Sari et al | Article 9686
J Pure Appl Microbiol. 2024;18(4):2288-2303. doi: 10.22207/JPAM.18.4.01
Received: 01 July 2024 | Accepted: 09 September 2024
Published Online: 05 October 2024

P-ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Phytoconstituent and *In silico* Screening of Active Compounds from Red Ginger (*Zingiber officinale var.* rubrum Theilade) Rhizome and Avocado (*Persea americana* Mill.) Leaves Extracts as Novel Inhibitors of MRSA

Ni Kadek Yunita Sari[1,2]*, Putu Angga Wiradana[2], Anak Agung Ayu Putri Permatasari[2], I Gede Widhiantara[2], Novaria Sari Dewi Panjaitan[3], Arif Nur Muhammad Ansori[4], Komang Januartha Putra Pinatih[5], I Made Jawi[6] and Ketut Suastika[7]

[1]Doctoral Study Program of Medical Science, Faculty of Medicine, Universitas Udayana (UNUD), Denpasar City, Bali Province - 80232, Indonesia.
[2]Research Group of Biological Health, Study Program of Biology, Faculty of Health and Science, Universitas Dhyana Pura, Jalan Raya Padangluwih, Dalung, North Kuta, Badung Regency, Bali Province - 80351, Indonesia.
[3]Center for Biomedical Research Organization for Health, National Research and Innovation Agency (BRIN), Cibinong – Bogor, Indonesia.
[4]Postgraduate School, Universitas Airlangga, Kampus B, Jalan Airlangga, Surabaya, East Java (60286), Indonesia.
[5]Microbiology Department, Faculty of Medicine, Universitas Udayana, Jalan P.B. Sudirman, Denpasar, Bali Province - 80232, Indonesia.
[6]Department of Pharmacology, Faculty of Medicine, Universitas Udayana, Jalan P.B. Sudirman, Dangin Puri Klod, Denpasar City, Bali Province - 80232, Indonesia.
[7]Department of Internal Medicine, Faculty of Medicine, Universitas Udayana, Jalan P.B. Sudirman, Dangin Puri Klod, Denpasar City, Bali Province - 80232, Indonesia.

***Correspondence:** yunitasari@undhirabali.ac.id

Citation: Sari NKY, Wiradana PA, Permatasari AAAP, et al. Phytoconstituent and In silico Screening of Active Compounds from Red Ginger (*Zingiber officinale var.* rubrum Theilade) Rhizome and Avocado (*Persea americana* Mill.) Leaves Extracts as Novel Inhibitors of MRSA. *J Pure Appl Microbiol.* 2024;18(4):2288-2303. doi: 10.22207/JPAM.18.4.01

Abstract

Red ginger rhizome (*Zingiber officinale* var. Rubrum) and avocado leaves (*Persea americana* Mill.) are empirically known as one of the medicinal plants used in Taro Village, Gianyar Regency, Bali which have great potential in treating infectious diseases caused by antibiotic resistance, such as MRSA. This study aims to analyze the phytoconstituents and anti-MRSA potential contained in red ginger rhizome and avocado leaves extracts by assessing their inhibitory effects on three proteins related to MRSA resistance and virulence (PBAP2a, transglycosylase, and glycosyltransferase). Phytoconstituents of avocado leaf and red ginger extracts were analyzed using GC-MS. Molecular docking was performed in silico to determine the similarity properties of predicted drugs, bioactivity, toxicity, identification of active sites and validation of protein structures, and docking simulations were performed between compounds found in the extract and their target proteins. Phytoconstituent analysis revealed that avocado leaves and red ginger extracts as a whole have 43 types of compounds and 10 bioactive compounds each with beneficial drug-like properties. The compound 6,11-hexadecadien-1-ol from avocado leaves extracts was predicted to have hepatotoxic properties. There were at least 3 compounds, namely beta-bisabolene from avocado leaves extract, zingiberenol and gamma-curcumene from red ginger rhizome extract, showing the lowest *binding affinity* for the target protein. Red ginger rhizome and avocado leaves extracts showed valuable potential as anti-MRSA agents through the mechanism of inhibition of three resistance-related proteins, as predicted by *in silico* analysis.

