Open Acess
Jurnal Biota Vol. 10 No. 1 (2024) https://doi.org/10.19109/Biota.v10i1.20038

Research Article

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, Indonesia
[2] Department of Biological Education, Faculty of Teacher Training and Education, Universitas PGRI Madiun, Indonesia
[3] Department of Pharmacy, Faculty of Medicine, Ibrahimy University Situbondo, Indonesia
*Email: dewiratihtirtosari@ibrahimy.ac.id

Received: 06/11/2023	Revised: 25/11/2023	Accepted: 27/11/2023

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 [1]–[3]. Infectious conditions can reduce the body's condition, resulting in the potential for secondary infections [4], [5]. Secondary infections are a pathogenic factor that can reduce the body's immunity [4].

The use of immunomodulatory supplements can increase the body's resistance, thereby reducing the risk of secondary infections [6]–[8]. Efforts to increase the body's immune system are often carried out using vitamins, supplements and immunostimulant ingredients [9]. Herbal medicines have been widely used to increase the body's immunity, this is because herbal medicines are more effective, efficient and safe [10]–[13].

Elaeocarpus ganitrus, known as genitri, is widely used in medical therapy worldwide [14]–[16]. This plant grows abundantly in Indonesia, especially in Java, Sulawesi, Bali, Timor, Kalimantan and

Sumatra [17]. Ganitri plants in Central Java are found in the districts of Cilacap, Kebumen, Kendal, Brebes, Purworejo, Banjarnegara, Wonosobo, Banyumas, Temanggung, Semarang and Karanganyar [18]. 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% [19].

Research results have identified various Elaeocarpaceae phytochemicals, namely carbohydrates, proteins [20], alkaloids, flavonoids, glycosides, fatty acids, triterpene tannins, steroids and saponins [21]. 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 [22], [23]. The pericarp of genitri fruit contains saponins, anthocyanins, tannins, alkaloids, phenols, flavonoids [19], [24], [25].

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 [26], [27]. Genitri leaves contain routine compounds as antibacterial agents [28]. Quercetin, phytosterol, rudrakin, and elaeocarpidin, performed as antioxidants, anti-inflammatory, and anti-hypertension. 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

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 70^0C. The leaves, fruit and seeds are separately ground to make a fine powder.

1. 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.

2. 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 [29]. 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 [30]. 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 [29], [31]. 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 [29], [32]. Quinones was evaluated by mixing 1 ml of extract with 1 ml of concentrated sulphuric acid. Red color indicated presence of quinones [29], [33]. 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 [29], [34]. 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 [35]. 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 [29]. 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 [29]. Anthocyanin was identified by heating 1 mL of extract with HCl for 2 minutes. A positive result shows a red color change [36].

3. *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 [37];

$$Effectiveness\ (\%) = \frac{Inhibitory\ zone\ diameter\ of\ the\ genitri\ leaves\ (mm)}{Diameter\ of\ the\ antibiotic\ inhibition\ zone\ (mm)}\ x\ 100\% \qquad (1)$$

4. *In silico evaluation of antibacterial activity*

Seventy two compounds from *Elaeocarpus* in previous study was predicted their bioavaibility using PASS way2drug online program [38]. 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 [39]. Seven compounds and D-alanine ligase were docked by Molegro virtual docker at active sites [40]. 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 [41]–[44]. Previous study also reported that *Elaeocarpus sphaericus* Schum Fruit has 72 compounds, which was classified as terpenoids, alkaloids, flavonoids, steroids, tannin, and saponins [45].

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	+	+	+

The phytochemistry of *Elaeocarpus floribundus* fruit contains cardiac glycosides, anthraquinone glycosides, steroids, terpenoids and quinines in fruits [24], [46]. 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 [47]. Genitri seeds contain alkaloids, flavonoids, phytosterols, tannins, carbohydrate, and protein compounds [42]. The secondary metabolite components of *Elaeocarpus recurvatus* leaves and stems are proanthocyanidins, phenolics, flavonoids [24]. *Ealeocarpus serratus* and *Elaeocarpus variabilis* leaves flavonoids, saponins, tannins, glycosides, flavonoids tannins, steroids, tannins, terpenoids Phenols, flavonoids, sterols, amino acids, terpenoids and alkaloids [48].

Based on various research results, *Elaeocarpus* phytochemicals have potential as constituents, which can be used to treat microbial infections [49]–[51]. Flavonoids, saponins, tannins, and alkaloids from *Elaeocarpus* seed extract performed antibacterial activity [42], [52]. 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 [53]–[56]. Peptidoglycan is part of the bacterial cell wall which is polar, making it easier for secondary metabolite activities to enter the bacterial cell wall [57], [58]. 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 [59], [60].

Flavonoids are secondary metabolite compounds of genitri seed extract which have the function of inhibiting bacterial cell membranes [61], [62]. 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 [62], [63]. Saponins performed enzyme inhibition activity and disrupt bacterial metabolism [64]. The effectiveness of the inhibition activity of *Elaeocarpus ganitrus* leaves extract was presented at Table 2 and Effectiveness of the inhibitory power of genitri extract against bacteria (%) table 3. *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%.

