Jurnal Biota Prodi Biologi Universitas Islam Negeri Raden Fatah Palembang

ACCEPTED MANUSCRIPT • OPEN ACCESS

Phytochemical screening, in vitro and in silico antibacterial investigation of *Elaeocarpus ganitrus* extract

Primiani, C.N. (2023). Phytochemical screening, in vitro and in silico antibacterial investigation of *Elaeocarpus ganitrus* extract. Jurnal Biota. In press
http://jurnal.radenfatah.ac.id/index.php/biota/article/view/20038

Phytochemical screening, in vitro and in silico antibacterial investigation of *Elaeocarpus ganitrus* extract

Cicilia Novi Primiani[1], Weka Sidha Bhagawan[1], Pujiati[2], Dewi Ratih Tirto Sari[3]*

[1]Department of Pharmacy, Faculty of Health and Science, Universitas PGRI Madiun, Jl. Setia Budi 85 Madiun 63 118, East Java, Indonesia.

[2]Department of Biological Education, Faculty of Teacher Training and Education, Universitas PGRI Madiun, Jl. Setia Budi 85 Madiun 63118, East Java, Indonesia

[3]Department of Pharmacy, Faculty of Medicine, Ibrahimy University, Jl. KHR. Syamsul Arifin No.1-2, Situbondo 68374 , East Java, Indonesia

*Email: dewiratihtirtosari@ibrahimy.ac.id

Abstract

This study evaluated phytochemical composition, and in vitro and in silico antibacterial activity of *Elaeocarpus ganitrus* extract. *Elaeocarpus ganitrus* leaves, seed and fruit powder were extracted with absolute ethanol. Then, the extract was identified phytochemical compounds qualitatively and evaluated the antibacterial activity through in vitro against *Staphylococcus aureus* and *E. coli.* Molecular docking was conducted to evaluate the antibacterial mechanism of *Elaeocarpus ganitrus* extract. *Elaeocarpus ganitrus* leaves, seeds, and fruits extract presented positive tannin, saponin, cardiac glycoside, quinone, steroids, terpenoids, and anthocyanins. In vitro analysis performed *Elaeocarpus ganitrus* leaves strong inhibited *Staphylococcus aureus* growth and medium inhibition against *E. coli.* structure activity relationship revealed 14 of 72 compounds have high antibacterial activities. molecular docking of 7 compounds showed inhibition activity of D-alanin ligase of *Staphylococcus aureus.* Those compounds blocked the activity of D-alanine ligase at inhibitor sites of enzyme, and might be disrupted the cell wall synthesis. In conclusion, *Elaeocarpus ganitrus* contained several phytochemical compounds and has antibacterial activity both in vitro and in silico investigation.

Keywords: *Antibacterial activity; D-alanine ligase; Elaeocarpus ganitrus; Phytochemical*

Introduction

Microbial infections, including bacteria and viruses, cause a spectrum of clinical manifestations, including mild to severe impacts on the body's biological systems (Deets & Vance, 2021; Freer & Pistello, 2018; Kularatne & Dalugama, 2022). Infectious conditions can reduce the body's condition, resulting in the potential for secondary infections (Calder et al., 2020; Netea et al., 2016). Secondary infections are a pathogenic factor that can reduce the body's immunity (Calder et al., 2020).

The use of immunomodulatory supplements can increase the body's resistance, thereby reducing the risk of secondary infections (Allegra et al., 2022; Pecora et al., 2020; Samad et al., 2021). Efforts to increase the body's immune system are often carried out using vitamins, supplements and immunostimulant ingredients (Dong et al., 2015). Herbal medicines have been widely used to increase the body's immunity, this is because herbal medicines are more effective, efficient and safe (Chavda et al., 2022; Frazzoli et al., 2023; Phu et al., 2020; Shaito et al., 2020).

Elaeocarpus ganitrus, known as genitri, is widely used in medical therapy worldwide (Krishna et al., 2013; Prasannan et al., 2020; Vijayaraghavan et al., 2018). This plant grows abundantly in Indonesia, especially in Java, Sulawesi, Bali, Timor, Kalimantan and Sumatra (Brambach et al., 2016). Ganitri plants in Central Java are found in the districts of Cilacap, Kebumen, Kendal, Brebes, Purworejo, Banjarnegara, Wonosobo, Banyumas, Temanggung, Semarang and Karanganyar (Rohandi & Gunawan, 2015). Habitat Elaeocarpaceae grows at an altitude of up to 2.000 meters above sea level, prefers soil with a pH of 5 to 6.5, an average temperature of 20°C, and a humidity level of around 80% (Hardainiyan, Nandy, & Chaudhary, 2015).

Research results have identified various Elaeocarpaceae phytochemicals, namely carbohydrates, proteins (Joshi et al., 2012), alkaloids, flavonoids, glycosides, fatty acids, triterpene tannins, steroids and saponins (Das & Medhabati, 2014). Additionally, hydrocarbon compounds, alcohols, sesquiterpenes, diterpenoids, triterpene alcohols, phytosterols, fatty acids, and pheophytin have been identified in Elaeocarpus ganitrus, along with steroids, tannins, glycosides, alkaloids, quinones, coumarins, phenols, and flavonoids (Jain et al., 2019; Wu et al., 2008). The pericarp of genitri fruit contains saponins, anthocyanins, tannins, alkaloids, phenols, flavonoids (Deivasigamani et al., 2018; Hardainiyan, Nandy, & Chaudhary, 2015; Hardainiyan, Nandy, & Saxena, 2015).

The diversity of Elaeocarpaceae phytochemical composition provides biological and pharmacological activity in the body. *Elaeocarpus ganitrus* seeds have shown anti-

inflammatory, analgesic, hypoglycemic, antihypertensive, hydrocholeretic, smooth muscle relaxant, antiulcerogenic and antimicrobial effects (Xie et al., 2018; Yashwant Kumar et al., 2011). Genitri leaves contain routine compounds as antibacterial agents (Makhouri & Ghasemi, 2017). Quercetin, phytosterol, rudrakin, and elaeocarpidin, performed as antioxidants, anti-inflammatory, and anti-hypertension (Dubey, 2018). However, the exploration of *Elaeocarpus ganitrus* phytochemical compounds and antibacterial activity was limited. Therefore, this study evaluated the phytochemical screning and antibacterial activity through *in vitro* and *in silico* studies.

Materials and methods

Materials

Genitri leaves and fruit are picked from the forest edge of the Karangploso Malang area in fresh condition, not wilted, the color of the fruit is purplish blue. Plant identification was carried out at the Malang Materia Medika Laboratory, with identification numbers: 067/1046/102.20/2023. Leaves and fruit were washed with water and cut into pieces. The fruit is peeled to separate the seeds. Then all ingredients were dried separately in an oven at 70oC. The leaves, fruit and seeds are separately ground to make a fine powder.

Methods

Herbal Plant Extraction

Leaves, fruits and seeds of genitri powder 100 g was dissolved in 1000 ml of 96% ethanol and soak for 24 hours at room temperature. The homogenate was filtered by 0.45 micron filter paper. The filtrate was evaporated by a rotary evaporator at a temperature of 55°C at a speed of 120 rpm. Then, dried by oven at 50°C for three days.