Keywords: Antimicrobial Resistance, Penicillin-binding Protein-2a, Transglycosylase, Glycosyltransferase, MRSA

INTRODUCTION

Antimicrobial resistance is one of the most significant public health issues facing the globe today (AMR). Antimicrobial resistance (AMR) is expected to cause significant clinical losses, severe economic consequences, and the loss of 10 million lives annually by 2050, according to the highly cited review on antimicrobial resistance.[1] According to a recent systematic investigation, AMR bacteria were responsible for 4.95 million deaths in 2019, and 1.27 million of them were directly related to AMR.[2] Based on the results of the Global Burden of Disease, Injuries, and Risk Factors (GBD) research, AMR was reported as the third most common cause of death after ischemic heart disease and stroke.[3] The stages of the process of bacterial resistance to antibiotics include; (i) genetic mutations in bacteria; (ii) overuse of broad-spectrum antibiotics; and (iii) bacteria form a biofilm which functions as a protector so that the bacteria are resistant to antibacterials.[4-6]

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the second most common cause of antibiotic-resistant bacterial infections in many European countries,[7] America,[8] Africa,[9] Australia[7] to Asian countries[10] and including in the Southeast Asian region such as Indonesia.[11-13] The prevalence of MRSA infection in the world varies from 1% to 50% in each country. Asian countries have the highest prevalence of MRSA in the world, with about 50% of these bacteria causing circulatory infections.[4] Research conducted in the Asia-Pacific region shows that the population with MRSA carriers reaches 23.5%.[15] The prevalence of MRSA infection in Indonesia was reported around 0.3%-51% with the highest prevalence found in Aceh (50%)[16] and Jakarta reaching 47%.[11]

MRSA can survive and develop well after being captured by phagocytic cells.[17] The invading cells actually protect the bacteria from the bactericidal action of commercial antibiotics, thus causing resistance to infection. The current problem is that the treatment of intracellular infections requires long-term and intensive administration of antibiotics, however, most antibiotics are reported to fail to kill intracellular bacteria due to low intracellular accumulation, short retention, or reduced antibacterial action in cells. Interestingly, MRSA is also capable of producing a series of virulence factors that trigger infection, such as the penicillin-binding protein 2a (PBP2a) receptor, transglycosylase and glycosyltransferase. These receptors are known to have an important role as an inhibitor of the

activity of β-lactam antibiotics in the resistance mechanism of MRSA bacteria.[18]

Natural antibacterial drugs have emerged as a replacement solution for conventional antibiotics in the treatment of drug-resistant intracellular bacteria. Natural bactericides have the advantage of being easily accessible and having a wider range of use than standard antibiotics.[19,20] It should be noted that natural antibacterials exhibit bactericidal effects through multiple pathways, making the development of resistance an interesting challenge to be further studied through in silico studies on these virulence factor-associated proteins in MRSA. Gingerol, is an important component of red ginger rhizome extract which is popular as a natural antibacterial compound. Gingerol has high biocompatibility as a "green" bactericide and its antibacterial activity had been documented.[21] Several previous studies stated that gingerol specifically inhibited the expression of several pore-forming toxins which are important components of bacterial virulence factors. However, the single antibacterial activity exerted by red ginger rhizome extracts is lower than other popular antibiotics, thereby reducing its practical use and efficacy.

The combination of traditional Balinese medicine (Usaddha) to prevent and treat infectious diseases has recently attracted increasing attention. Therapy using a combination of natural ingredients was known as polyherbal therapy which had a tendency to produce synergistic therapeutic effects,[22] which was caused by the action between the active ingredients contained in each ingredient.[23] The ingredients stated refers to natural ingredients that have been used traditionally by people in Indonesia, especially in Taro Village, Gianyar Regency, Bali Province, whom for generations have used red ginger infusion combined with boiled avocado leaves which can empirically be effective in providing a therapeutic effect. The extracts of avocado leaves contain active antibacterial compounds such as alkaloids, saponins and flavonoids.[24]