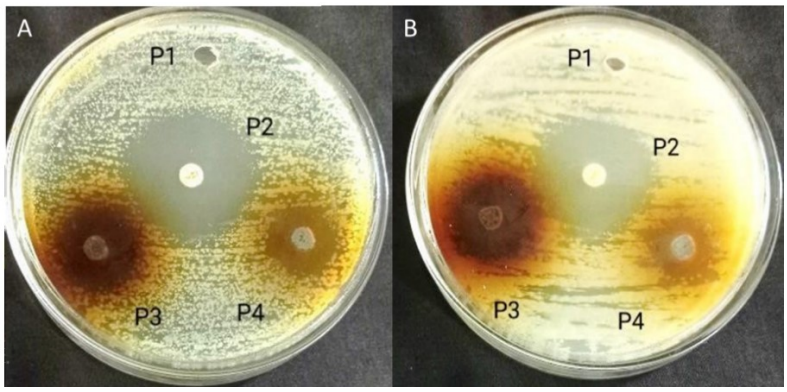

Figure 1. Inhibition zone of bacterial growth against *Elaeocarpus ganitrus* leaves extract, A. *Staphylococcus aureus,* B. *Escherichia coli.* P1: Negative control treatment with sterile distilled water; P2: Treatment with cloramphenicol 30 mg; P3: Treatment with 20% *Elaeocarpus ganitrus* leaf extract;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

Notes: 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.

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.

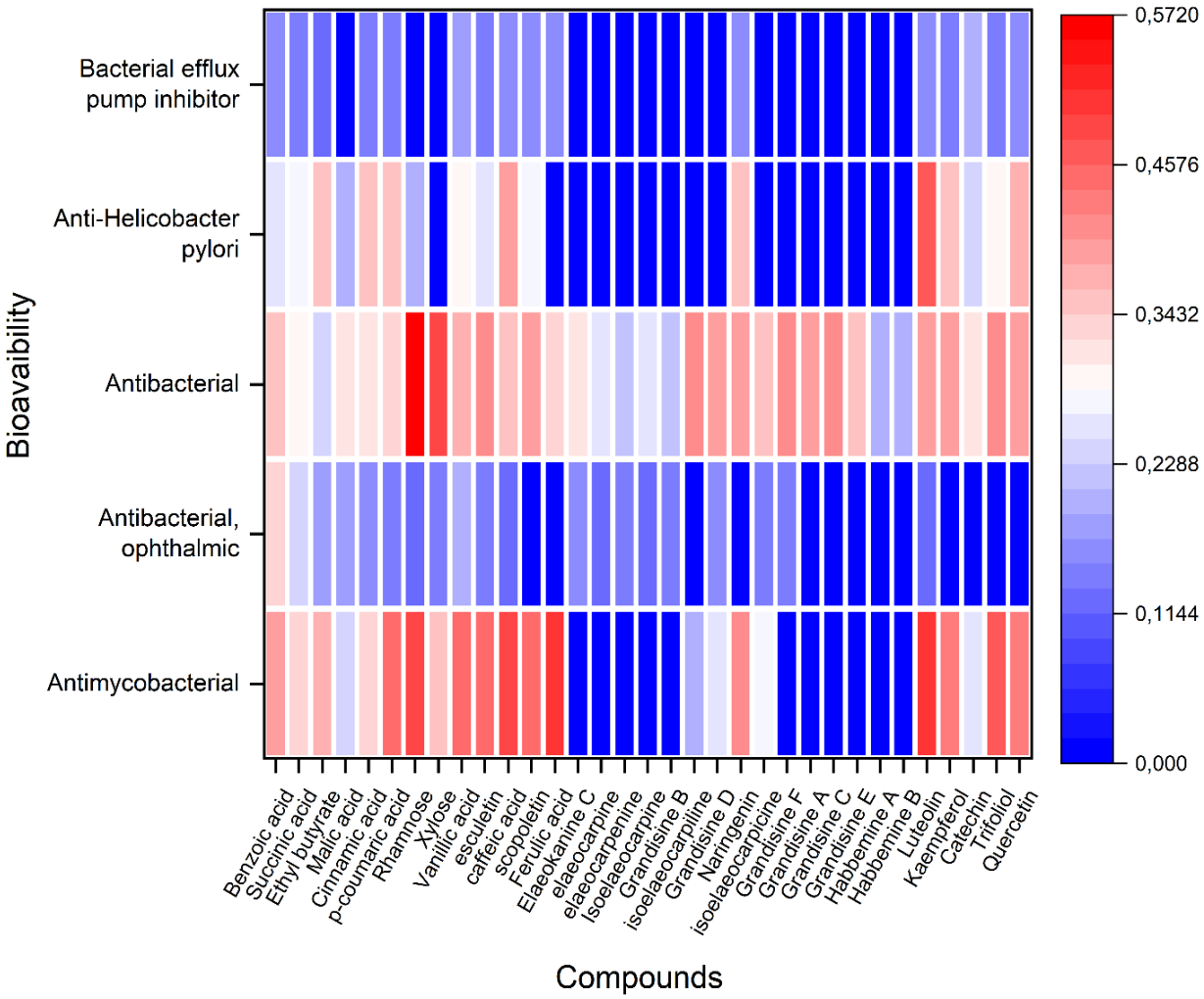

Figure 2. Antibacterial activity mechanism of *Elaeocarpus ganitrus* compounds

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.

