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ARTICLE

POTENTIAL ANTIBACTERIAL ACTIVITY OF *Sapindus rarak* DC FRUIT AGAINST ESBL (EXTENDED SPECTRUM BETA LACTAMASE) PRODUCING *Escherichia coli*: IN SILICO AND IN VITRO STUDIES

[*Potensi Aktivitas Antibakteri Buah Sapindus rarak DC Terhadap Escherichia coli Penghasil ESBL (Extended Spectrum Beta Lactamase): Studi In Siliko dan In Vitro*]

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ABSTRACT

The increasing Antimicrobial Resistance (AMR) drives the need of new antibacteria as drug candidate to eradicate resistant bacteria such as ESBL (Extended Spectrum Beta-Lactamase) producing *Escherichia coli*. Extract and fraction of *Sapindus rarak* fruit contains numerous saponin compounds, which exert potential antibacterial effect by binding to the bacterial cell membrane. The purpose of this research is to analyze and evaluate *Sapindus rarak* fruit extract and fraction antibacterial effects through *in silico* and *in vitro* approach. Molecular docking is conducted to identified compounds contained in *Sapindus rarak* fruit against Penicillin Binding Protein (PDB ID: 6NTZ) using Autodock Tools. Antibacterial testing of *Sapindus rarak* fruit extract and fraction is also conducted with disc diffusion and microdilution. *In silico* docking study resulted in potential activity from raraoside A and rarasaponin V, which showed affinity tinggi through their binding energy whilst interacting with the active site of the receptor protein. No inhibition zone was detected on extract and fraction, yet lowest minimum inhibition concentration (MIC) was detected at 2.5×10^4µg/mL on water and n-hexane fraction *Sapindus rarak* fruit ethanol extract. This weak antibacterial effect tends to happen to crude extract than to compound isolates. Hence, *Sapindus rarak* fruit fraction exhibits weak antibacterial effect on ESBL producing *E. coli*, but certain isolates might be potential to be further researched. *Sapindus rarak* fruit isolates such as raraoside A and rarasaponin V is potential to solve antimicrobial resistance by acting as a plant based antibiotic.

Keywords: antimicrobial resistance, *Sapindus rarak* fruit, saponin, *Escherichia coli*, ESBL

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ABSTRAK

Peningkatan resistensi antimikroba mendorong kebutuhan terhadap antibakteri baru sebagai kandidat obat untuk eradikasi bakteri seperti Escherichia coli penghasil ESBL (Extended Spectrum Beta-Lactamase). Ekstrak dan fraksi buah Sapindus rarak mengandung banyak senyawa saponin yang memiliki potensi efek antibakteri dengan mengikat dinding sel bakteri. Tujuan dari penelitian ini adalah untuk menganilisis dan mengevaluasi efek antibakteri ekstrak dan fraksi buah Sapindus rarak melalui pendekatan in silico dan in vitro. Penambatan molekuler dilakukan terhadap senyawa buah Sapindus rarak yang telah teridentifikasi terhadap Penicillin Binding Protein (PDB ID: 6NTZ) dengan menggunakan Autodock Tools. Studi in silico menghasilkan aktivitas potensial dari raraosida A dan rarasaponin V, yang menunjukkan afinitas tinggi melalui energi ikatan selagi berinteraksi dengan sisi aktif protein reseptor. Tidak terdeteksi zona hambat pada ekstrak dan fraksi, namun nilai konsentrasi hambat minimum (KHM) yang ditemukan ditemukan pada $2,5\times10^4$µg/mL pada fraksi air dan n-heksan ekstrak etanol Sapindus rarak fruit. Oleh karena itu, fraksi Sapindus rarak menunjukkan efek antibakteri lemah pada E. coli penghasil ESBSL, namun isolat tertentu mungkin berpotensi untuk diteliti lebih lanjut. Isolat buah Sapindus rarak seperti raraosida A dan raraponin V berpotensi sebagai solusi resistensi antimikroba sebagai antibiotik berbasis tanaman.

***Kata kunci**: resistensi antimikroba, buah S.rarak, saponin, Escherichia coli, ESBL*

INTRODUCTION

Extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* is one of resistant priority pathogen listed by WHO (World Health Organization) which renders beta lactams ineffective (Sunarno *et al.*, 2023; Gach *et al.*, 2024). This bacterium caused highest case of urinary tract infection with resistance against third generation beta lactams such as ceftriaxone (64.7%), ceftazidime (56.4%), and cefotaxime (80.0%) (Sunarno *et al.*, 2023). This problem escalates as carbapenem usage as last line antibiotics causes emergence of carbapenemase producing bacteria (Gach *et al.*, 2024; Kadariswantiningsih *et al.*, 2025). To address this issue, WHO suggested the antibiotic pipeline strategy, aiming to eradicate resistant bacteria with new antimicrobials to counter resistance pattern (WHO, 2023).