However, there are still no reports that reveal the phytoconstituent components of red ginger and avocado leaves and the effectiveness of their active compounds in inhibiting proteins that produce virulence factors in MRSA. Molecular docking using an in silico approach is a computational method used for the discovery of new drug candidates.[25] This makes it possible to discover and identify key compounds with therapeutic potential, namely evaluation of effectiveness, prediction of molecular interactions, and drug toxicity.[26] Some *in silico* studies have reported the effectiveness of certain traditional medicines, such as *Stachytarpheta jamaicensis* which could be found in Indonesia, as traditional plants with antibacterial active compounds. Based on previous report, docking *in silico* using Autodock Vina integrated with PyRx 8.0 showed that *S. jamaicensis*, a wild plant from the Verbenanceae, has the best *binding affinity* with luteolin-G1mS complex. Therefore, in this study, the extracts of red ginger rhizome and avocado leaves were used to screen their phytoconstituent composition using GC-MS and several phytochemicals were selected for *in silico* screening and evaluated for their interactions on the penicillin-binding protein 2a (PBAP2a), transglycosylase and glycosyltransferase receptors in MRSA. This research is very useful for revealing new phytochemicals from local plants that can play a role in the development of natural antibacterials through inhibitor mechanisms.

MATERIALS AND METHODS

Plant sample extraction and phytoconstituent profiling

The red ginger and avocado leaves used in this research came from the Satya Kencana Banjar Tebuana Farmers Group Garden, Taro Village, Tegalalang District, Gianyar Regency, Bali Province. The voucher specimens were preserved in the "Eka Karya" Bali-BRIN Botanical Garden Characteristics Laboratory (accession no: ELSA 35877 and ELSA 35901). Fresh red ginger rhizomes and avocado leaves were washed with clean water to remove foreign contaminants or organic matter. The samples were dried at room temperature to remove water before being dried for 24 hours in a 50°C oven. To obtain powder preparations, the dried samples (simplisia) were pulverized using a grinder and sieved with a 20-mesh sieve. The extraction process was carried out using a maceration method using ethanol 96% in a ratio of 1:10 w/v (200 grams of simplicia powder with 2000

mL of solvent) for 3 × 24 hours. It was evaporated using a vacuum rotary evaporator until a thick extract was produced,[27] which was then combined.

The phytoconstituent profile of red ginger rhizome and avocado leaves extracts was evaluated using GC-MS (QP 2010, Shimadzu). The bioactive compounds contained in the extract were identified by comparing the retention time and patterns of mass peak with reference to the database of the National Institute of Standards and Technology (NIST) and the Wiley Registry of Mass Spectral Data, New York.[28] Compounds were identified by comparing sample MS spectra with the WILEY229 Library and the NIST62 database.[29,30]

In Silico analysis

Ligand preparation

The chemical compounds used in this research came from the results of chromatography with ethanol solvent on avocado leaves consisting of benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS), zingiberene- (CAS), E,E-alpha-farnese, beta-bisabolene- (CAS), beta-sesquiphellandrene- (CAS), neophytadiene, tetradecanoic acid, ethyl ester- (CAS), 6,11-hexadecadien-1-ol, 9,12-octadecadienoic acid, methyl ester, (E,E)- (CAS), and ethyl oleate. Meanwhile, red ginger rhizome extracts consist of octanal (CAS), endo-borneol, decanal-(CAS), 2,6-octadienal, 3,7-dimethyl-, (Z)-, geraniol, gamma-curcumene, widdrene, zingiberenol, d-nerolidol, and trans-6-shogaol. Ligand sample preparation was carried out through the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to obtain several information such as CID, compound link, and 3D structure with structure data format (sdf) files.[31]

Protein preparation

The targets in this research are several proteins from MRSA consisting of penicillin-binding protein 2a (PBP2a) (RCSB ID: 5M18), transglycosylase (RCSB ID: 3VMT), and glycosyltransferase (RCSB: 6FTB). PBP2a in MRSA has an important role as an inhibitor of β-lactam antibiotic activity in the resistance mechanism. The activity of transglycosylase and glycosyltransferase enzymes plays a role in cell wall synthesis in MRSA, both of which have a relationship in the resistance mechanism, which triggers bacteria to adapt to various environments including antibiotics.[32] The 3D structure of each target was obtained from RCSB PDB (https://www.rcsb.org/) with *pdb* files.