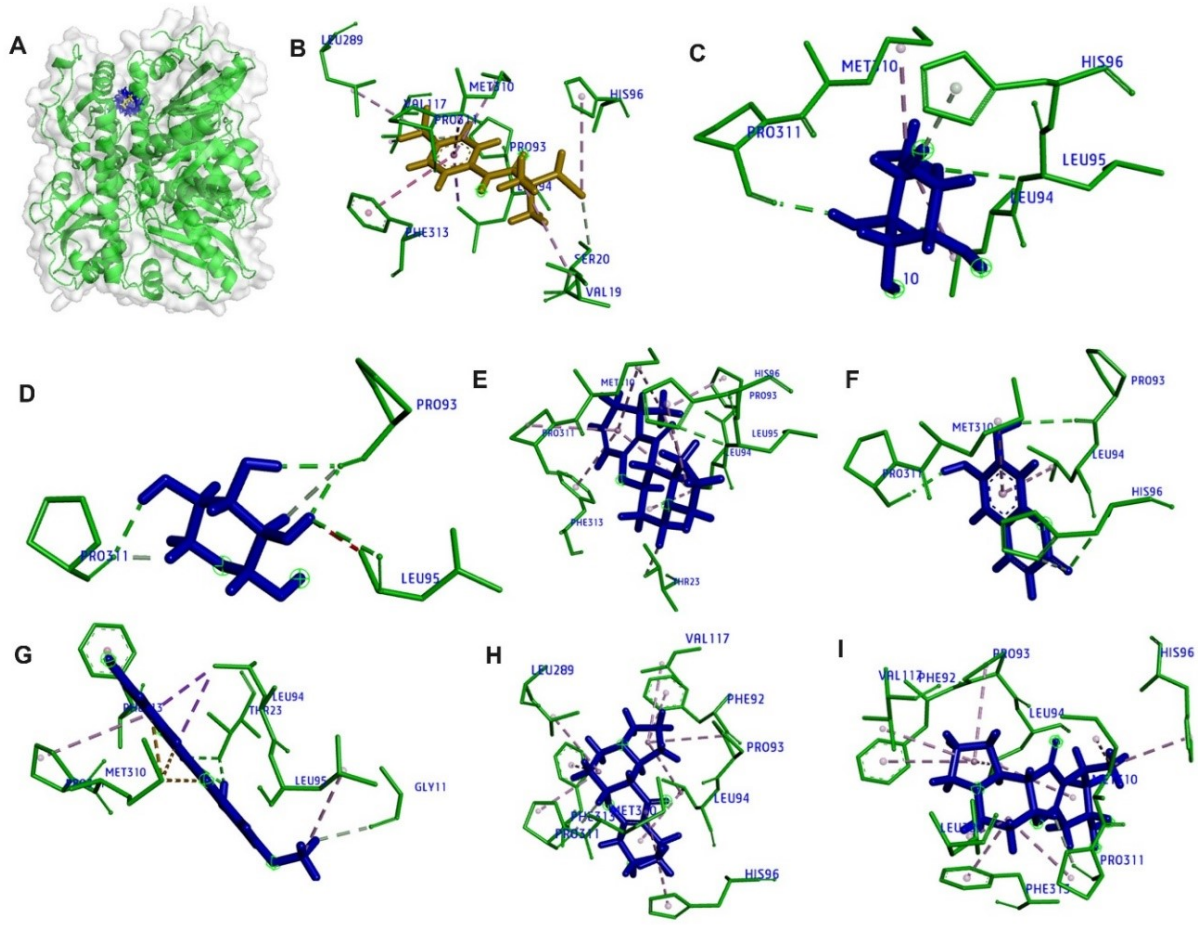

Figure 3. Three dimensional structure of *Elaeocarpus ganitrus* compounds – D-alanine ligase complex, A. superimpossed of ligands – protein complex, B. control inhibitor compound, C. L-Rhamnose, D. D-Xylose, E. Isoelaeocarpiline, F. Esculetin, G. Trifoliol, H. Grandisine C, I. Grandisine F. D-alanine 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 [65]–[71].

Table 3. Effectiveness of the inhibitory power of genitri extract against bacteria (%) (*Eq. 1*)

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

Notes: Hamzah, 2019 and Pouva et al., 2008

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 [22], [39], [72]–[74]. 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 [22], [73], [74].