Phytochemical screening

Phytochemical identification of *Elaeocarpus ganitrus* was conducted by qualitative assays. Tannin was tested by reacting 1 ml of extract with 2 ml of 5% Ferric chloride. The positive reaction was marked by dark blue or greenish black solution (Roghini & Vijayalakshmi, 2018). Saponins was observed by mixing 2 ml of extract and 2 ml of distilled water, then shaken vigorously for 15 min. Foam layer in solution was represented saponin (Patel et al., 2016). Glycosides was detected by making solution containing 3 ml of chloroform, 10% ammonia solution, and 2 ml of extract. Pink color indicated presence of glycosides (Roghini & Vijayalakshmi, 2018; Soulef et al., 2014). Cardiac Glycosides was identified by

mixing 0,5 ml of the extract with 2 ml of glacial acetic acid and few drops of ferric chloride. Then the solution was added by 1 ml of concentrated sulphuric acid. Brown ring at the interface indicates the presence of cardiac glycosides (Morsy, 2017; Roghini & Vijayalakshmi, 2018). Quinones was evaluated by mixing 1 ml of extract with 1 ml of concentrated sulphuric acid. Red color indicated presence of quinones (J. Kaur, 2014; Roghini & Vijayalakshmi, 2018). Anthraquinones was identified by adding 10 ml of benzene to 1 ml of extract, then filter and add 5 ml of 10% (v/v) ammonia to the extract and shake well. A positive indication is that the solution changes to pink (S. Kaur et al., 2019; Roghini & Vijayalakshmi, 2018). Steroid was observed by adding 10 ml chloroform and 10 ml H2SO4 slowly to 1 ml extract. Changes occur in upper layer turns red and sulphuric acid layer showed yellow with green fluorescence (Al-Snafi, 2015). Terpenoid was identified by mixing 0.5 ml of the extract with 2 ml of chloroform and concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of terpenoids (Roghini & Vijayalakshmi, 2018). Alkaloid was determined qualitatively by adding 2 ml of extract with 2 ml of concentrated hydrochloric acid. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicated the presence of alkaloids (Roghini & Vijayalakshmi, 2018). Anthocyanin was identified by heating 1 mL of extract with HCl for 2 minutes. A positive result shows a red color change (Fatchiyah et al., 2020).

Antibacterial evaluation by in vitro

Nutrient Agar (NA) media was prepared by dissolving 11.5 g of NA in 500 ml of distilled water using an Erlenmeyer flask. Then homogenize using a magnetic stirrer. The homogenized NA media was sterilized in an autoclave at 121°c for 15 minutes and left for ±30 minutes at room temperature until it solidified. *Staphylococcus aureus* and *Escherichia coli* isolate was inoculated on 10 ml of physiological water and equate the turbidity level with the Mc Fraland turbidity standard. Bacterial turbidity equalization was carried out using a vortex. Antibacterial testing uses the disk diffusion method. Staphylococcus aureus and Escherichia coli bacterial suspensions were inoculated on NA medium. The paper disc that had been soaked in the genitri leaf extract sample for 2 hours was taken with tweezers and placed in NA medium. Incubate at 37°C for 24 hours. Observe the formation of a clear zone and measure in mm. The Effectiveness of inhibition was calculated by following formula (Chachad et al., 2016);

$$Effectiveness\ (\%) = \frac{Inhibitory\ zone\ diameter\ of\ the\ genitri\ leaves\ (mm)}{Diameter\ of\ the\ antibiotic\ inhibition\ zone\ (mm)} x\ 100\%$$

In silico evaluation of antibacterial activity

Seventy two compounds from *Elaeocarpus* in previous study was predicted their bioavaibility using PASS way2drug online program (Filimonov et al., 2014). Then, the bioavaibility of compounds were presented using Heatmap analysis. Seven of 72 compounds that have high antibacterial activity, L-Rhamnose, D-Xylose, Isoelaeocarpiline, Esculetin, Trifoliol, Grandisine C, and Grandisine F was selected for molecular docking. The compound structure were retrieved from PubChem NCBI database. Targeted protein for antibacterial against *Staphylococcus aureus* D-alanine ligase was carried out from Protein Data Bank with accession code 2I80 (Liu et al., 2006). Seven compounds and D-alanine ligase were docked by Molegro virtual docker at active sites (Bitencourt-Ferreira & de Azevedo, 2019). The docking center was X = 26.67; Y=13.65; Z = 32.7; Radius 11. Setting evaluator was set init string : crop distance = 0; grid resolution = 0.30; ligands = false; sp2sp2 bond = false; internal h-bond = false; h-bond 90 = true; Displace Water = false. MolDock SE optimizer was used for setting optimizer. Setting init string: population size = 50; cavity = true; creation Energy Threshold = 100; pose Generator = 10,10,30; recombine = true; max simplex = 750; simplex steps = 300; simplex distance factor = 1; cluster threshold = 1.00; keep max poses = 5. The binding poses and three dimensional structure of complex were analyzed by PyMol 2.3 and Discovery studio version 21.1.1.

Results and Discussion

The phytochemical screening of *Elaeocarpus ganitrus* extract showed positive tannin, saponin, cardiac glycoside, quinone, steroids, alkaloid, terpenoids, and anthocyanins (Table 1). Glycoside and anthraquinone did not detect on leaves, seed and fruit of *Elaeocarpus ganitrus.* Alkaloids was identified on leaves and fruit extract of *Elaeocarpus ganitrus.* The phytochemical compounds of *Elaeocarpus* leaves and and seeds was reported in several studies. Leaves and seeds of *Elaeocarpus* genus contained geranin, alkaloids, glycosides, saponins, phytosterols, flavonoids, tannin, 3-4-5 trimethoxy geranin, grandisinin, and quercetin (Singh et al., 2018; Sudradjat & Timotius, 2022; Talukdar et al., 2017; Tripathy et al., 2016). Previous study also reported that *Elaeocarpus sphaericus* Schum Fruit has 72 compounds, which was classified as terpenoids, alkaloids, flavonoids, steroids, tannin, and saponins (Primiani et al., 2022).

The phytochemistry of *Elaeocarpus floribundus* fruit contains cardiac glycosides, anthraquinone glycosides, steroids, terpenoids and quinines in fruits (Deivasigamani et al., 2018; Lakshmi et al., 2014). The phytochemical components of *Elaeocarpus tuberculatus*

leaves, fruit and seeds are carbohydrates, proteins and amino acids, alkaloids, flavonoids, tannins, phenols, terpenoids, steriods, triterpenoids, coumarin, saponins, quinine, glycosides(Rastogi & Sinha, 2009). Genitri seeds contain alkaloids, flavonoids, phytosterols, tannins, carbohydrate, and protein compounds (Tripathy et al., 2016). The secondary metabolite components of *Elaeocarpus recurvatus* leaves and stems are proanthocyanidins, phenolics, flavonoids (Deivasigamani et al., 2018). *Ealeocarpus serratus* and *Elaeocarpus variabilis* leaves flavonoids, saponins, tannins, glycosides, flavonoids tannins, steroids, tannins, terpenoids Phenols, flavonoids, sterols, amino acids, terpenoids and alkaloids (Sumana et al., 2015).