Recent researches have shown potential antimicrobial activity from plant derived antimicrobial to combat antimicrobial resistance (Keita *et al*., 2022). Saponin based metabolites has shown antimicrobial activity derived from plants such as *Quillaja saponaria*, *Chenopodium quinoa*, and *Melanthera elliptica* were effective against Gram positive and negative bacteria (Sewlikar and D'Souza, 2017; Tagousop *et al.*, 2018; Dong *et al.*, 2020). Potential source of saponin, especially in Indonesia is *Sapindus rarak* (locally known as "lerak") fruit which is known as natural detergent (Pratiwi and Nurlaeni, 2022). This fruit contains saponins such as rarasaponin, raraoside A, and mukuroziosides IIb (Chung *et al.*, 1997; Asao *et al.*, 2009; Morikawa *et al.*, 2009; Abdallah *et al.*, 2023). To explain this, saponins are known to disrupt bacterial cell wall integrity which leads to cell death (Abushaheen *et al.*, 2020; Abdallah *et al.*, 2023). This shows that *Sapindus rarak* as a novel source of antibacteria against ESBL producing *E. coli*. Furthermore, *Sapindus rarak* has shown to have antibacterial activity against pathogenic *E. coli*, yet specific compound which responsible to the activity was not examined (Puspitaningrum and Silviani, 2013; Silviani and Puspitaningrum, 2015; Murni *et al.*, 2023).

In vitro testing of antibacteria was known as "gold standard" gives reliable result, but this method doesn't show which of the component of plant extract contributes the most antibacterial activity (Khan *et al*., 2019; Alsheikh *et al.*, 2020). Recently developed *in silico* approach has the capacity to determine compound binding energy and mechanism involved through receptor and ligand interaction (Dyas *et al.*, 2023). This method is time and cost efficient while having reliable correlation with *in vivo* results (Kurhekar *et al*., 2019). To combine *in silico* and *in vitro* method could comprehensively determine *Sapindus rarak* antibacterial activity in short amount of time. Furthermore, *in vitro* testing is to be conducted on extract and fraction to give specificity on *Sapindus rarak's*'s antibacterial properties.

This study aims to explore the antibacterial potential of *Sapindus rarak* fruit against ESBL-producing *Escherichia coli* using both laboratory testing and computer-based analysis. Laboratory *in vitro* experiments will assess the effectiveness of the fruit extracts and their specific fractions in inhibiting bacterial growth. In parallel, *in silico* computer simulations will be used to predict how key compounds like rarasaponin, raraoside A, and mukurozioside IIb—interact with bacterial proteins involved in resistance. By combining these two methods, the research seeks to identify which compounds are most active and understand mechanism underlying antibacterial activity which ultimately supporting the development of *Sapindus rarak* as a natural antibacterial agent against drug resistant bacteria.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

All chemicals and reagents used were: ethanol 96%; dimethyl Sulfoxide (DMSO)(EMSURE®ACS, Germany); ethyl acetate; iodine; n-hexane; McFarland standard solution (HiMedia™Laboratories, India); Mueller-Hinton Agar (MHA)(HiMedia™Laboratories, India); Mueller-Hinton Broth (MHB) (HiMedia™Laboratories, India); and ampicillin (Bernofarm, Indonesia); meropenem (Interbat Pharmacetical Industry, Indonesia); safranin; violet crystal; and Sterile water for injection (WFI)(Intralab Ekatama,Indonesia) were used.

Software and Databases

AutoDock Tools 1.5.7 with AutoDock 4.2.6, Discovery Studio 2024, PubChem, and RCSB PDB databases.

Bacterial Strains

E. coli ATCC 25922 (ESBL -) and *E. coli* ESBL (ESBL +) isolates were obtained from Tanjungpura University Pontianak and the National Research and Innovation Agency (BRIN).

Methods

This research employed an experimental approach comprising *in-silico* and *in-vitro* antibacterial testing of *Sapindus rarak* fruit extract and its fractions against *Escherichia coli* ESBL-producing strains. *In silico* study aimed to analyze potential bioactive compounds through molecular docking simulations, while *in vitro* tests such as disc diffusion and microdilution assessed antibacterial efficacy.

