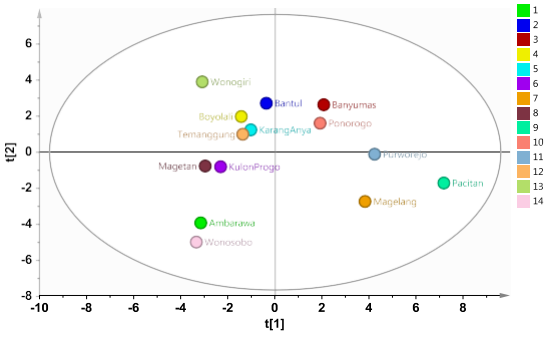

Figure 2. OPLS DA for Discriminating Compounds Inside Emprit Ginger.

The compounds influencing the MIC_{50} against *Staphylococcus aureus* bacteria with GCMS variables include *Z-Citral; Geranyl asetat; Zingiberenol; Beta-Myrcene; (1S)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene; and (2R)-1,7,7-trimethylbicyclo [2.2.1] heptan-2-ol.*
The graphical results are presented in Figure 3:

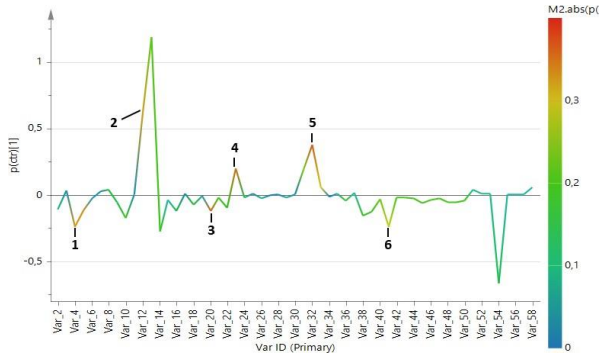

Figure 3. OPLS of *Staphylococcus aureus* Bacteria with GCMS Variables.

Correlation of Essential Oil Profiles from Ginger with Growth Location and its Antibacterial Activity

The antibacterial activity of *Staphylococcus aureus* on essential oils from the rhizomes of fingerroot ginger, grown at varying locations was discerned. The results of the antibacterial activity test of *Staphylococcus aureus* on the essential oil of emprit ginger rhizome from Boyolali area has the highest MIC_{50} value of 0.2011% v/v.

STRENGTH AND LIMITATION

The study on emprit ginger rhizomes presents a commendable breadth of analysis, encompassing essential oil yield data sourced from 14 distinct regions, complemented by the rigorous analytical technique of *Gas Chromatography Mass Spectrometry* (GCMS) applied in duplicate. This methodological depth is further enriched by the exploration of the antibacterial activity of the oils, particularly against *Staphylococcus aureus*, thus offering a functional perspective alongside compositional insights. Additionally, the employment of *Principal Component Analysis* (PCA) sheds light on the intricate relationships between different samples based on their essential oil profiles, with the identification of key compounds serving as a pivotal contribution to understanding the growth and antibacterial properties of emprit ginger. However, while the research is thorough, it is not without limitations. The open-air drying method employed for the rhizomes before distillation may have compromised the oil yield, introducing potential bias. This is coupled with a detectable variability in the compounds observed between the two GCMS replications. Moreover, the singular focus on *Staphylococcus aureus* limits the study's antibacterial scope, and the absence of comparative MIC_{50} values to a reference makes discerning the potency challenging. Furthermore, the lack of detailed results from the PCA and potential absence of controls in antibacterial testing call for cautious interpretation. Lastly, while the study provides valuable regional insights, external factors such as soil quality, climatic conditions, and specific farming practices were not considered, possibly impacting the essential oil yield and composition. Overall, the study stands as a significant contribution to the domain, but considerations regarding its limitations are crucial for a holistic understanding.

CONCLUSIONS

The essential oil from the ginger rhizome, derived from 14 different geographic locations, demonstrates a significant variation in MIC_{50} values against *Staphylococcus aureus* bacteria. The results of the antibacterial activity test

of *S. aureus* on ginger emprit rhizome essential oil derived from Boyolali area has the highest MIC_{50} value of 0.2011% v/v. Analysis of antibacterial activity of compounds that showed significant discriminatory effects on *Staphylococcus aureus* bacteria are *Z-Citral; Geranyl asetat; Zingiberenol; Beta-Myrcene; (1S)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene; and (2R)-1,7,7-trimethylbicyclo [2.2.1] heptan-2-ol*. These have shown impactful effects on the *Staphylococcus aureus* bacteria.

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CONFLICT OF INTEREST

All authors have no conflict of interest

AUTHOR CONTRIBUTION

AAS: Conceptualization, Methodology, Software, Resources, Data Curation, and Writing Original Draft; **P**: Conceptualization, Methodology, Formal analysis, Writing, Review and Editing, and Supervision; RA**S**: Writing, Review and Editing, Visualization, and Supervision; **AW**: Writing, Review and Visualization; **AR**: Writing, Review and Supervision.