Drug-likeness assay

The similarity of the activity of the query compound with the drug molecule is predicted via the SCFBio server (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) using the Lipinski Rule of Five's method. These rules refer to physicochemical parameters consisting of molecular mass, lipophilicity, donor-acceptor hydrogen bonds, and molar refractivity. Compounds with positive prediction results are categorized as drug-like molecule.[33]

Prediction of bioactivity and toxicity probabilities

The bioactivity test in this study refers to the probability of being antibacterial, the test was carried out via the PASS Online server (http://www.pharmaexpert.ru/passonline/). This prediction refers to an activation probability $Pa \geq 0.3$ to trigger the emergence of antibacterial activity of the query compound and the *Pa* value must be greater than the inhibition probability (*Pi*).[34] Toxicity predictions for compounds with antibacterial activity values, namely $Pa \geq 0.3$, are carried out via the ProTox-II server (http://tox.charite.de/protox_II/), the toxicity prediction results obtained are the possible carcinogenicity, hepatoxicity and LD_{50} values of the query compounds.[35]

Molecular docking simulation

Molecular docking simulation Ligands in *sdf* format were minimized for increased structural flexibility and conversion of sdf files into protein databank format (PDB) via OpenBabel v2.3.2 software. The energy minimization process is included in the preparation stage for molecular docking simulations with specific targets. Sterilization of target proteins was carried out in this study using PyMOL v.2.5.2 software (Schrodinger, Inc., USA) with an academic license. Sterilization of 3D structures refers to the removal of water molecules on the target for preparation and optimization of molecular docking. Docking analysis aims to identify the inhibitory activity of the ligand on its target. This refers to the binding affinity value. The increasingly negative binding affinity value triggers an increase in the binding

Table 1. Phytoconstituents of red ginger rhizome and avocado leaf extracts were identified by GC-MS analysis

No.	Chemical compound	Retention Time	Peak Area (%)		Formula
			Red Ginger	Avocado leave	
1	Octanal (CAS)	6.120	2.01	-	$C_8H_{16}O$
		6.111	-	-	
2	endo-Borneol	9.765	1.19	-	$C_{10}H_{18}O$
3	Decanal (CAS)	10.441	4.51	-	$C_{10}H_{20}O$
4	2,6-Octadienal, 3,7-dimethyl-, (Z)-	11.436	1.26	-	C10H16O
5	GERANIOL	11.958	2.41	-	$C_{10}H_{18}O$
6	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate (CAS)	15.147	1.85	-	$C_{12}H_{20}O_2$
7	gamma-curcumene	17.790	2.10	-	$C_{15}H_{24}$
		17.775			
8	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	17.971	6.67	3.38	$C_{15}H_{22}$
9	trans-Caryophyllene	18.390	13.75	-	$C_{15}H_{24}$
10	Thujopsene	18.594	8.46	-	$C_{15}H_{24}$
11	alpha-Himachalene	18.668	3.28	-	$C_{15}H_{24}$
12	(+)-Aromadendrene	19.112	10.84	-	$C_{15}H_{24}$
13	Elemol	19.841	0.63	-	$C_{15}H_{26}O$
14	d-Nerolidol	20.751	1.54	-	$C_{15}H_{26}O$
15	zingiberenol	21.345	1.15	-	$C_{15}H_{26}O$
16	1,2-diethoxy-4-ethylbenzene	22.887	20.93	-	$C_{12}H_{18}O_2$
17	alpha-Bisabolol	23.263	1.47	-	$C_{15}H_{26}O$
18	6,10-Dodecadien-1-yn-3-ol, 3,7, 11-trimethyl- (CAS)	23.480	0.99	-	$C_{15}H_{24}O$
19	Campherenone	26.153	1.32	-	$C_{15}H_{24}O$
20	9,10-Dimethyltricyclo[4.2.1.1 (2,5)]decane-9,10-diol	27.525	1.10	-	$C_{12}H_{20}O_2$
21	Ethyl myristate	29.444	2.34	-	$C_{16}H_{32}O_2$
22	Oleic acid	32.839	3.54	-	$C_{20}H_{38}O_2$
23	(E)-4-(2',6',6'-Trimethyl-1',2'-epoxycyclohexyl)-3-penten-2-one	34.356	1.82	-	$C_{14}H_{22}O_2$
24	Shogaol	35.669	2.77	-	$C_{17}H_{24}O_3$
24	Zingiberene (CAS)	18.156	-	3.33	$C_{15}H_{24}$
26	Alpha-Faresenne	18.405	-	1.03	$C_{15}H_{24}$
27	beta-Bisabolene (CAS)	18.487	-	1.66	$C_{15}H_{24}$
28	beta-Sesquiphellandrene (CAS)	18.922	-	2.67	$C_{15}H_{24}$
29	Neophytadiene	26.139	-	0.54	$C_{20}H_{38}$
30	Tetradecanoic acid, ethyl ester (CAS)	29.464	-	29.12	$C_{16}H_{32}O_2$
31	6,11-Hexadecadien-1-ol	31.096	-	3.12	$C_{16}H_{30}O$
32	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	32.727	-	3.84	$C_{19}H_{34}O_2$
33	Ethyl Oleate	32.864	-	36.28	$C_{20}H_{38}O_2$
34	Dicyclohexyl-4,4'-diol	33.102	-	0.49	$C_{12}H_{22}O_2$
35	Heptadecanoic acid, ethyl ester (CAS)	33.303	-	4.27	$C_{19}H_{38}O_2$
36	2,5-Furandione, 3-(dodecenyl)dihydro-	34.372	-	1.26	$C_{16}H_{26}O_2$
37	Hexadecadienoic acid, methyl ester (CAS)	34.500	-	1.22	$C_{17}H_{30}O_2$
38	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	35.240	-	1.78	$C_{35}H_{68}O_5$
39	Hexadecanoic acid, ethyl ester (CAS)	36.850	-	0.90	$C_{18}H_{36}O_2$
40	D-Mannitol	36.885	-	0.55	$C_{28}H_{58}O_{12}$
41	cis-9-Hexadecenal	37.559	-	0.65	$C_{16}H_{30}O$
42	13-Octadecenal, (Z)-	38.319	-	2.79	$C_{18}H_{34}O$
43	9-Eicosynee	39.369	-	1.09	$C_{20}H_{38}$