Molecular Docking

Docking parameter method validation was performed by redocking 6NTZ's native ligand (MXR (meropenem tautomer)) into its the active site using AutoDock 4.2.6. The Penicillin Binding Protein (PDB ID: 6NTZ) was obtained from the RCSB Protein Data Bank. Water molecules and the native ligand were removed using Discovery Studio. The protein was then processed in AutoDock Tools 1.5.7 by adding polar hydrogens and Gasteiger charges. The parameter used was: grid box size of 40 × 40 × 40 Å with 0.375 Å spacing; Lamarckian Genetic Algorithm (GA) with 100 runs; population size of 150; and other parameters as default. Root Mean Square Deviation (RMSD) values below 2 Å considered valid (Morris *et al.*, 2010; Amrulloh *et al.*, 2023; Dyas *et al*., 2023).

After the validation, testing ligands were used to analyze each binding energy. Ligands including raraoside A, rarasaponin I to VI, and mukurozioside IIb were downloaded from the PubChem database. The structures were optimized in AutoDock Tools with added charges and rotatable bonds for torsion. Each ligand was docked individually into the active site of 6NTZ protein using the validated parameters. The docking results were analyzed based on binding affinity (Kcal/mol) to determine the most stable conformation (Amrulloh *et al.*, 2023). Docked ligand-protein complexes were visualized using Discovery Studio. Interactions such as hydrogen bonds,

hydrophobic contacts, and involved amino acid residues were identified. The interactions were compared to native ligand.

Preparation of Extract and Fractions

Sapindus rarak fruits were obtained from Situbondo region in East Jawa Province. The sample was determined by Bandung Institute of Technology through issued document of "Surat Keputusan No. 1471/IT1.C11.2/TA.00/2022" that the sample was indeed *Sapindus rarak* DC from Sapindaceae family. The fruit was then dried at 40°C, ground, and extracted using Soxhlet extraction with ethanol 96%.(Murni *et al.*, 2023; Fitria *et al.*, 2024) The extract was concentrated using a rotary evaporator then dried in an oven at 50°C until all of the solvent had evaporated. Fractionation was performed using a separating funnel with n-hexane and ethyl acetate respectively after diluted with water (1:3) with two repetitions.(Manalu *et al*., 2022)

Disc Diffusion Assay

The antibacterial activity of the extract and its fractions was evaluated using the disc diffusion method on Mueller-Hinton Agar (MHA). Bacterial suspensions of non-producing ESBL *E. coli* (ESBL -) and ESBL producing *E. coli* (ESBL +) were adjusted to 0.5 McFarland standard (1–2 × 10^8 CFU (Colony Forming Unit)/mL) then spread evenly on the media. Sterile 6 mm paper discs were soaked in extract or fraction solutions at concentrations of 100% (w/v) in 10% DMSO, then placed on MHA plates. Meropenem and penicillin solution were used as positive control standard following the susceptibility testing from CLSI M02-A11 (Clinical and Laboratory Standard Institute).(CLSI, 2012) Negative control used was 10% DMSO. Plates were incubated at 37°C for 18–24 hours, and inhibition zones were measured in millimeters by calipers.

Microdilution assay

Minimum Inhibitory Concentration (MIC) was determined using a two-fold serial dilution method in 96-well microplates with Mueller-Hinton Broth (MHB). Extracts and fractions were diluted in media, and bacterial inoculum was added to reach a final concentration of 5 × 10^5 CFU/mL as instructed in CLSI M07-A9.(CLSI, 2012b). Each well contained 100 µL of mixture between media and sample then 10 µL of inoculum was added. Controls included media only, sample only, and bacteria without treatment. Plates were incubated at 35 ± 2°C for 16–20 hours. MIC was determined at the lowest concentration of row with no visible turbidity by unassisted eye.