Table 1. **Phytochemical screening of *Elaeocarpus ganitrus* extract**

Phytochemicals	Part of a plant		
	leaves	seeds	fruit
Tannin	+	+	+
Saponin	+	+	+
Glycoside	-	-	-
Cardiac glycoside	+	+	+
Quinone	+	+	+
Anthraquinone	-	-	-
Steroid	+	+	+
Terpenoid	+	+	+
Alkaloid	+	-	+
Anthocyanins	+	+	+

Based on various research results, *Elaeocarpus* phytochemicals have potential as constituents, which can be used to treat microbial infections (Chockalingam et al., 2021; Sharma et al., 2016; V C et al., 2018). Flavonoids, saponins, tannins, and alkaloids from *Elaeocarpus* seed extract performed antibacterial activity (Dalei, 2016; Tripathy et al., 2016). Antibacterial activity of *Elaeocarpus ganitrus* was tested against *Staphylococcus aureus* and *Escherichia coli*. *Elaeocarpus ganitrus* leaves extract showed strong antibacterial activity against S*taphylococcus aureus* both of 20% and 60% extract concentration (Figure 1. Table 2). Comparing against cloramphenicol 30 mg, *Elaeocarpus ganitrus* leaves extract has lower antibacterial activity. The antibacterial activity of *Elaeocarpus ganitrus* leaves extract both 20% and 60% against *Escherichia coli* performed lower inhibition zone than cloramphenicol 30 mg. the category of antibacterial activity was medium.

Staphylococcus aureus is a gram-positive bacterium that has a thick peptidoglycan structure which facilitates the diffusion of antibacterial compounds into cells (Cui et al., 2006; Fisher & Mobashery, 2021; Szweda et al., 2012). Peptidoglycan is part of the bacterial cell wall

which is polar, making it easier for secondary metabolite activities to enter the bacterial cell wall (Hasan et al., 2022; Rizki et al., 2023). Gram-negative bacteria (*Escherichia coli*) have cell walls containing non-polar lipopolysaccharides, so it is more difficult for phytochemicals to penetrate the bacterial cell walls (Aelenei et al., 2016; Muchtaromah et al., 2020).

Flavonoids are secondary metabolite compounds of genitri seed extract which have the function of inhibiting bacterial cell membranes (Salem et al., 2014; Tagousop et al., 2018). Flavonoids also inhibited the bacterial growth by several mechanism, involved disrupting nucleic acid synthesis, inhibiting cytoplasmic membrane function, blocking energy metabolism, blocking bacterial attachment and biofilm formation, inhibiting porins in cell membranes, changing membrane permeability (Shamsudin et al., 2022; Tagousop et al., 2018). Saponins performed enzyme inhibition activity and disrupt bacterial metabolism (Mabhiza et al., 2016). The effectiveness of the inhibition activity of *Elaeocarpus ganitrus* leaves extract was presented at Table 2. *Elaeocarpus ganitrus* leaves extract both 20% and 60% showed high effective inhibition in *Staphylococcus aureus,* more than 90%. While, the effectivity of extract in *Escherichia coli* was lower, less than 70%.

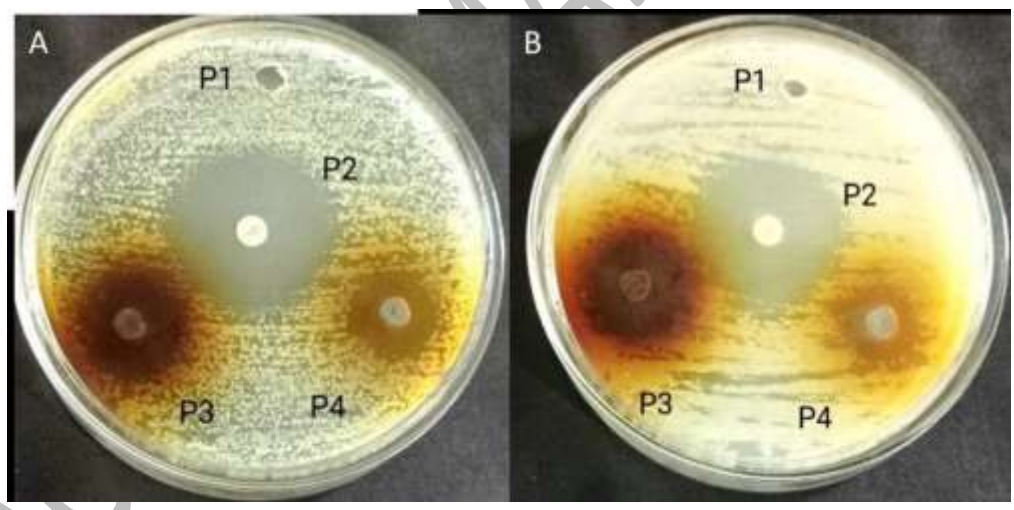

Figure 1. Inhibition zone of bacterial growth against *Elaeocarpus ganitrus* leaves extract, A. *Staphylococcus aureus,* B. *Escherichia coli.* P1: Negative control treatment with sterile distilled water; P2: Treatment with cloramphenicol 30 mg; P3: Treatment with 20% *Elaeocarpus ganitrus* leaf extract; P4: Treatment with 60% *Elaeocarpus ganitrus* leaf extract

Table 2. **Antibacterial activity of *Elaeocarpus ganitrus* leaves extract**

Species	Treatment	Average diameter (mm)	Inhibitory response *
Staphylococcus aureus	P1	0,00±0,00	None
	P2	27 ±0,03	Strong
	P3	24,3±0,02	Strong
	P4	25,6±0,01	Strong
Escherichia coli	P1	0±0,00	None
	P2	28,3±0,03	Strong
	P3	15,6±0,04	Medium
	P4	19±0,02	Medium

……: P1: Negative control treatment with sterile distilled water; P2: Treatment with cloramphenicol 30 mg; P3: Treatment with 20% *Elaeocarpus ganitrus* leaf extract; P4: Treatment with 60% *Elaeocarpus ganitrus* leaf extract. *Greenwood et al., 1995.

Table 3. Effectiveness of the inhibitory power of genitri extract against bacteria (%) **(Hamzah, 2019 and Pouva et al., 2008)**

Treatment	Effectiveness of inhibition (%)	
	Staphylococcus aureus	*Escherichia coli*
P3	90	55,12
P4	94,81	67,13

To proposed the antibacterial mechanism of Elaeocarpus ganitrus extract against *Staphylococcus aureus and Eschericia coli,* structure activity relationship of compounds was conducted. The 72 identified compounds by LCMS/MS was screened the bioavaibility as antibacterial mechanism (Figure 2). The parameter was set to filter the bioavaibility, including antimycobacterial, antibacterial opthalmic, antibacterial, anti-Helicobater pylori, and becterial efflux pum inhibitor. Out of 72 compounds, 14 had high antimycobacterial activity, one was high in antibacterial opthalmic, 18 had high performance in antibacterial, 8 compounds in anti-Helicobacter activities.