Table 2. Ligand samples of red ginger rhizome and avocado leaves accessed from the PubChem database

Sample name	Compounds	PubChem CID	Link
Avocado leaves	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)	577053	https://pubchem.ncbi.nlm.nih.gov/compound/577053
	Zingiberene (CAS)	92776	https://pubchem.ncbi.nlm.nih.gov/compound/92776
	E,E-Alpha-Farnesene	5281516	https://pubchem.ncbi.nlm.nih.gov/compound/5281516
	beta-Bisabolene (CAS)	10104370	https://pubchem.ncbi.nlm.nih.gov/compound/beta-Bisabolene
	beta-Sesquiphellandrene (CAS)	519764	https://pubchem.ncbi.nlm.nih.gov/compound/beta-Sesquiphellandrene
	Neophytadiene	10446	https://pubchem.ncbi.nlm.nih.gov/compound/Neophytadiene
	Tetradecanoic acid, ethyl ester (CAS)	31283	https://pubchem.ncbi.nlm.nih.gov/compound/31283
	6,11-Hexadecadien-1-ol	6440740	https://pubchem.ncbi.nlm.nih.gov/compound/6440740
	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	3931	https://pubchem.ncbi.nlm.nih.gov/compound/9_12-Octadecadienoic-acid
	Ethyl Oleate	5363269	https://pubchem.ncbi.nlm.nih.gov/compound/Ethyl-oleate
Red Ginger	Octanal (CAS)	454	https://pubchem.ncbi.nlm.nih.gov/compound/454
	endo-Borneol	6552009	https://pubchem.ncbi.nlm.nih.gov/compound/6552009
	Decanal (CAS)	8175	https://pubchem.ncbi.nlm.nih.gov/compound/8175
	2,6-Octadienal, 3,7-dimethyl-, (Z)-	8843	https://pubchem.ncbi.nlm.nih.gov/compound/8843
	Geraniol	637566	https://pubchem.ncbi.nlm.nih.gov/compound/637566
	gamma-curcumene	12304273	https://pubchem.ncbi.nlm.nih.gov/compound/12304273
	Widdrene	442402	https://pubchem.ncbi.nlm.nih.gov/compound/442402
	Zingiberenol	13213649	https://pubchem.ncbi.nlm.nih.gov/compound/13213649
	d-Nerolidol	5356544	https://pubchem.ncbi.nlm.nih.gov/compound/5356544
	trans-6-shogaol	11152	https://pubchem.ncbi.nlm.nih.gov/compound/11152

strength of the ligand to the target. This research uses PyRx v1.0.0 software (Scripps Research, USA) with an academic license for molecular docking simulations carried out with a grid position covering all targets at the XYZ center position and dimensions.[36]