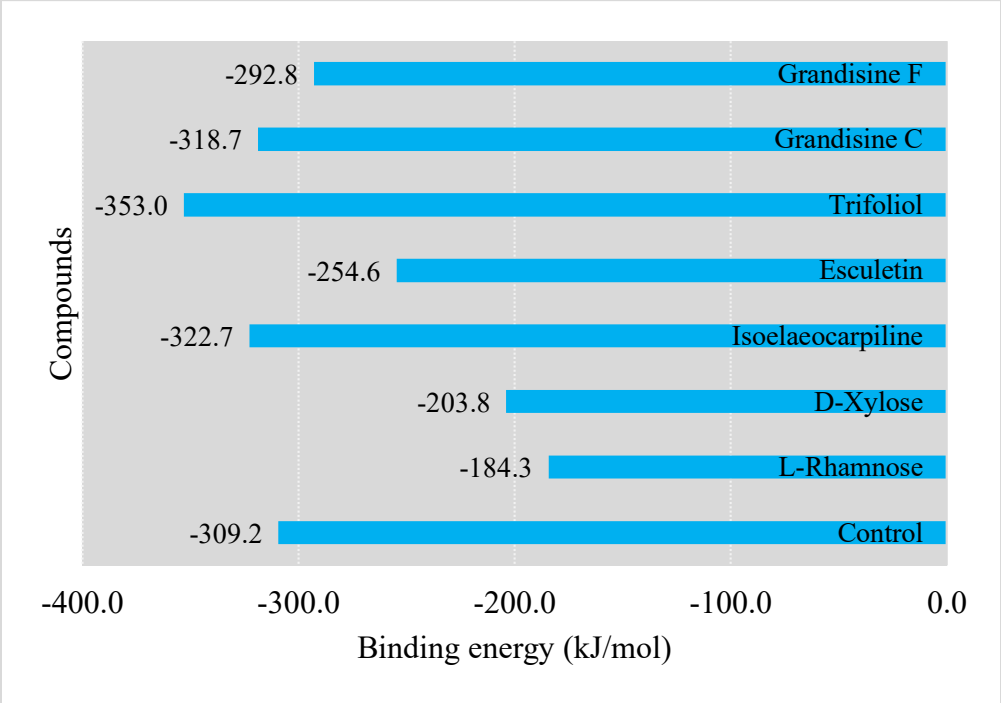

Figure 4. Binding energy of *Elaeocarpus ganitrus* compounds – D-alanine ligase complex

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

[1] G. Freer and M. Pistello, "Varicella-zoster virus infection: natural history, clinical manifestations, immunity and current and future vaccination strategies.," *New Microbiol.*, vol. 41, no. 2, pp. 95–105, Apr. 2018.

[2] S. A. Kularatne and C. Dalugama, "Dengue infection: Global importance, immunopathology and management.," *Clin. Med. Lond. Engl.*, vol. 22, no. 1, pp. 9–13, Jan. 2022, doi:10.7861/clinmed.2021-0791.

[3] K. A. Deets and R. E. Vance, "Inflammasomes and adaptive immune responses.," *Nat. Immunol.*, vol. 22, no. 4, pp. 412–422, Apr. 2021, doi:10.1038/s41590-021-00869-6.

[4] P. C. Calder, A. C. Carr, A. F. Gombart, and M. Eggersdorfer, "Optimal Nutritional Status for a Well-Functioning Immune System Is an Important Factor to Protect against Viral Infections.," *Nutrients*, vol. 12, no. 4, Apr. 2020, doi:10.3390/nu12041181.

[5] M. G. Netea *et al.*, "Trained immunity: A program of innate immune memory in health and disease.," *Science*, vol. 352, no. 6284, p. aaf1098, Apr. 2016, doi:10.1126/science.aaf1098.

[6] F. Pecora, F. Persico, A. Argentiero, C. Neglia, and S. Esposito, "The Role of Micronutrients in Support of the Immune Response against Viral Infections.," *Nutrients*, vol. 12, no. 10, Oct. 2020, doi:10.3390/nu12103198.

[7] N. Samad *et al.*, "Fat-Soluble Vitamins and the Current Global Pandemic of COVID-19: Evidence-Based Efficacy from Literature Review," *J. Inflamm. Res.*, vol. 14, no. null, pp. 2091–2110, May 2021, doi:10.2147/JIR.S307333.

[8] A. Allegra, G. Mirabile, R. Ettari, G. Pioggia, and S. Gangemi, "The Impact of Curcumin on Immune Response: An Immunomodulatory Strategy to Treat Sepsis.," *Int. J. Mol. Sci.*, vol. 23, no. 23, Nov. 2022, doi:10.3390/ijms232314710.

[9] Y. Dong, D. Dekens, P. De Deyn, P. Naudé, and U. Eisel, *Targeting of Tumor Necrosis Factor Alpha Receptors as a Therapeutic Strategy for Neurodegenerative Disorders*, vol. 4, no. 4. 2015. doi:10.3390/antib4040369.

[10] H. T. Phu, D. T. B. Thuan, T. H. D. Nguyen, A. M. Posadino, A. H. Eid, and G. Pintus, "Herbal Medicine for Slowing Aging and Aging-associated Conditions: Efficacy, Mechanisms and Safety.," *Curr. Vasc. Pharmacol.*, vol. 18, no. 4, pp. 369–393, 2020, doi:10.2174/1570161117666190715121939.