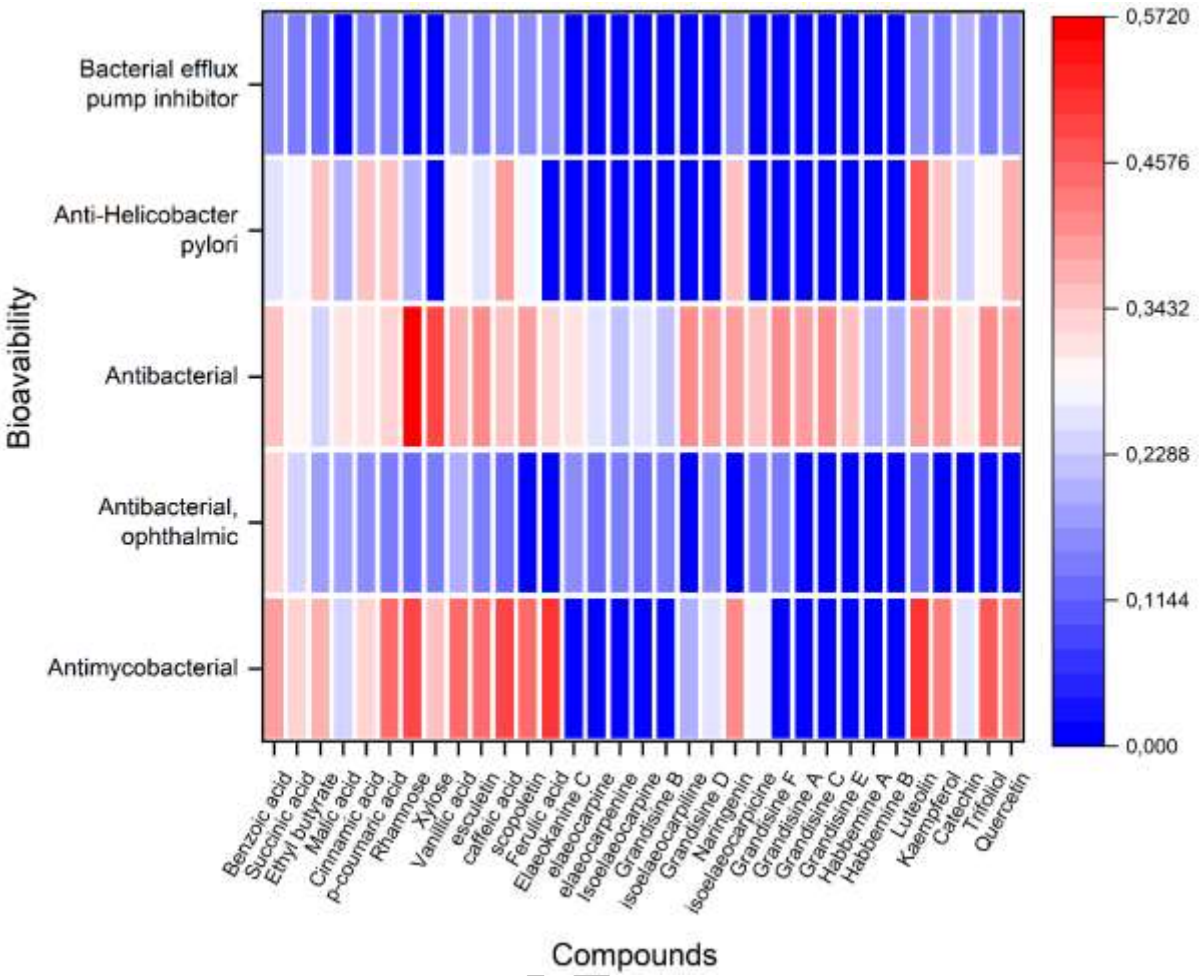

Figure 2. Antibacterial activity mechanism of *Elaeocarpus ganitrus* compounds

Molecular docking was conducted to evaluate the inhibitory mechanism of *Elaeocarpus ganitrus* compounds against *Staphylococcus aureus* D-alanine ligase. Inhibitor compound, 3-Chloro-2,2-Dimethyl-N-[4-(Trifluoromethyl) Phenyl] Propanamide, was used as a docking control. The binding poses of *Elaeocarpus ganitrus* compounds against *Staphylococcus aureus* D-alanine ligase presented same binding pose with control (Figure 3). The inhibitor control bound to D-alanine ligase by 2 hydrogen bonds at residus SER20 and PRO93. Furthermore, 8 hydrophobic interactions also performed in inhibitor control with D-alanine ligase. The residues that attached by hydrophobic interaction were LEU94, PHE313, MET310, PRO311, VAL117, LEU289, VAL19, and HIS96. Several binding sites of inhibitor compound were identified on *Elaeocarpus ganitrus* binding sites. PRO311 was identified in all compounds active sites. Pro93 was identified at D-Xylose, Isoelaeocarpiline, Esculetin, Grandisine C, and Grandisine F binding sites. LEU94 was detected at L-Rhamnose, Isoelaeocarpiline, Esculetin, Trifoliol, Grandisine C, and Grandisine F. The PHE313 was found at active site of Isoelaeocarpiline, Trifoliol, Grandisine C, and Grandisine F. All compounds was showed

MET310 as active site, except L-Rhamnose. VAL117 and LEU289 was detected at Grandisine C and Grandisine F.

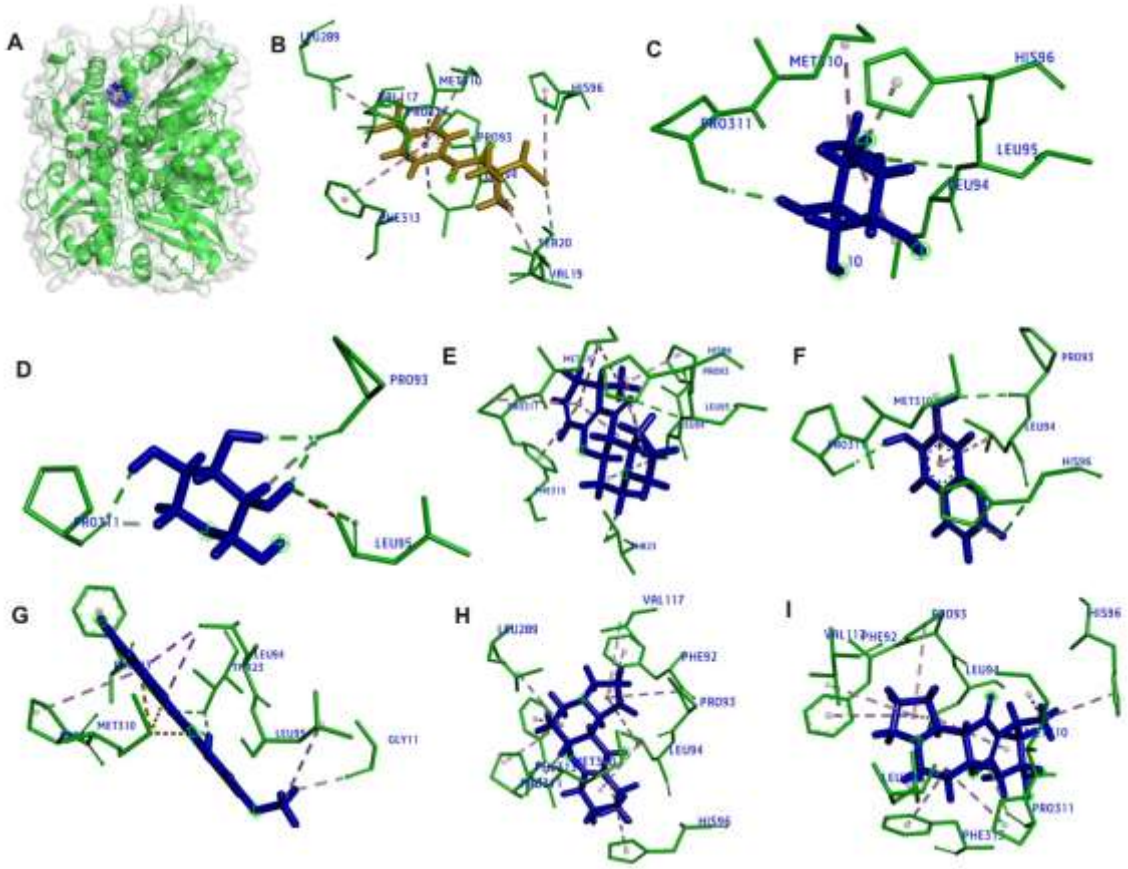

Figure 3. Three dimensional structure of *Elaeocarpus ganitrus* compounds – D-alanine ligase complex, A. superimpossed of ligands – protein complex, B. control inhibitor compound, C. L-Rhamnose, D. D-Xylose, E. Isoelaeocarpiline, F. Esculetin, G. Trifoliol, H. Grandisine C, I. Grandisine F. D-alanine ligase was presented in green cartoon, inhibitor compound was showed in yellow, and *Elaeocarpus ganitrus* compounds were in blue color.