RESULTS

Molecular Docking

Table 1. Validation of molecular docking parameter (*Validasi parameter penambatan molekuler*)

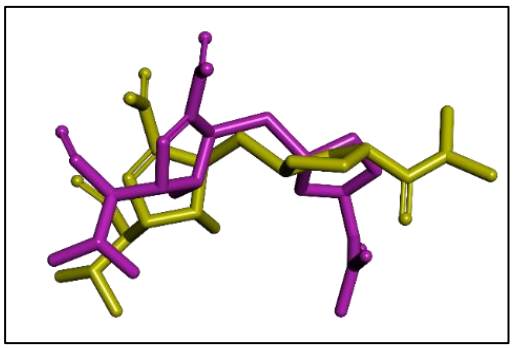
Protein	**Protein PDB ID**	**Native Ligand** (*Ligan Asal*)	**RMSD (Å)**	**Visualization** (*Visualisasi*)
Penicillin Binding Protein (PBP)	6NTZ	MXR (PubChem CID: 137349769)	1.94	 Native ligand (magenta) and redocked ligand (yellow)

Table 2. Binding energy of *Sapindus rarak* compounds with Penicillin Binding Protein (PBP) (*Energi ikatan antara senyawa Sapindus rarak dengan Penicillin Binding Protein (PBP)*)

Ligand (*Ligan*)	**Binding Energy (Kcal/mol)** (*Energi Ikatan (Kkal/mol)*)	**Number of Hydrogen Bond** (*Jumlah Ikatan Hidrogen*)	**Amino Acid Residue** (*Residu Asam Amino*)	
Raraoside A	-7.31	9	HIS 245	LYS 242
			SER 115	THR 243
			ASN 141	LEU 225
			SER 116	ARG 227
			SER 139	GLN 224
			SER 73	
Rarasaponin I	-7.18	4	HIS 245	SER 116
			SER 139	ARG 277
Rarasaponin II	-7.14	7	HIS 245	ARG 227
			LEU 225	SER 116
Rarasaponin III	-7.54	4	LEU 225	ASN 141
			ARG 227	
Rarasaponin IV	-7.22	5	HIS 245	GLN 224
			ASN 141	SER 116
Rarasaponin V	-6.90	7	SER 73	LEU 225
			HIS 245	ARG 227
			SER 116	
Rarasaponin VI	-4.75	3	ARG 227	
			SER 73	
			SER 139	

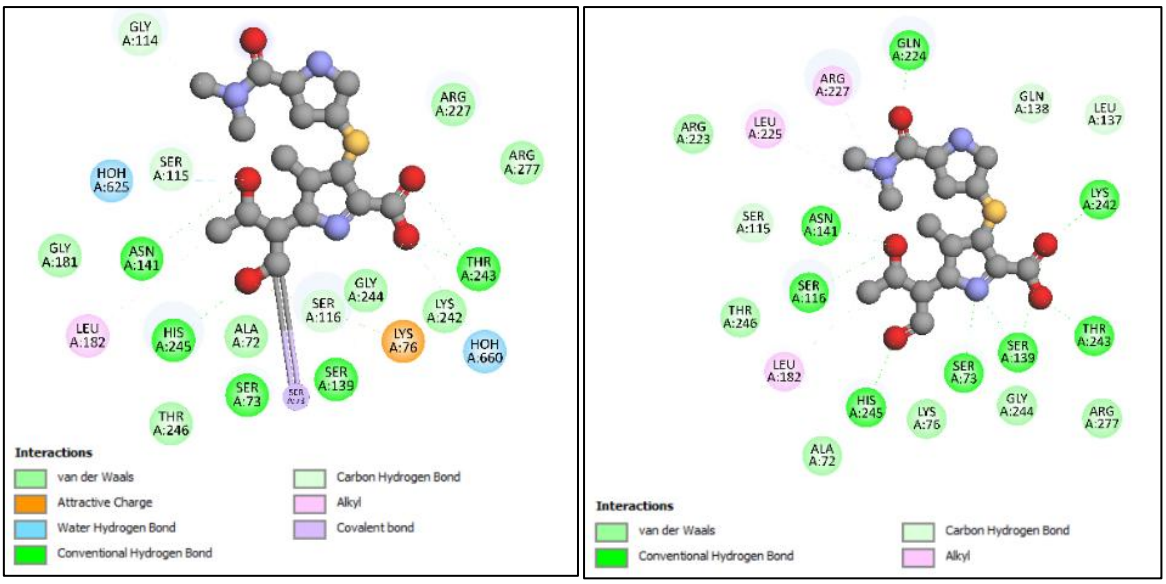


Figure 1. Interaction Between Ligand and Amino Acid Residue Visualization in 2 dimensions: raraoside A (a) and rarasaponin V (b) (*Visualisasi interaksi antara ligan dan asam amino dalam dua dimensi : raraosida A(a), rarasaponin V (b), rarasaponin VI (c)*).