Chemical bond interactions

Identification of the position and type of chemical bond interactions in the ligand-protein complex was carried out using LigPlot +v.2.2 software. Weak bonds such as hydrogen and hydrophobic can be formed when a ligand binds to the target domain, this aims to trigger a biological response such as inhibition of activity. The existence of these bonds can affect the stability of drug candidates.[37]

Visualization of 3D structure

The 3D structure from the molecular docking simulation results is displayed in the form of cartoons, transparent surfaces, and sticks with color selection using PyMOL v.2.5.2 software (Schrodinger, Inc., USA) with an academic license. Molecular visualization aims to display the 3D structure of ligand-protein with a representative appearance through structural and color selection methods with publication standards.[38] Table 1 below shows the detailed identification and reported concentrations of chemicals in each red ginger and avocado leaves extract solvent.

RESULTS

In this research, the extraction was carried out using ethanol solvent to evaluate the impact of solvent polarity on the bioactivity produced from each extract. GC-MS analysis was used to determine the bioactive compound profile of each extract. In general, 43 chemical components were found with the following phytochemical content of red ginger rhizome extract: 1,2-diethoxy-4-ethylbenzene (20.93%), trans-caryophyllene (13.75%), (+)-aromadendrene (10.84%), thujopsene (8.46%), benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (6.67%), decanal (4.51%), oleic acid (zingiberenol (1.15%)) and gamma-curcumin (2.10%). Meanwhile, in the extracts of avocado leaves, the main elemental composition is ethyl oleate (36.28%), tetradecanoic acid, ethyl ester (29.12%), 9,12-octadecadienoic acid, methyl ester, (E,E)- (3.84%), and benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (3.38%).

The inclusion criteria for phytochemical compounds used as bioactive compounds must meet pharmacological and pharmacodynamic criteria. Based on their similarities as candidate medicinal ingredients, there are ten compounds each that meet the criteria of avocado leaves and red ginger rhizome extracts (Table 2). The target proteins used in this study were PBP2a (RCSB ID: 5M18), Transglycosylase (RCSB ID: 3VMT), and Glycosyltransferase (RCSB: 6FTB). 3D structure

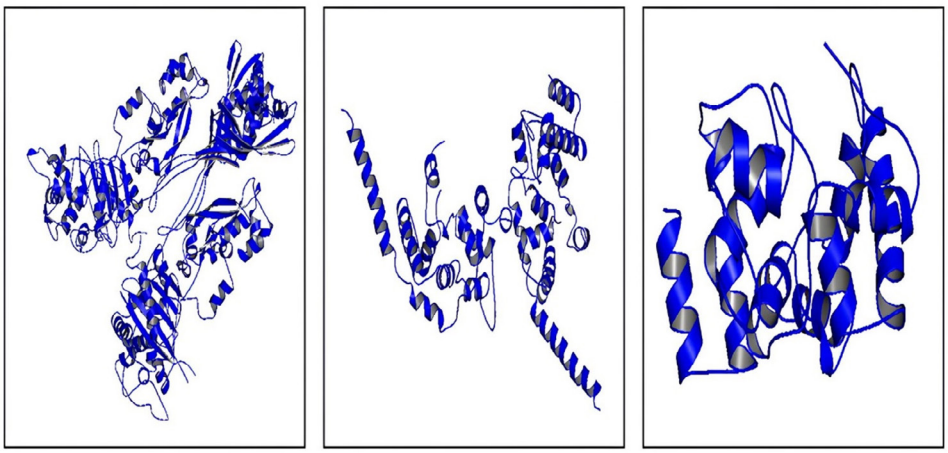

Figure 1. Visualization of target 3D structures in MRSA bacteria. (A) PBAP2a; (B) Transglycosylase; (C) Glycosyltransferase