L-Rhamnose bound to D-alanine ligase by three hydrogen bonds and two hydrophobic interactions. Those residues were LEU95, PRO311, HIS96, LEU94, and MET310. D-Xylose showed interaction in some residues active sites of D-alanine ligase. PRO93 bound to H7, H8, and H2 by hydrogen bonds. LEU95 bound to H8 of D-xylose by hydrogen bonds, similar with PRO311 also bound to H9 and H5 by hydrogen bonds. Isoelaeocarpiline interacted with D-alanine ligase by two hydrogen bonds (LEU95 and THR23) and 9 hydrophobic interactions (LEU94, MET310, PRO311, LEU94, PRO93, LEU94, MET310, HIS96, and PHE313). Esculetin showed interaction at HIS96, PRO311, PRO93, and HIS96 by gydrogen bonds,

LEU94 and MET310 by Hydrophobic interactions. Trifoliol showed interaction by 4 Hydrogen Bonds and 5 Hydrophobic, while grandisine C showed one Hydrogen Bond and 10 Hydrophobic interactions as well as grandisine F. Binding energy of ligands – protein complexes were presented at Figure 4. Trifoliol revealed the lowest binding energy against D-alanine ligase, with binding energy -353.0 kJ/mol, followed by isoelaeocarpiline and grandisine C. Low binding energy indicated tight interaction between ligands and targeted protein. The binding energy of ligands – protein complex was affected by several factors, including the number of hydrogend bond, hydrophobic interaction, the complex structure both of ligands and protein (Bare et al., 2022, 2023; Krisnamurti & Sari, 2023; Sari, Dewi Ratih Tirto; Krisnamurti, 2021; Sari et al., 2020, 2022; Sari & Krisnamurti, 2022).

D-alanine D alanine ligase is an essential enzyme for bacterial cell wall synthesis. This enzyme catalyzed D-alanine D alanine dipeptide formation by using one D-alanine as substrate and second D-alanine to complete a reaction. D-alanine ligase also contributing for developing new antibiotic, mutation on D-alanine ligase revealed antibiotic resistance (Liu et al., 2006; May et al., 2005; Pederick et al., 2020; Wu et al., 2008; Yang et al., 2018). In this study, *Elaeocarpus ganitrus* compounds bound to D-alanine D alanine ligase at inhibitor sites, as well as inhibitory control. Blocking mechanisms caused weakening the bacterial cell wall leading to the cell wall lysis (May et al., 2005; Wu et al., 2008; Yang et al., 2018).

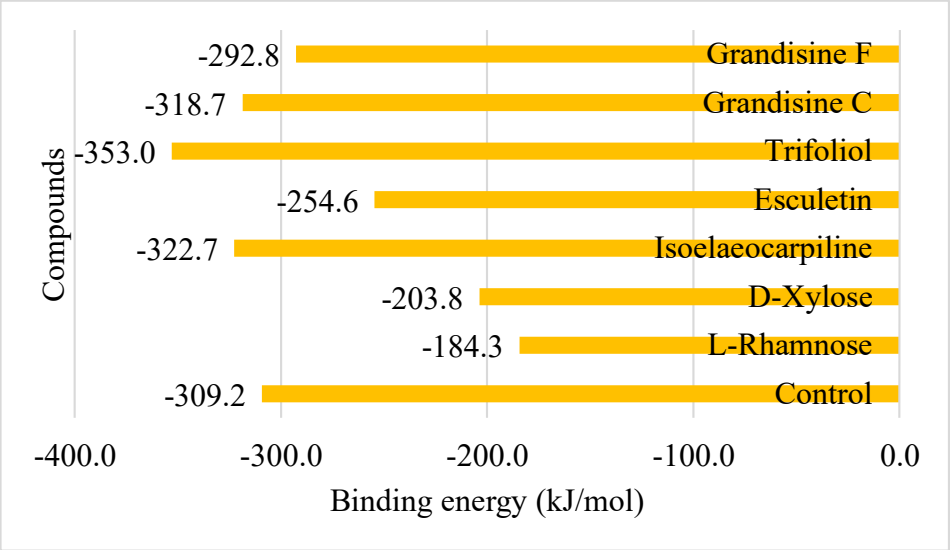

Figure 4. Binding energy of *Elaeocarpus ganitrus* compounds – D-alanine ligase complex

Conclusions

The phytochemical compounds that identified on leaves, seed and fruit of *Elaeocarpus ganitrus* extract were tannin, saponin, cardiac glycoside, quinone, steroids, terpenoids, and

anthocyanins. In vitro observation, *Elaeocarpus ganitrus* leaves extract performed that strong inhibition activity against *Staphylococcus aureus* and medium inhibition against *E. coli.* Molecular docking revealed seven compounds of *Elaeocarpus ganitrus,* L-Rhamnose, D-Xylose, Isoelaeocarpiline, Esculetin, Trifoliol, Grandisine C, and Grandisine F have high bioavaibity in antibacterial and blocked D-alanine ligase in cell wall synthesis in S. aureus.

Acknowledgments

We sincerely acknowledge the financial assistance from the National Innovation Research Agency (BRIN) RIIM Programme and Education Fund Management Institution (LPDP) No. B-1468/II.7/FR/10/2022.

References

Aelenei, P., Miron, A., Trifan, A., Bujor, A., Gille, E., & Aprotosoaie, A. C. (2016). Essential Oils and Their Components as Modulators of Antibiotic Activity against Gram-Negative Bacteria. *Medicines (Basel, Switzerland)*, *3*(3). https://doi.org/10.3390/medicines3030019

Al-Snafi, A. E. (2015). The Pharmacological Importance of Bellis Perennis - A Review. *Inter. J. of Phytotherapy*, *5*(2), 63–69. www.phytotherapyjournal.com

Allegra, A., Mirabile, G., Ettari, R., Pioggia, G., & Gangemi, S. (2022). The Impact of Curcumin on Immune Response: An Immunomodulatory Strategy to Treat Sepsis. *International Journal of Molecular Sciences*, *23*(23). https://doi.org/10.3390/ijms232314710

Bare, Y., Sari, D. R. T., Mogi, M. C., & Nurak, M. M. D. (2022). Fucodiphlorethol Dan Phloroglucinol Alga Coklat Sebagai Inhibitor Lipase Secara In Silico. *Florea: Jurnal Biologi Dan Pembelajarannya*, *9*(1), 53–59.