Extraction and Fractionation

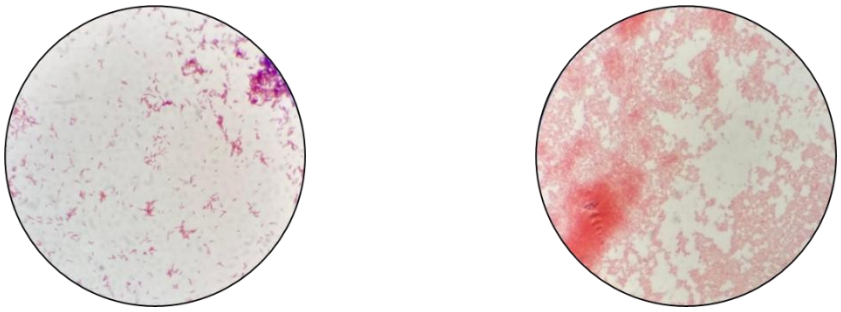
Table 3. *Sapindus rarak* fruit extraction yield. (*Rendemen ekstraksi buah Sapindus rarak*)

Dried Fruit Weight (g) (*Berat Buah Kering (g)*)	**Viscous Extract Weight (g)** (*Berat Ekstrak Kental (g)*)	**Yield (%w/w)** *Rendemen* (*%b/b)*)
200	90.57	45.28

Table 4. *Sapindus rarak* fruit extract fractionation yield. (*Rendemen fraksinasi ekstrak buah Sapindus rarak*)

Fraction (*Fraksi*)	**Yield (%w/w)** (*Rendemen (%b/b)*)
Water (*air*)	39.22%
Ethyl Acetate (*Etil Asetat*)	2.24%
N-Hexane (*N-Heksan*)	10.64%

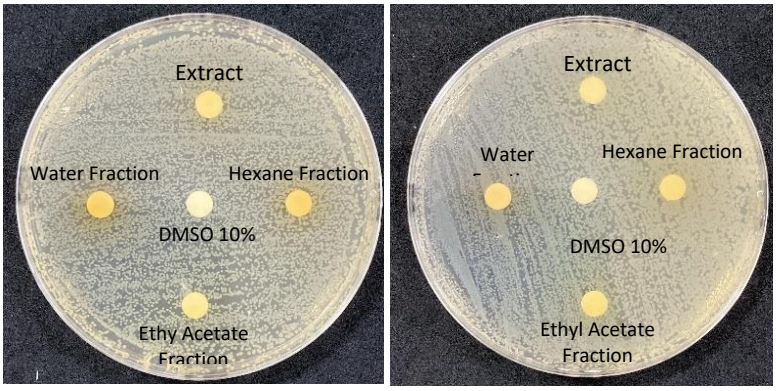
Gram Staining



(a) (b)

Figure 2. Gram staining of *E. coli*: ESBL **-** (a) and ESBL + (b) (*Pewarnaan Gram E.coli : ESBL – (a) dan ESBL + (b)*).

Disc Diffusion Assay



(a) (b)

Figure 3. Disc diffusion assay result of *Sapindus rarak* extract and fraction against *E. coli*: ESBL – (a) and ESBL + (b) (*Hasil uji difusi cakram ekstrak dan fraksi buah Sapindus rarak terhadap E.coli ESBL – (a) dan ESBL + (b)*).

Table 5. Zone of inhibition of *Sapindus rarak* extract and fraction against *E. coli*: ESBL – (a) and ESBL + (b) (*Zona hambat ekstrak dan fraksi Sapindus rarak terhadap E.coli: ESBL-(a) dan ESBL + (b)*).

Groups (*Kelompok*)	**Zone of Inhibition (mm)±SD** (*Zona Hambat (mm)*±SD)	
	***E. coli* ESBL (-)**	***E. coli* ESBL (+)**
Extract (*Ekstrak*)	0.00±0.00	0.00±0.00
Water Fraction (*Fraksi Air*)	0.00±0.00	0.00±0.00
Ethyl Acetate Fraction (*Fraksi Etil Asetat*)	0.00±0.00	0.00±0.00
N-Hexane Fraction (*Fraksi N-Heksan*)	0.00±0.00	0.00±0.00
Meropenem (Positive Control) (*Meropenem (Kontrol Positif)*)	29.09 ± 0.69	35.09 ± 0.69
Ampicillin (Positive Control) (*Ampisilin (Kontrol Negatif)*)	31.53 ± 0.85	5.52 ± 2.29
DMSO 10% (Negative Control) (*DMSO 10% (Kontrol Negatif)*)	0.00±0.00	0.00±0.00