Table 3. The results of druglikeness prediction

Source	Compounds	MM (<500 Dalton)	LogP (<5)	HBD (<5)	HBA (<10)	MR (40-130)	Probable
Avocado leaves	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)	204.000	4.924	0	0	68.282	Drug-like molecule
	Zingiberene (CAS)	204.000	4.891	0	0	68.832	Drug-like molecule
	E,E-ALPHA-FARNESENE	204.000	5.201	0	0	70.992	Drug-like molecule
	beta-Bisabolene (CAS)	204.000	5.035	0	0	68.902	Drug-like molecule
	beta-Sesquiphell-andrene (CAS)	204.000	4.891	0	0	68.832	Drug-like molecule
	Neophytadiene	278.000	7.167	0	0	94.055	Drug-like molecule
	Tetradecanoic acid, ethyl ester (CAS)	256.000	5.250	0	2	77.710	Drug-like molecule
	6,11-Hexadecadien-1-ol	280.000	5.582	0	2	86.756	Drug-like molecule
	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	280.000	5.884	1	2	86.993	Drug-like molecule
	Ethyl Oleate	310.000	5.705	0	2	108.268	Drug-like molecule
Red Ginger	Octanal (CAS)	128.000	2.545	0	1	39.439	Drug-like molecule
	endo-Borneol	154.000	2.193	1	1	45.235	Drug-like molecule
	Decanal (CAS)	156.000	3.325	0	1	48.673	Drug-like molecule
	2,6-Octadienal, 3,7-dimethyl-, (Z)-	152.000	2.877	0	1	48.485	Drug-like molecule
	GERANIOL	154.000	2.671	1	1	49.507	Drug-like molecule
	gamma-curcumene	204.000	5.035	0	0	68.902	Drug-like molecule
	Widdrene	204.000	4.559	0	0	64.652	Drug-like molecule
	Zingiberenol	222.000	4.086	1	1	70.316	Drug-like molecule
	d-Nerolidol	222.000	4.396	1	1	72.476	Drug-like molecule
	trans-6-shogaol	276.000	4.038	1	3	81.268	Drug-like molecule

rendered via PyMOL v.2.5.2 (Schrodinger, Inc., USA) with an academic license with ster (Figure 1).

Lipinski Rule's of Five plays a role in identifying the similarity of query compounds with drug molecules through physicochemical parameters. These rules state that a query compound that is categorized as a drug-like molecule must fulfill at least two rules of five. These rules refer to the molecular mass must be below 500 Daltons, the high lipophilicity (LogP) must have a value smaller than 5, the number of donor hydrogen bonds must be less than 5 and the molar refractivity must have a value between 40-130.[33] The drug-likeness prediction results for *query* ligands from avocado leaves and red ginger show that all chemical compound samples are drug-like molecules because they fulfill at least two rules in the Lipinski Rule's of Five (Table 3).

Bioactivity prediction in this study refers to the probability level of antibacterial activity ability of the query compound which is indicated by the values of *Pa* dan *Pi*.[34] Compounds with values of $Pa \geq 0.3$ and $Pa \geq Pi$ show computationally proven antibacterial capabilities. The results of identifying bioactivity and toxicity in compounds from avocado leaves showed zingiberene (CAS), E,E-alpha-farnesene, beta-bisabolene (CAS), beta-sesquiphellandrene (CAS), neophytadiene, 9,12-octadecadienoic acid, methyl ester, (E,E)- (CAS), and compounds from red ginger extracts such as 2,6-octadienal, 3,7-dimethyl-, (Z)-, geraniol, gamma-curcumene, zingiberenol, and d-nerolidol have antibacterial activity and do not have carcinogenicity and hepatoxicity type toxins. The compound 6,11-hexadecadien-1-ol from the extract of avocado leaves was actually antibacterial but not used for further analysis because it had hepatoxicity type toxin activity (Table 4).