[11] A. Shaito *et al.*, "Herbal Medicine for Cardiovascular Diseases: Efficacy, Mechanisms, and Safety.," *Front. Pharmacol.*, vol. 11, p. 422, 2020, doi:10.3389/fphar.2020.00422.

[12] V. P. Chavda *et al.*, "Herbal Remedies, Nutraceuticals, and Dietary Supplements for COVID-19 Management: An Update.," *Clin. Complement. Med. Pharmacol.*, vol. 2, no. 1, p. 100021, Mar. 2022, doi:10.1016/j.ccmp.2022.100021.

[13] C. Frazzoli, G. Grasso, D. C. Husaini, D. N. Ajibo, F. C. Orish, and O. E. Orisakwe, "Immune System and Epidemics: The Role of African Indigenous Bioactive Substances.," *Nutrients*, vol. 15, no. 2, Jan. 2023, doi:10.3390/nu15020273.

[14] J. Vijayaraghavan *et al.*, "Structural studies and molecular dynamics simulations suggest a processive mechanism of exolytic lytic transglycosylase from Campylobacter jejuni," *PLoS ONE*, vol. 13, no. 5, pp. 1–30, 2018, doi:10.1371/journal.pone.0197136:

[15] P. Prasannan, Y. Jeyaram, A. Pandian, R. Raju, and S. Sekar, "A Review on Taxonomy, Phytochemistry, Pharmacology, Threats and Conservation of Elaeocarpus L. (Elaeocarpaceae)," *Bot. Rev.*, vol. 86, Aug. 2020, doi:10.1007/s12229-020-09229-9.

[16] P. S. Krishna *et al.*, "In -silico molecular docking analysis of prodigiosin and cycloprodigiosin as COX-2 inhibitors," *SpringerPlus*, vol. 2, no. 1, pp. 1–6, 2013, doi:10.1186/2193-1801-2-172.

[17] F. Brambach, M. Coode, S. Biagioni, and H. Culmsee, "Elaeocarpus firdausii (Elaeocarpaceae), a new species from tropical mountain forests of Sulawesi.," *PhytoKeys*, no. 62, pp. 1–14, 2016, doi:10.3897/phytokeys.62.7548.

[18] A. Rohandi and G. Gunawan, "Sebaran Populasi dan Potensi Tanaman Ganitri (*Elaeocarpus ganitrus* Roxb) di Jawa Tengah," *J. Ilmu Kehutan.*, vol. 8, no. 1, p. 25, 2015, doi:10.22146/jik.8550.

[19] S. Hardainiyan, B. Nandy, and K. Chaudhary, "*Elaeocarpus Ganitrus* (Rudraksha): A Reservoir Plant with their Pharmacological Effects," *Int. J. Pharm. Sci. Rev. Res.*, vol. 34, pp. 55–64, Oct. 2015.

[20] S. Joshi, P. Gupta, N. Kumar, N. Rai, P. Gautam, and A. Thapliyal, "A comprehensive report on therapeutic potential of *Elaeocarpus ganitrus* Roxb. (Rudraksha)," *Environ. Conserv. J.*, vol. 13, pp. 147–150, Dec. 2012, doi:10.36953/ECJ.2012.130324.

[21] K. R. Das and K. Medhabati, "The Potential of Dark Purple Scented Rice- From Staple Food to Nutraceutical," vol. 9, no. 3, pp. 867–876, 2014, doi:10.12944/CWE.9.3.38.

[22] D. Wu *et al.*, "Enzymatic characterization and crystal structure analysis of the D-alanine-D-alanine ligase from Helicobacter pylori.," *Proteins*, vol. 72, no. 4, pp. 1148–1160, Sep. 2008, doi:10.1002/prot.22009.

[23] C. Jain, S. Khatana, and R. Vijayvergia, "Bioactivity of secondary metabolites of various plants: A review," *Int. J. Pharm. Sci. Res.*, vol. 10, no. 2, pp. 494–504, 2019, doi:10.13040/IJPSR.0975-8232.10(2).494-04.

[24] R. Deivasigamani, M. S. Devi, and T. Nadu, "Phytochemical analysis of elaeocarpus floribundus blume," vol. 7, no. 10, pp. 1452–1457, 2018, doi:10.20959/wjpps201810-12506.

[25] S. Hardainiyan, B. Nandy, and R. Saxena, "Phytochemical investigation of fruit extract of Elaeocarpus ganitrus," *Int. J. Pharm. Pharm. Sci.*, vol. 7, pp. 415–418, Jun. 2015.

[26] A. Yashwant Kumar, K. Nandakumar, M. Handral, S. Talwar, and D. Dhayabaran, "Hypoglycaemic and anti-diabetic activity of stem bark extracts *Erythrina indica* in normal and alloxan-induced diabetic rats," *Saudi Pharm. J.*, vol. 19, no. 1, pp. 35–42, 2011, doi:10.1016/j.jsps.2010.10.001.