Microdilution Assay

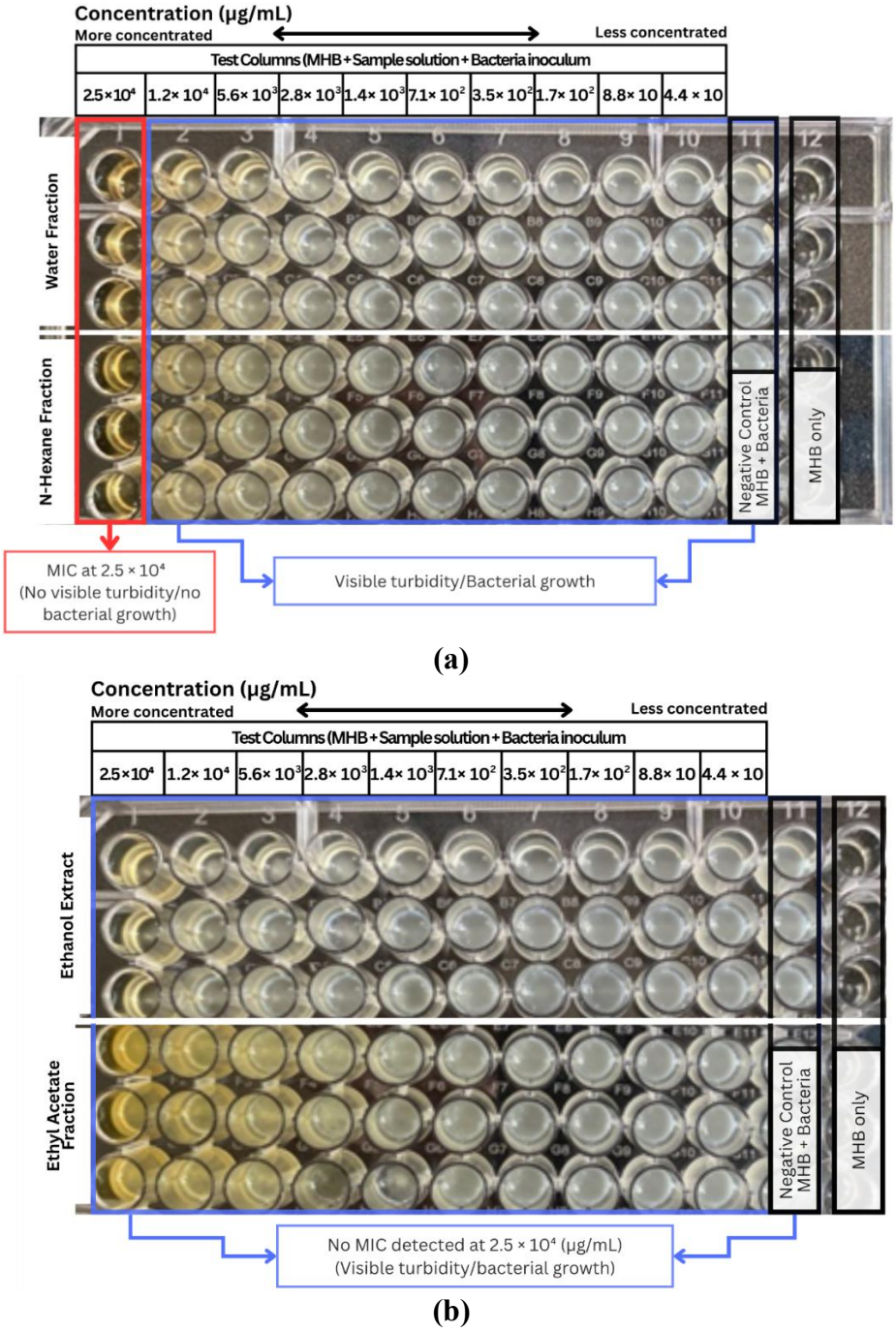


Figure 4. Microdilution Assay Result Illustration of *Sapindus rarak* fruit extract and fraction against *E. coli* ESBL +: water and n-hexane fraction (a); ethanol extract and ethyl acetate fraction (b) (*Ilustrasi hasil uji mikrodilusi ekstrak dan fraksi buah Sapindus rarak terhadap E.coli ESBL +: fraksi air dan n-heksan (a); ekstrak etanol dan fraksi etil asetat (b)*).