Bare, Y., Timba, F. N. S., Nurak, M. M. D., & Sari, D. R. T. (2023). Analisis In silico Heksosa, D-Manitol dan Asam Malat Kulit Kopi sebagai Penghambat Infeksi Virus Corona. *Biota : Jurnal Ilmiah Ilmu-Ilmu Hayati*, *8*(2), 41–48. https://doi.org/10.24002/biota.v8i2.5970

Bitencourt-Ferreira, G., & de Azevedo, W. F. J. (2019). Molegro Virtual Docker for Docking. *Methods in Molecular Biology (Clifton, N.J.)*, *2053*, 149–167. https://doi.org/10.1007/978-1-4939-9752-7_10

Brambach, F., Coode, M., Biagioni, S., & Culmsee, H. (2016). Elaeocarpus firdausii (Elaeocarpaceae), a new species from tropical mountain forests of Sulawesi. *PhytoKeys*, *62*, 1–14. https://doi.org/10.3897/phytokeys.62.7548

Calder, P. C., Carr, A. C., Gombart, A. F., & Eggersdorfer, M. (2020). Optimal Nutritional Status for a Well-Functioning Immune System Is an Important Factor to Protect against Viral Infections. *Nutrients*, *12*(4). https://doi.org/10.3390/nu12041181

Chachad, D. P., Talpade, M. B., & Jagdale, S. P. (2016). Antimicrobial Activity of Rhizomes of Curcuma zedoaria Rosc. *International Journal of Science and Research*, *5*(11), 938–940. https://doi.org/10.21275/ART20162324

Chavda, V. P., Patel, A. B., Vihol, D., Vaghasiya, D. D., Ahmed, K. M. S. B., Trivedi, K. U., & Dave, D. J. (2022). Herbal Remedies, Nutraceuticals, and Dietary Supplements for COVID-19 Management: An Update. *Clinical Complementary Medicine and Pharmacology*, *2*(1), 100021. https://doi.org/10.1016/j.ccmp.2022.100021

Chockalingam, J., Monica, R., & Ramasamy, S. (2021). Antibiofilm Activity Of Elaeocarpus Ganitrus Against Methicillin Resistant Staphylococcus Species (Mrss) Causing Bovine Mastitis. *Plant Cell Biotechnology and Molecular Biology*, 339–355.

Cui, L., Iwamoto, A., Lian, J.-Q., Neoh, H., Horikawa, Y., & Hiramatsu, K. (2006). Novel Mechanism of Antibiotic Resistance Originating in Vancomycin-Intermediate. *Antimicrobial Agents and Chemotherapy*, *50*, 428. https://doi.org/10.1128/AAC.50.2.428-438.2006

Dalei, J. (2016). Evaluation Of Antimicrobial Activity And Phytochemical Screening Of Epicarp And Endocarp Parts Of Elaeocarpus Ganitrus. *International Journal of Pharma and Bio Sciences*, *7*, 265–269.

Das, K. R., & Medhabati, K. (2014). *The Potential of Dark Purple Scented Rice- From Staple Food to Nutraceutical*. *9*(3), 867–876. https://doi.org/10.12944/CWE.9.3.38

Deets, K. A., & Vance, R. E. (2021). Inflammasomes and adaptive immune responses. *Nature Immunology*, *22*(4), 412–422. https://doi.org/10.1038/s41590-021-00869-6

Deivasigamani, R., Devi, M. S., & Nadu, T. (2018). *Phytochemical analysis of elaeocarpus floribundus blume*. *7*(10), 1452–1457. https://doi.org/10.20959/wjpps201810-12506

Dong, Y., Dekens, D., De Deyn, P., Naudé, P., & Eisel, U. (2015). Targeting of Tumor Necrosis Factor Alpha Receptors as a Therapeutic Strategy for Neurodegenerative Disorders. In *Antibodies* (Vol. 4, Issue 4). https://doi.org/10.3390/antib4040369

Fatchiyah, F., Sari, D. R. T., Safitri, A., & Cairns, J. R. K. (2020). Phytochemical compound and nutritional value in black rice from Java Island, Indonesia. *Systematic Reviews in Pharmacy*, *11*(7). https://doi.org/10.31838/srp.2020.7.61

Filimonov, D. A., Lagunin, A. A., Gloriozova, T. A., Rudik, A. V., Druzhilovskii, D. S., Pogodin, P. V., & Poroikov, V. V. (2014). Prediction of the biological activity spectra of

organic compounds using the pass online web resource. *Chemistry of Heterocyclic Compounds*, *50*(3), 444–457. https://doi.org/10.1007/s10593-014-1496-1

Fisher, J. F., & Mobashery, S. (2021). β-Lactams against the Fortress of the Gram-Positive Staphylococcus aureus Bacterium. *Chemical Reviews*, *121*(6), 3412–3463. https://doi.org/10.1021/acs.chemrev.0c01010

Frazzoli, C., Grasso, G., Husaini, D. C., Ajibo, D. N., Orish, F. C., & Orisakwe, O. E. (2023). Immune System and Epidemics: The Role of African Indigenous Bioactive Substances. *Nutrients*, *15*(2). https://doi.org/10.3390/nu15020273

Freer, G., & Pistello, M. (2018). Varicella-zoster virus infection: natural history, clinical manifestations, immunity and current and future vaccination strategies. *The New Microbiologica*, *41*(2), 95–105.

Greenwood, D., Finch, R., Davey, P., & Wilcox, M. (1995). Antibiotics, susceptibility (Sensitivity) test antimicrobial and chemoterapy. *United State of America: Mc Graw Hill Company*.

Hamzah A. In Vitro Analysis of Dragon Scale Leaves (Drymoglossum pilosellaoides) Antibacterial Activity Against Vibrio harveyi and Vibrio parahaemolyticus Bacteria. J Aquac Fish Heal. 2019; 8(2):86-91. 33.

Hardainiyan, S., Nandy, B., & Chaudhary, K. (2015). Elaeocarpus Ganitrus (Rudraksha): A Reservoir Plant with their Pharmacological Effects. *International Journal of Pharmaceutical Sciences Review and Research*, *34*, 55–64.

Hardainiyan, S., Nandy, B., & Saxena, R. (2015). Phytochemical investigation of fruit extract of Elaeocarpus ganitrus. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*, 415–418.

Hasan, N., Rachmayanti, A., & Masaenah, E. (2022). Antibacterial Activity Test of Meniran Herb Extract (Phyllanthus Niruri L.) against Staphylococcus Epidermidis and Klebsiella Pneumoniae. *Science Midwifery*, *10*, 3876–3885. https://doi.org/10.35335/midwifery.v10i5.927

Jain, C., Khatana, S., & Vijayvergia, R. (2019). Bioactivity of secondary metabolites of various plants: A review. *International Journal of Pharmaceutical Sciences and Research*, *10*(2), 494–504. https://doi.org/10.13040/IJPSR.0975-8232.10(2).494-04

Joshi, S., Gupta, P., Kumar, N., Rai, N., Gautam, P., & Thapliyal, A. (2012). A comprehensive report on therapeutic potential of Elaeocarpus ganitrus Roxb. (Rudraksha). *Environment Conservation Journal*, *13*, 147–150. https://doi.org/10.36953/ECJ.2012.130324

Kaur, J. (2014). A comprehensive review on metabolic syndrome. *Cardiology Research and*

Practice, *2014*(March). https://doi.org/10.1155/2014/943162

Kaur, S., Gupta, S., & Gautam, P. (2019). *Phytochemical analysis of Eucalyptus leaves extract*. *8*, 2442–2446.