[27] L. Xie, H. Su, C. Sun, X. Zheng, and W. Chen, "Recent advances in understanding the anti-obesity activity of anthocyanins and their biosynthesis in microorganisms," *Trends Food Sci. Technol.*, vol. 72, no. September 2017, pp. 13–24, 2018, doi:10.1016/j.tifs.2017.12.002.

[28] F. R. Makhouri and J. B. Ghasemi, "In Silico Studies in Drug Research Against Neurodegenerative Diseases," *Curr. Neuropharmacol.*, vol. 16, no. 6, pp. 664–725, 2017, doi:10.2174/1570159x15666170823095628.

[29] R. Roghini and K. Vijayalakshmi, "Phytochemical Screening, Quantitative Analysis of Flavonoids and Minerals in Ethanolic Extract of Citrus Paradisi," *Int. J. Pharm. Sci. Res.*, vol. 9, no. 11, p. 4859, 2018,

doi:10.13040/IJPSR.0975-8232.9(11).4859-64.

[30] R. Patel, P. K. Shukla, A. Verma, and M. P. Singh, "Pharmacognostical, phytochemical evaluation and insilico lead finding of *Callicarpa macrophylla* with hepatoprotective potentials," *J. Chem. Pharm. Res.*, vol. 8, no. 3, pp. 383–393, 2016.

[31] K. Soulef, Y. Abdelouahab, and B. Dalal, "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," *J. Sci. Innov. Res.*, vol. 3, pp. 28–34, Feb. 2014, doi:10.31254/jsir.2014.3106.

[32] N. Morsy, "Cardiac Glycosides in Medicinal Plants," 2017. doi:10.5772/65963.

[33] J. Kaur, "A comprehensive review on metabolic syndrome," *Cardiol. Res. Pract.*, vol. 2014, no. March, 2014, doi:10.1155/2014/943162.

[34] S. Kaur, S. Gupta, and P. Gautam, "Phytochemical analysis of Eucalyptus leaves extract," vol. 8, pp. 2442–2446, Jan. 2019.

[35] A. E. Al-Snafi, "The Pharmacological Importance of Bellis Perennis - A Review," *Inter J Phytother.*, vol. 5, no. 2, pp. 63–69, 2015.

[36] F. Fatchiyah, D. R. T. Sari, A. Safitri, and J. R. K. Cairns, "Phytochemical compound and nutritional value in black rice from Java Island, Indonesia," *Syst. Rev. Pharm.*, vol. 11, no. 7, 2020, doi:10.31838/srp.2020.7.61.

[37] D. P. Chachad, M. B. Talpade, and S. P. Jagdale, "Antimicrobial Activity of Rhizomes of Curcuma zedoaria Rosc.," *Int. J. Sci. Res.*, vol. 5, no. 11, pp. 938–940, 2016, doi:10.21275/ART20162324.

[38] D. A. Filimonov *et al.*, "Prediction of the biological activity spectra of organic compounds using the pass online web resource," *Chem. Heterocycl. Compd.*, vol. 50, no. 3, pp. 444–457, 2014, doi:10.1007/s10593-014-1496-1.

[39] S. Liu *et al.*, "Allosteric inhibition of *Staphylococcus aureus* d-alanine:d-alanine ligase revealed by crystallographic studies," *Proc. Natl. Acad. Sci.*, vol. 103, no. 41, pp. 15178–15183, Oct. 2006, doi:10.1073/pnas.0604905103.

[40] G. Bitencourt-Ferreira and W. F. J. de Azevedo, "Molegro Virtual Docker for Docking.," *Methods Mol. Biol. Clifton NJ*, vol. 2053, pp. 149–167, 2019, doi:10.1007/978-1-4939-9752-7_10.

[41] K. Singh, M. Bhori, Y. A. Kasu, G. Bhat, and T. Marar, "Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity – Exploring the armoury of obscurity," *Saudi Pharm. J.*, vol. 26, no. 2, pp. 177–190, 2018, doi:10.1016/j.jsps.2017.12.013.

[42] S. Tripathy, A. Mida, and S. Swain, "Phytochemical Screening And Thin Layer Chromatographic Studies Of *Elaeocarpus Ganitrus* Seed The Magical Electromagnetic Bead (Rudraksha)," *Int. J. Pharm. Biol. Sci.*, vol. 6, pp. 16–24, Jul. 2016, doi:10.21276/ijpbs.2016.6.3.3.

[43] P. R. Talukdar, S. Rathi, K. Pathak, S. K. Chetia, and R. N. Sarma, "Population Structure and Marker-Trait Association in Indigenous Aromatic Rice," *Rice Sci.*, vol. 24, no. 3, pp. 145–154, 2017, doi:10.1016/j.rsci.2016.08.009.

[44] S. E. Sudradjat and K. H. Timotius, "Pharmacological properties and phytochemical components of Elaeocarpus: A comparative study," *Phytomedicine Plus*, vol. 2, no. 4, p. 100365, 2022, doi:10.1016/j.phyplu.2022.100365.