Table 6. Microdilution assay of *Sapindus rarak*'s extract and fraction against *E. coli*: ESBL – (a) and ESBL + (b) (*Zona hambat ekstrak dan fraksi Sapindus rarak terhadap E.coli: ESBL-(a) dan ESBL + (b)*).

Groups (*Kelompok*)	**Minimum Inhibition Concentration (µg/mL)** (*Konsentrasi Hambat Minimum (µg/mL)*)	
	***E. coli* ESBL (—)**	***E. coli* ESBL (+)**
Extract (*Ekstrak*)	$>2{,}27\times10^{4}$	$>2{,}27\times10^{4}$
Water Fraction (*Fraksi Air*)	$2{,}27\times10^{4}$	$2{,}27\times10^{4}$
Ethyl Acetate Fraction (*Fraksi Etil Asetat*)	$>2{,}27\times10^{4}$	$>2{,}27\times10^{4}$
N-Hexane Fraction (*Fraksi N-Heksan*)	$2{,}27\times10^{4}$	$2{,}27\times10^{4}$
Meropenem (Positive Control) (*Meropenem (Kontrol Positif)*)	$5{,}68\times10^{-2}$	$5{,}68\times10^{-2}$
Ampicillin (Positive Control) (*Ampisilin (Kontrol Negatif)*)	$1{,}45\times10$	$>1{,}86\times10^{3}$

DISCUSSION

Molecular Docking

Native ligand redocking into the Penicillin Binding Protein (PDB ID: 6NTZ) was important to validate the docking protocol. The RMSD value was 1.94 Å which is < 2.0 Å, affirming the docking parameters were appropriate and the method was reliable for further ligand docking. This also ensures that the binding pose predicted by AutoDock is consistent with the crystallized ligand structure (Amrulloh *et al.*, 2023; Dyas *et al*., 2023).

All compounds showed negative binding energies except mukurozioside IIb. This is explained because it has the biggest molecular weight of giving bigger interaction to the incative site of the protein (Terefe and Ghosh, 2022). Whilst other ligand that were docked are further analyzed for binding energy lower than −6 kcal/mol, suggesting potential biological activity for hydrolase enzyme protein such as PBP as seen in Table 1 (Shityakov and Förster, 2014; Ivanova and Karelson, 2022). The lower the binding energy indicates that the interaction between the ligand and protein is more stable and rational. However, strong interaction doesn't always equal to strong pharmacological activity as the interaction between the amino acid residue and ligand need to be analyzed further as it may not specifically interacts with the protein's binding site (Taghizadeh *et al.*, 2022).

Raraosida A and rarasaponin V shows great potential as the both compound not only interacted with amino acid residue in PBP's active site, they also exhibit binding energy lower than −6 kcal/mol. SER 73 amino acid residue plays important part in the antibacterial mechanism of the native ligand (meropenem). This amino acid specifically binds to meropenem to form strong covalent bond, inhibiting the bacteria's growth (Caveney *et al.*, 2019). Other residues related to the active site of the protein was also interacting with these ligand such as SER 139, ASN 141, dan HIS 245 with varying affinities. These findings support the hypothesis that saponin compounds from *Sapindus rarak* have the potential to inhibit PBP, which is the target of β-lactam antibiotics. The similar interaction profile of raraosida A and rarasaponin V to the native ligand strengthens its potential as a marker compound.

Extraction and Fractionation

Extraction of *Sapindus rarak* fruit by soxhlet extraction with ethanol yielded of 45.28% (w/w) is considered numerous as it is more than 10%. This percentage reflects the abundance of extractable compounds, especially saponin as polar compounds which is dominant in this plant. The solvent used plays important part in this method. Ethanol is known to be semipolar solvent with neutral, safe, and nontoxic nature (Subaryanti *et al*., 2022). Fractionation conducted on the ethanol extract producing three fractions shows that the water fraction had the highest yield at 39.22%, followed by ethyl acetate and n-hexane. This shows that the majority of active constituents in the extract are polar, aligning with the solubility profile of saponins and supporting their presence as major bioactive components in *Sapindus rarak*.

Gram Staining

Gram staining is a quick and simple method is used to observe the identity of the bacteria's identity through its stain, morphology, and arrangement (Paray *et al.*, 2023). This method confirmed that both *E. coli* ESBL **-** and *E. coli* ESBL + are negative Gram bacteria. Microscopically, the cells appeared pink with rod-shaped (bacil) morphology after counterstained with safranin. This result is consistent with the known characteristics of *E. coli* (Tasyakusuma *et al.*, 2023). This validates that the bacterial strains used in the study were appropriate for antibacterial activity evaluation.