REFERENCE

1. Dolok TNS, Nursaadar E, Ariefa Primairyani. Keanekaragaman Hayati Tumbuhan Obat Tradisional dan Pemanfaatanya. BIOEDUSAINS:Jurnal Pendidikan Biologi dan Sains. 2023;6(2):2598–7453.
2. Rahmawati A, Kuswandi B, Retnaningtyas Y. Deteksi Gelatin Babi pada Sampel Permen Lunak Jelly Menggunakan Metode Fourier Transform Infra Red (FTIR) dan Kemometrik. Jurnal Pustaka Kesehatan. 2015;3(2):278–83.
3. Styawan AA, Susidarti RA, Purwanto P, Irnawati I, Rohman A. The use of pattern recognition for classification of Indonesian ginger (*Zingiber officinale* var. *Amarum*) based on antioxidant activities and FTIR spectra. J Appl Pharm Sci. 2023 Apr 1;13(4):149–56.
4. Rosidi A, Khomsan A, Setiawan C, Riyadi H, Briawan D. Potensi temulawak (Curcuma xanthoriza Roxb) sebagai antioksidan. IPB. 2014
5. Otto M. Staphylococcus Aureus Toxins. Current Opinion in Microbiology. 2014;17:32–7.
6. Styawan AA, Susidarti RA, Purwanto, Windarsih A, Rahmawati N, Sholikhah IKM, et al. Review on ginger (*Zingiber officinale* Roscoe): phytochemical composition, biological activities and authentication analysis. Food Res. 2022 Aug 1;6(4):443–54.
7. Syahputri ER, Selaras .H, Farma SA. Manfaat Tanaman Jahe *(Zingiber officinale)* Sebagai Obat Obatan Tradisional *(Traditional Medicine).* Prosiding Seminar Nasional Biologi.. 2021;1(1):_ 579-86.

8. Rohaetia E, Rafi M, Syafitric UD, Heryanto R. Fourier transform infrared spectroscopy combined with chemometrics for discrimination of *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber cassumunar*. J Chem Inf Model. 2015.
9. Moreira Da Silva T, Pinheiro CD, Puccinelli Orlandi P, Pinheiro CC, Soares Pontes G. Zerumbone from Zingiber zerumbet (L.) smith: A potential prophylactic and therapeutic agent against the cariogenic bacterium Streptococcus mutans. BMC Complement Altern Med. 2018 Nov 13;18(1).
10. Tomat B, Sulistyowati A, Sedyadi E, Prabawati SY. Pengaruh Penambahan Ekstrak Jahe (*Zingiber officinale*) Sebagai Antioksidan Pada Edible Film Pati Ganyong (*Canna edulis*) Dan Lidah *Buaya (Aloe vera . L)* Terhadap Masa Simpan. 2019;4(01):1–12.
11. Assya AA, Ikhlas O, Putri NP, Niawanti H. Pengaruh Pengeringan Terhadap Kadar Tanin Teh Herbal Daun Belimbing Wuluh *(Averrhoa Bilimbi).* Jurnal Atmosphere. 2022;3(1):1-7.
12. Jasmansyah J, Fitriyani P, Sujono H, Aisyah LS. Uji Aktivitas Antimikroba Minyak Atsiri Tanaman Pegagan (*Centella asiatica* (L.) Urb). Jurnal Kartika Kimia. 2020 May 31;3(1).
13. Ali S, Baharuddin M, Kimia J, Sains F, Makassar UINA. Pengujian aktivitas antibakteri minyak atsiri Jahe (*Zingiber officinale* Roscoe) Terhadap Bakteri *Staphylococcus aureus* Dan *Escherichia coli*. 2019 :18–31.
14. Aristyawan AD, Sugijanto NE, Suciati S. Potensi Antibakteri dari Ekstrak Etanol Spons Agelas cavernosa. Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia. 2018;4(1):39.
15. Houdkova M, Rondevaldova J, Doskocil I, Kokoska L. Evaluation Of Antibacterial Potential and Toxicity of Plant Volatile Compounds Using New Broth Microdilution Volatilization Method and Modified Mtt Assay. Fitoterapia. 2017;118: 56-62.
16. Kolarević S, Milovanović D, Avdović M, Oalde M, Kostić J, Sunjog K. Optimisation of the microdilution method for detection of minimum inhibitory concentration values in selected bacteria. Bot Serb. 2016;40(1):29–36.
17. Farmakope Herbal Indonesia Edisi II. Kementerian Kesehatan Republik Indonesia. 2017.
18. Dacosta M , Sudirga , SK. Ketut Muksin I. Perbandingan Kandungan Minyak Atsiri Tanaman Sereh Wangi (*Cymbopogon nardus* L. Rendle) Yang Ditanam Di Lokasi Berbeda Comparison Plant Contains Oil Of Citronella (*Cymbopogon nardus* Rendle L.) Grown In Different Locations. SIMBIOSIS Journal of Biological Sciences. 2017; 5(1),
19. Wahyuningsih S. Digital Repository Universitas Jember Penerapan Standard Repository Universitas Universitas Jember Jember. 2018. 1–49
20. Wang Y, Cheng R, Wu X, Miao M. Neuroprotective and Neurotrophic Effects of Ginkgetin and Bilobalide on MPTP-Induced Mice with Parkinson's Disease. Die Pharmazie-An International Journal of Pharmaceutical Sciences 2021;76(1), 27-33.
21. Ghozali MT. Is integrating video into tech-based patient education effective for improving medication adherence? - A review. Paladyn [Internet]. 2023 Jan 1 [cited 2023 Jul 23];14(1). Available from: https://www.degruyter.com/document/doi/10. 1515/pjbr-2022-0109/html

22. Rohman A. Spektroskopi Inframerah dan Kemometrika untuk Analisis Farmasi. Yogyakarta: Pustaka Pelajar; 2014.