[45] C. N. Primiani, Pujiati, and M. A. Setiawan, " Bioactive Compounds Profile of Alkaloid on Elaeocarpus sphaericus Schum Seeds by Liquid Chromatography-Mass Spectrometry

," *Proc. 2nd Int. Conf. Educ. Technol. ICETECH 2021*, vol. 630, no. Icetech 2021, pp. 120–125, 2022, doi:10.2991/assehr.k.220103.019.

[46] S. R. Lakshmi, T. S. Kumar, and M. V Rao, "Phytochemical Analysis, Histochemical localization and Antioxidant Activity of Hoya wightii ssp. palniensis and *Elaeocarpus recurvatus*," *Am. J. Phytomedicine Clin. Ther.*, vol. 2, no. 3, pp. 357–366, 2014.

[47] R. P. Rastogi and R. P. Sinha, "Review article : Apoptosis : Molecular Mechanisms And Pathogenicity," *Excli J.*, vol. 8, pp. 155–181, 2009.

[48] S. Sumana, S. K A., J. D., C. Raviswamy, M. Seema, and D. Ns, *Antimicrobial Assay of Elaeocarpus Species of Western Ghats of Karnataka.* 2015.

[49] A. K. Sharma, M. Gangwar, D. Kumar, G. Nath, A. S. Kumar Sinha, and Y. B. Tripathi, "Phytochemical characterization, antimicrobial activity and reducing potential of seed oil, latex, machine oil and presscake of Jatropha curcas.," *Avicenna J. Phytomedicine*, vol. 6, no. 4, pp. 366–375, 2016.

[50] D. V C, S. Sumathi, F. Athikkavil, and K. Elyas, "Isolation And Identification Of Endophytic Fungi With Antimicrobial Activities From The Leaves Of *Elaeocarpus Sphaericus* (Gaertn.) K. Schum. And Myristica Fragrans Houtt.," *Int. J. Pharm. Sci. Res.*, Jul. 2018, doi:10.13040/IJPSR.0975-8232.9(7).2783-91.

[51] J. Chockalingam, R. Monica, and S. Ramasamy, "Antibiofilm Activity Of *Elaeocarpus ganitrus* Against Methicillin Resistant *Staphylococcus species* (MRSS) Causing Bovine Mastitis," *Plant Cell Biotechnol. Mol. Biol.*, pp. 339–355, Jan. 2021.

[52] J. Dalei, "Evaluation Of Antimicrobial Activity And Phytochemical Screening Of Epicarp And Endocarp Parts Of Elaeocarpus Ganitrus," *Int. J. Pharma Bio Sci.*, vol. 7, pp. 265–269, Apr. 2016.

[53] L. Cui, A. Iwamoto, J.-Q. Lian, H. Neoh, Y. Horikawa, and K. Hiramatsu, "Novel Mechanism of Antibiotic Resistance Originating in Vancomycin-Intermediate," *Antimicrob. Agents Chemother.*, vol. 50, p. 428, Jan. 2006, doi:10.1128/AAC.50.2.428-438.2006.

[54] P. Szweda, M. Schielmann, R. Kotlowski, G. Gorczyca, M. Zalewska, and S. Milewski, "Peptidoglycan hydrolases-potential weapons against *Staphylococcus aureus*.," *Appl. Microbiol. Biotechnol.*, vol. 96, no. 5, pp. 1157–1174, Dec. 2012, doi:10.1007/s00253-012-4484-3.

[55] J. F. Fisher and S. Mobashery, "β-Lactams against the Fortress of the Gram-Positive *Staphylococcus aureus* Bacterium.," *Chem. Rev.*, vol. 121, no. 6, pp. 3412–3463, Mar. 2021, doi:10.1021/acs.chemrev.0c01010.

[56] I. Kandida, M. Tari, and A. Fatiqin, "Effectiveness of the Combination of Green Betel Leaf Extract (*Piper betle*) and Mint Leaf (*Mentha piperita*) as Antibacterials against *Streptococcus mutans*," *Bioactivities*, vol. 1, no. 1, pp. 32–38, Jun. 2023, doi:10.47352/bioactivities.2963-654X.184.

[57] N. Hasan, A. Rachmayanti, and E. Masaenah, "Antibacterial Activity Test of Meniran Herb Extract (*Phyllanthus Niruri* L.) against Staphylococcus Epidermidis and Klebsiella Pneumoniae," *Sci. Midwifery*, vol. 10, pp. 3876–3885, Nov. 2022, doi:10.35335/midwifery.v10i5.927.

[58] M. Rizki, U. Harahap, and P. Sitorus, "Phytochemical Screening of *Phaleria macrocarpa* (Scheff.) Boerl.) and Antibacterial Activity Test

of Ethanol Extract Against *Staphylococcus aureus* Bacteria," *Int. J. Sci. Technol. Manag.*, vol. 4, no. 2, pp. 422–427, 2023, doi:10.46729/ijstm.v4i2.781.

[59] P. Aelenei, A. Miron, A. Trifan, A. Bujor, E. Gille, and A. C. Aprotosoaie, "Essential Oils and Their Components as Modulators of Antibiotic Activity against Gram-Negative Bacteria.," *Med. Basel Switz.*, vol. 3, no. 3, Jul. 2016, doi:10.3390/medicines3030019.