Disc Diffusion Assay

No inhibition zones were observed for the ethanolic extract nor its fractions at concentrations of 100% against both *E. coli* strain as shown in Figure 3. In contrast, the meropenem and penicillin produced measurable inhibition zones, confirming that the bacterial strains were susceptible and the method functioned properly as seen in Table 3. The negative control (DMSO 10%) did not show any inhibition, showing no interference from the solvent used.

The absence of inhibition zones could be explained by some suspected mechanism. First, the compound might not diffuse efficiently through the agar as a known interaction between saponin compounds and cellulose in the diffusion disc which contains glycosidic components is capable of forming hydrogen bonds with cellulose of the filter paper discs, thus failing the test (Bundjaja *et al.*, 2020). Second, the saponins it self, might be degraded by some microbes causing it to hydrolyse the saponin producing sugar and aglycone. Third, certain low concentration of saponin increased bacterial cell permeability to allow more glucose from the media used to be absorbed by the bacteria, enhancing its growth (Sen *et al.*, 2018). Fourth, a study conducted on negative and positive Gram bacteria, shows that saponin is more effective on the positive one. This happened because negative Gram bacteria have double layered structure causing it be more resistant to be more resistant to saponin than the single layered positive Gram bacteria (Zhao *et al.*, 2020). Despite the problem faced in the disc diffusion method, broth media would address the antibacterial activity as discussed in the next part.

Microdilution Assay

The microdilution method was used to determine the minimum inhibitory concentration (MIC) of *Sapindus rarak* extract and its fractions against *E. coli* ESBL + Among all samples tested, only the n-hexane and water fractions showed inhibitory activity, each with an MIC value of 2.5×10^4 µg/mL on both strains tested as seen in Figure 4. This shows that the antibacterial effect of *Sapindus rarak* is not affected by the presence of beta lactamase enzyme, proves the assumption deduced. The slight activity observed in the water and n-hexane fractions may be attributed to more stable dispersion of their active constituents. A study claimed that any MIC value above 1×10^3 µg/mL is considered inactive (Holetz *et al.*, 2002). However, the overall high MIC values suggest that further purification of the active compounds would be necessary to enhance antibacterial potency as there would be no interference with other constituents. A study shows that isolated saponin compounds from *Melanthera elliptica* exhibits an MIC at 16-32 µg/mL, significantly reduced MIC

from the crude extract alone (Tagousop *et al*., 2018). Another study on saponin isolate from *Camellia sinensis* exhibits higher MIC of one fraction has lower MIC than other fraction and its crude saponin mixture (Khan *et al.*, 2018). Both of these research invovles further fractionation and purification such as defatting, complex extraction, and fractionation rather than crude liquid fractionation. Specific compound isolation such as raraoside A and rarasaponin V to be tested directly can be used to correlate specifically to the molecular docking conducted respective to the compound isolated.

CONCLUSION

This study evaluated the antibacterial potential of *Sapindus rarak* fruit ethanolic extract and its fractions against ESBL-producing *E. coli* using *in silico* and *in vitro* approaches. Molecular docking showed that saponin compounds such as raraosida A and rarasaponin V had affinity through binding energy to Penicillin Binding Protein, with binding energies -7.31 Kcal/mol and -6.90 Kcal/mol which are below −6 kcal/mol and key interactions at active site residues. These findings suggest a potential mechanism of antibacterial action through interference with bacterial cell wall synthesis. However, *in vitro* antibacterial testing did not show inhibition zones in the disc diffusion assay. This was likely due to the interaction between saponins and cellulose in the filter discs, which inhibited diffusion into the agar. Microdilution testing revealed weak antibacterial activity, with the lowest MIC observed at 2.5×10^4 µg/mL in the water and n-hexane fractions. Overall, while *Sapindus rarak* extract contains compounds with predicted antibacterial properties *in silico*, its *in vitro* efficacy against ESBL-producing *E. coli* was minimal. Further research is needed to isolate and purify the active compounds to improve antibacterial effect.

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AUTHOR CONTRIBUTIONS

FN: creating research concept, final revision of manuscript; HK: creating research concept, drafting research article revising article draft; HI: final revision of manuscript; IF: collecting research data and data analysis; SN: drafting research article revising article draft; A: collecting research data, drafting research article.

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