Krishna, P. S., Vani, K., Prasad, M. R., Samatha, B., Hima Bindu, N. S. V. S. L., Singara Charya, M. A., & Shetty, P. R. (2013). In -silico molecular docking analysis of prodigiosin and cycloprodigiosin as COX-2 inhibitors. *SpringerPlus*, *2*(1), 1–6. https://doi.org/10.1186/2193-1801-2-172

Krisnamurti, G. C., & Sari, D. R. T. (2023). Unveiling the Potency of Coriandrum sativum as Repellent for Antimalarial : In silico Study. *Proceeding International Conference on Religion, Science and Education*, *2*, 563–567.

Kularatne, S. A., & Dalugama, C. (2022). Dengue infection: Global importance, immunopathology and management. *Clinical Medicine (London, England)*, *22*(1), 9–13. https://doi.org/10.7861/clinmed.2021-0791

Lakshmi, S. R., Kumar, T. S., & Rao, M. V. (2014). Phytochemical Analysis, Histochemical localization and Antioxidant Activity of Hoya wightii ssp. palniensis and Elaeocarpus recurvatus. *American Journal of Phytomedicine and Clinical Therapeutics*, *2*(3), 357–366.

Liu, S., Chang, J. S., Herberg, J. T., Horng, M.-M., Tomich, P. K., Lin, A. H., & Marotti, K. R. (2006). Allosteric inhibition of Staphylococcus aureus d-alanine:d-alanine ligase revealed by crystallographic studies. *Proceedings of the National Academy of Sciences*, *103*(41), 15178–15183. https://doi.org/10.1073/pnas.0604905103

Mabhiza, D., Chitemerere, T., & Mukanganyama, S. (2016). Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa. *International Journal of Medicinal Chemistry*, *2016*, 6304163. https://doi.org/10.1155/2016/6304163

Makhouri, F. R., & Ghasemi, J. B. (2017). In Silico Studies in Drug Research Against Neurodegenerative Diseases. *Current Neuropharmacology*, *16*(6), 664–725. https://doi.org/10.2174/1570159x15666170823095628

May, J. J., Finking, R., Wiegeshoff, F., Weber, T. T., Bandur, N., Koert, U., & Marahiel, M. A. (2005). Inhibition of the D-alanine:D-alanyl carrier protein ligase from Bacillus subtilis increases the bacterium's susceptibility to antibiotics that target the cell wall. *The FEBS Journal*, *272*(12), 2993–3003. https://doi.org/10.1111/j.1742-4658.2005.04700.x

Morsy, N. (2017). *Cardiac Glycosides in Medicinal Plants*. https://doi.org/10.5772/65963

Muchtaromah, B., Safitri, E. S., Fitriasari, P. D., & Istiwandhani, J. (2020). Antibacterial

activities of Curcuma mangga Val. extract in some solvents to Staphylococcus aureus and Escherichia coli. *AIP Conference Proceedings*, *2231*(1), 30005. https://doi.org/10.1063/5.0002490

Netea, M. G., Joosten, L. A. B., Latz, E., Mills, K. H. G., Natoli, G., Stunnenberg, H. G., O'Neill, L. A. J., & Xavier, R. J. (2016). Trained immunity: A program of innate immune memory in health and disease. *Science (New York, N.Y.)*, *352*(6284), aaf1098. https://doi.org/10.1126/science.aaf1098

Patel, R., Shukla, P. K., Verma, A., & Singh, M. P. (2016). Pharmacognostical, phytochemical evaluation and insilico lead finding of Callicarpa macrophylla with hepatoprotective potentials. *Journal of Chemical and Pharmaceutical Research*, *8*(3), 383–393. https://www.scopus.com/inward/record.uri?eid=2-s2.0-84979475561&partnerID=40&md5=51e72a62640239ebd69b3149c42f87bc

Pecora, F., Persico, F., Argentiero, A., Neglia, C., & Esposito, S. (2020). The Role of Micronutrients in Support of the Immune Response against Viral Infections. *Nutrients*, *12*(10). https://doi.org/10.3390/nu12103198

Pederick, J. L., Thompson, A. P., Bell, S. G., & Bruning, J. B. (2020). d-Alanine-d-alanine ligase as a model for the activation of ATP-grasp enzymes by monovalent cations. *The Journal of Biological Chemistry*, *295*(23), 7894–7904. https://doi.org/10.1074/jbc.RA120.012936

Phu, H. T., Thuan, D. T. B., Nguyen, T. H. D., Posadino, A. M., Eid, A. H., & Pintus, G. (2020). Herbal Medicine for Slowing Aging and Aging-associated Conditions: Efficacy, Mechanisms and Safety. *Current Vascular Pharmacology*, *18*(4), 369–393. https://doi.org/10.2174/1570161117666190715121939

Pouvova D, Kokoskova B, Pavela R, Rysanek P. Effectivity of plant essential oils against Clavibacter michiganensis, in vitro. Zemdirbyste. 2008; 95(3):440-446.

Prasannan, P., Jeyaram, Y., Pandian, A., Raju, R., & Sekar, S. (2020). A Review on Taxonomy, Phytochemistry, Pharmacology, Threats and Conservation of Elaeocarpus L. (Elaeocarpaceae). *The Botanical Review*, *86*. https://doi.org/10.1007/s12229-020-09229-9

Primiani, C. N., Pujiati, & Setiawan, M. A. (2022). Bioactive Compounds Profile of Alkaloid on Elaeocarpus sphaericus Schum Seeds by Liquid Chromatography-Mass Spectrometry . *Proceedings of the 2nd International Conference on Education and Technology (ICETECH 2021)*, *630*(Icetech 2021), 120–125. https://doi.org/10.2991/assehr.k.220103.019

Rastogi, R. P., & Sinha, R. P. (2009). Review article : APOPTOSIS : MOLECULAR MECHANISMS AND PATHOGENICITY. *EXCLI Journal*, *8*, 155–181.

Rizki, M., Harahap, U., & Sitorus, P. (2023). Phytochemical Screening of Phaleria macrocarpa (Scheff.) Boerl.) and Antibacterial Activity Test of Ethanol Extract Against Staphylococcus aureus Bacteria. *International Journal of Science, Technology & Management*, *4*(2), 422–427. https://doi.org/10.46729/ijstm.v4i2.781

Roghini, R., & Vijayalakshmi, K. (2018). Phytochemical Screening, Quantitative Analysis of Flavonoids and Minerals in Ethanolic Extract of Citrus Paradisi. *International Journal of Pharmaceutical Sciences and Research*, *9*(11), 4859. https://doi.org/10.13040/IJPSR.0975-8232.9(11).4859-64

Rohandi, A., & Gunawan, G. (2015). Sebaran Populasi dan Potensi Tanaman Ganitri (Elaeocarpus ganitrus Roxb) di Jawa Tengah. *Jurnal Ilmu Kehutanan*, *8*(1), 25. https://doi.org/10.22146/jik.8550

Salem, E. G., El Hissewy, A., Agamy, N. F., & Abd El Barry, D. (2014). Assessment of the quality of bran and bran oil produced from some Egyptian rice varieties. *Journal of the Egyptian Public Health Association*, *89*(1), 29–34. https://doi.org/10.1097/01.EPX.0000443988.38424.9d

Samad, N., Dutta, S., Sodunke, T. E., Fairuz, A., Sapkota, A., Miftah, Z. F., Jahan, I., Sharma, P., Abubakar, A. R., Rowaiye, A. B., Oli, A. N., Charan, J., Islam, S., & Haque, M. (2021). Fat-Soluble Vitamins and the Current Global Pandemic of COVID-19: Evidence-Based Efficacy from Literature Review. *Journal of Inflammation Research*, *14*(null), 2091–2110. https://doi.org/10.2147/JIR.S307333

Sari, Dewi Ratih Tirto; Krisnamurti, G. C. (2021). 1-dehydrogingerdione , Senyawa Volatil Jahe sebagai Agen Sedatif subtitutif γ - aminobutyrate (GABA); Kajian Biokomputasi. *Prosiding Seminar Nasional Biologi*, *7*(1), 389–395. https://doi.org/https://doi.org/10.24252/psb.v7i1.24709

Sari, D. R. T., & Krisnamurti, G. C. (2022). In Silico Repositioning Strategies of Theobromine and Caffeine for Psychiatric and Neurological Disorders. *Proceeding International Conference on Religion, Science and Education*, *1*, 685–692.