[60] B. Muchtaromah, E. S. Safitri, P. D. Fitriasari, and J. Istiwandhani, "Antibacterial activities of Curcuma mangga Val. extract in some solvents to S*taphylococcus aureus* and *Escherichia coli*," *AIP Conf. Proc.*, vol. 2231, no. 1, p. 30005, Apr. 2020, doi:10.1063/5.0002490.

[61] E. G. Salem, A. El Hissewy, N. F. Agamy, and D. Abd El Barry, "Assessment of the quality of bran and bran oil produced from some *Egyptian rice* varieties," *J. Egypt. Public Health Assoc.*, vol. 89, no. 1, pp. 29–34, 2014, doi:10.1097/01.EPX.0000443988.38424.9d.

[62] C. N. Tagousop, J.-D. Tamokou, S. E. Ekom, D. Ngnokam, and L. Voutquenne-Nazabadioko, "Antimicrobial activities of flavonoid glycosides from *Graptophyllum grandulosum* and their mechanism of antibacterial action.," *BMC Complement. Altern. Med.*, vol. 18, no. 1, p. 252, Sep. 2018, doi:10.1186/s12906-018-2321-7.

[63] N. F. Shamsudin *et al.*, "Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation.," *Mol. Basel Switz.*, vol. 27, no. 4, Feb. 2022, doi:10.3390/molecules27041149.

[64] D. Mabhiza, T. Chitemerere, and S. Mukanganyama, "Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa.," *Int. J. Med. Chem.*, vol. 2016, p. 6304163, 2016, doi:10.1155/2016/6304163.

[65] Y. Bare, D. R. T. Sari, M. C. Mogi, and M. M. D. Nurak, "Fucodiphlorethol Dan Phloroglucinol Alga Coklat Sebagai Inhibitor Lipase Secara In Silico," *Florea J. Biol. Dan Pembelajarannya*, vol. 9, no. 1, pp. 53–59, 2022.

[66] G. C. Sari, Dewi Ratih Tirto; Krisnamurti, "1-dehydrogingerdione , Senyawa Volatil Jahe sebagai Agen Sedatif subtitutif γ - aminobutyrate (GABA); Kajian Biokomputasi," *Pros. Semin. Nas. Biol.*, vol. 7, no. 1, pp. 389–395, 2021, doi:10.24252/psb.v7i1.24709.

[67] G. C. Krisnamurti and D. R. T. Sari, "Unveiling the Potency of Coriandrum sativum as Repellent for Antimalarial : In silico Study," *Proceeding Int. Conf. Relig. Sci. Educ.*, vol. 2, pp. 563–567, 2023.

[68] D. R. T. Sari and G. C. Krisnamurti, "In Silico Repositioning Strategies of Theobromine and Caffeine for Psychiatric and Neurological Disorders," *Proceeding Int. Conf. Relig. Sci. Educ.*, vol. 1, pp. 685–692, 2022.

[69] Y. Bare, F. N. S. Timba, M. M. D. Nurak, and D. R. T. Sari, "Analisis In silico Heksosa, D-Manitol dan Asam Malat Kulit Kopi sebagai Penghambat Infeksi Virus Corona," *Biota J. Ilm. Ilmu-Ilmu Hayati*, vol. 8, no. 2, pp. 41–48, 2023, doi:10.24002/biota.v8i2.5970.

[70] D. R. T. Sari, A. Safitri, J. R. K. Cairns, and F. Fatchiyah, "Anti-Apoptotic Activity of Anthocyanins has Potential to inhibit Caspase-3 Signaling," *J. Trop. Life Sci.*, vol. 10, no. 1, pp. 15–25, 2020, doi:10.11594/jtls.10.01.03.

[71] D. R. T. Sari, M. E. Pranoto, and S. Z. Azkiyah, "Kajian

Farmakoinformatika Senyawa Alkaloid Anggur Laut (*Caulerpa racemose*) Sebagai Inhibitor Collagenase Dalam Mekanisme Antiaging," *Florea J. Biol. Dan Pembelajarannya*, vol. 9, no. 2, pp. 127–133, 2022, doi:10.25273/florea.v9i2.14434.

[72] J. L. Pederick, A. P. Thompson, S. G. Bell, and J. B. Bruning, "d-Alanine-d-alanine ligase as a model for the activation of ATP-grasp enzymes by monovalent cations.," *J. Biol. Chem.*, vol. 295, no. 23, pp. 7894–7904, Jun. 2020, doi:10.1074/jbc.RA120.012936.

[73] S. Yang *et al.*, "The Biological Properties and Potential Interacting Proteins of d-Alanyl-d-alanine Ligase A from *Mycobacterium tuberculosis*.," *Mol. Basel Switz.*, vol. 23, no. 2, Feb. 2018, doi:10.3390/molecules23020324.

[74] J. J. May *et al.*, "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.," *FEBS J.*, vol. 272, no. 12, pp. 2993–3003, Jun. 2005, doi:10.1111/j.1742-4658.2005.04700.x.