Sari, D. R. T., Pranoto, M. E., & Azkiyah, S. Z. (2022). Kajian Farmakoinformatika Senyawa Alkaloid Anggur Laut (Caulerpa racemose) Sebagai Inhibitor Collagenase Dalam Mekanisme Antiaging. *Florea : Jurnal Biologi Dan Pembelajarannya*, *9*(2), 127–133. https://doi.org/10.25273/florea.v9i2.14434

Sari, D. R. T., Safitri, A., Cairns, J. R. K., & Fatchiyah, F. (2020). Anti-Apoptotic Activity of

Anthocyanins has Potential to inhibit Caspase-3 Signaling. *Journal of Tropical Life Science*, *10*(1), 15–25. https://doi.org/10.11594/jtls.10.01.03

Shaito, A., Thuan, D. T. B., Phu, H. T., Nguyen, T. H. D., Hasan, H., Halabi, S., Abdelhady, S., Nasrallah, G. K., Eid, A. H., & Pintus, G. (2020). Herbal Medicine for Cardiovascular Diseases: Efficacy, Mechanisms, and Safety. *Frontiers in Pharmacology*, *11*, 422. https://doi.org/10.3389/fphar.2020.00422

Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Ali Shah, S. A., Khatib, A., Mukhtar, S., Alsharif, M. A., Parveen, H., & Zakaria, Z. A. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules (Basel, Switzerland)*, *27*(4). https://doi.org/10.3390/molecules27041149

Sharma, A. K., Gangwar, M., Kumar, D., Nath, G., Kumar Sinha, A. S., & Tripathi, Y. B. (2016). Phytochemical characterization, antimicrobial activity and reducing potential of seed oil, latex, machine oil and presscake of Jatropha curcas. *Avicenna Journal of Phytomedicine*, *6*(4), 366–375.

Singh, K., Bhori, M., Kasu, Y. A., Bhat, G., & Marar, T. (2018). Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity – Exploring the armoury of obscurity. *Saudi Pharmaceutical Journal*, *26*(2), 177–190. https://doi.org/https://doi.org/10.1016/j.jsps.2017.12.013

Soulef, K., Abdelouahab, Y., & Dalal, B. (2014). Effect of glycosides extract of the medicinal plant Glycyrrhiza glabra L. from the region of Mlilli (southeast of Algeria) on the growth of some human pathogenic bacteria. *Journal of Scientific and Innovative Research*, *3*, 28–34. https://doi.org/10.31254/jsir.2014.3106

Sudradjat, S. E., & Timotius, K. H. (2022). Pharmacological properties and phytochemical components of Elaeocarpus: A comparative study. *Phytomedicine Plus*, *2*(4), 100365. https://doi.org/https://doi.org/10.1016/j.phyplu.2022.100365

Sumana, S., K A., S., D., J., Raviswamy, C., Seema, M., & Ns, D. (2015). *Antimicrobial Assay of Elaeocarpus Species of Western Ghats of Karnataka.*

Szweda, P., Schielmann, M., Kotlowski, R., Gorczyca, G., Zalewska, M., & Milewski, S. (2012). Peptidoglycan hydrolases-potential weapons against Staphylococcus aureus. *Applied Microbiology and Biotechnology*, *96*(5), 1157–1174. https://doi.org/10.1007/s00253-012-4484-3

Tagousop, C. N., Tamokou, J.-D., Ekom, S. E., Ngnokam, D., & Voutquenne-Nazabadioko, L. (2018). Antimicrobial activities of flavonoid glycosides from Graptophyllum grandulosum and their mechanism of antibacterial action. *BMC Complementary and*

Alternative Medicine, *18*(1), 252. https://doi.org/10.1186/s12906-018-2321-7

Talukdar, P. R., Rathi, S., Pathak, K., Chetia, S. K., & Sarma, R. N. (2017). Population Structure and Marker-Trait Association in Indigenous Aromatic Rice. *Rice Science*, *24*(3), 145–154. https://doi.org/10.1016/j.rsci.2016.08.009

Tripathy, S., Mida, A., & Swain, S. (2016). Phytochemical Screening And Thin Layer Chromatographic Studies Of Elaeocarpus Ganitrus Seed The Magical Electromagnetic Bead (Rudraksha). *International Journal of Pharmacy and Biological Sciences*, *6*, 16–24. https://doi.org/10.21276/ijpbs.2016.6.3.3

V C, D., Sumathi, S., Athikkavil, F., & Elyas, K. (2018). Isolation And Identification Of Endophytic Fungi With Antimicrobial Activities From The Leaves Of Elaeocarpus Sphaericus (Gaertn.) K. Schum. And Myristica Fragrans Houtt. *International Journal of Pharmaceutical Sciences and Research*. https://doi.org/10.13040/IJPSR.0975-8232.9(7).2783-91

Vijayaraghavan, J., Kumar, V., Krishnan, N. P., Kaufhold, R. T., Zeng, X., Lin, J., & Akker, F. Van Den. (2018). Structural studies and molecular dynamics simulations suggest a processive mechanism of exolytic lytic transglycosylase from Campylobacter jejuni. *PLoS ONE*, *13*(5), 1–30. https://doi.org/https://doi.org/10.1371/ journal.pone.0197136 Editor:

Wu, D., Zhang, L., Kong, Y., Du, J., Chen, S., Chen, J., Ding, J., Jiang, H., & Shen, X. (2008). Enzymatic characterization and crystal structure analysis of the D-alanine-D-alanine ligase from Helicobacter pylori. *Proteins*, *72*(4), 1148–1160. https://doi.org/10.1002/prot.22009

Xie, L., Su, H., Sun, C., Zheng, X., & Chen, W. (2018). Recent advances in understanding the anti-obesity activity of anthocyanins and their biosynthesis in microorganisms. *Trends in Food Science and Technology*, *72*(September 2017), 13–24. https://doi.org/10.1016/j.tifs.2017.12.002

Yang, S., Xu, Y., Wang, Y., Ren, F., Li, S., Ding, W., Ma, Y., & Zhang, W. (2018). The Biological Properties and Potential Interacting Proteins of d-Alanyl-d-alanine Ligase A from Mycobacterium tuberculosis. *Molecules (Basel, Switzerland)*, *23*(2). https://doi.org/10.3390/molecules23020324

Yashwant Kumar, A., Nandakumar, K., Handral, M., Talwar, S., & Dhayabaran, D. (2011). Hypoglycaemic and anti-diabetic activity of stem bark extracts Erythrina indica in normal and alloxan-induced diabetic rats. *Saudi Pharmaceutical Journal*, *19*(1), 35–42. https://doi.org/10.1016/j.jsps.2010.10.001