The molecular docking method used in this research is a blind type, ignoring the active

Table 4. Bioactivity and toxicity prediction results

Source	Compound	Antibacterial Activity		Toxicity Information		
		Pa	*Pi*	Carcinogenicity	Hepatoxicity	LD_{50} (mg/kg)
Avocado leaves extracts	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)	-	-	-	-	-
	Zingiberene (CAS)	0.416	0.026	Inactive	Inactive	1680
	E,E-Alpha-Farnesene	0.459	0.021	Inactive	Inactive	3650
	beta-Bisabolene (CAS)	0.413	0.027	Inactive	Inactive	4440
	beta-Sesquiphellandrene (CAS)	0.441	0.023	Inactive	Inactive	5000
	Neophytadiene	0.363	0.040	Inactive	Inactive	5050
	Tetradecanoic acid, ethyl ester (CAS)	-	-	-	-	-
	6,11-Hexadecadien-1-ol	0.310	0.056	Inactive	Active	1190
	9,12-Octadecadienoic acid, methyl ester, (E,E)- (CAS)	0.335	0.047	Inactive	Inactive	10000
	Ethyl Oleate	-	-	-	-	-
Red ginger extracts	Octanal (CAS)	-	-	-	-	-
	endo-Borneol	-	-	-	-	-
	Decanal (CAS)	-	-	-	-	-
	2,6-Octadienal, 3,7-dimethyl-, (Z)-	0.371	0.038	Inactive	Inactive	500
	Geraniol	0.424	0.025	Inactive	Inactive	2100
	gamma-curcumene	0.367	0.039	Inactive	Inactive	1680
	Widdrene	-	-	-	-	-
	Zingiberenol	0.463	0.020	Inactive	Inactive	2340
	d-Nerolidol	0.462	0.020	Inactive	Inactive	5000
	trans-6-shogaol	-	-	-	-	-

site to screen for other potential binding sites on the target. Ligand activity is shown through the binding affinity value. Binding affinity refers to the negative binding energy formed in a ligand-protein complex. This energy works based on Gibbs' law, namely, the more negative it is, the stronger the bonding interactions will trigger and trigger stability in the molecular complex formed. Ligands with the most negative binding affinity values can trigger inhibitory activity on targets.[39,40] Grid docking plays a role in directing ligand binding to the target; the grid position in this study consists of PBP2a center (Å) X: 6.162 Y: -13.287 Z: -50.318 Dimension (Å) X: 115.233 Y: 92.017 Z: 134.318, transglycosylase center (Å) X: -22.275 Y: -2.201 Z: -3.133 Dimension (Å) X: 76.625 Y: 82.946 Z: 109.053 and glycosyltransferase center (Å) X: -35.030 Y: -27.001 Z: 62.281 Dimension (Å) X: 47.826 Y: 52.426 Z: 51.292.

Visualization of ligand-target protein interactions is displayed by staining proteins with different ligands. The chemical bond interactions formed in the complex resulting from docking are weak bonds such as hydrogen and hydrophobic which play a role in triggering biological responses, for example target inhibitory activity by ligands.[41,42] The results of the research show that all antibiotic candidate compounds from the extracts of avocado leaves and red ginger, namely beta-bisabolene (CAS), zingiberenol, and gamma-curcumene can form weak bonds such as hydrogen and hydrophobic; this triggers inhibitory activity at the target receptor on MRSA (Figure 2).

DISCUSSION

Several chemicals found in high concentrations in each extract material can be investigated for their potential as compound identities in an effort to standardize materials through the use of finding compound identity markers. Several compounds from each extract

were screened to determine their effectiveness in silico in inhibiting receptors that generate virulence factors in MRSA. Several results of previous studies reported something similar to these results. Monoterpene and sesquiterpene hydrocarbon compounds were found to dominate the chemical composition of wild ginger extract.[43]

The bioactive compounds, including geranial, zingiberene, and -sesquiterpene, have been shown to be the main components in ginger plants, ranging from 10-60%.[44,45] Apart from avocado leaves extracts, previous research also revealed that the bioactive compounds extracted from avocado seed powder are mostly terpenes and fatty acid derivative esters which have been proven to have bioactivity to alleviate nephrotoxicity and hepatoprotective properties induced by cyclosporine-A (CsA).[46,47] The marker compound for avocado seed extract is known to be flavon C-glycoside based on its metabolite characteristics. Naringenin is one of the main flavanones detected together with its glycosides

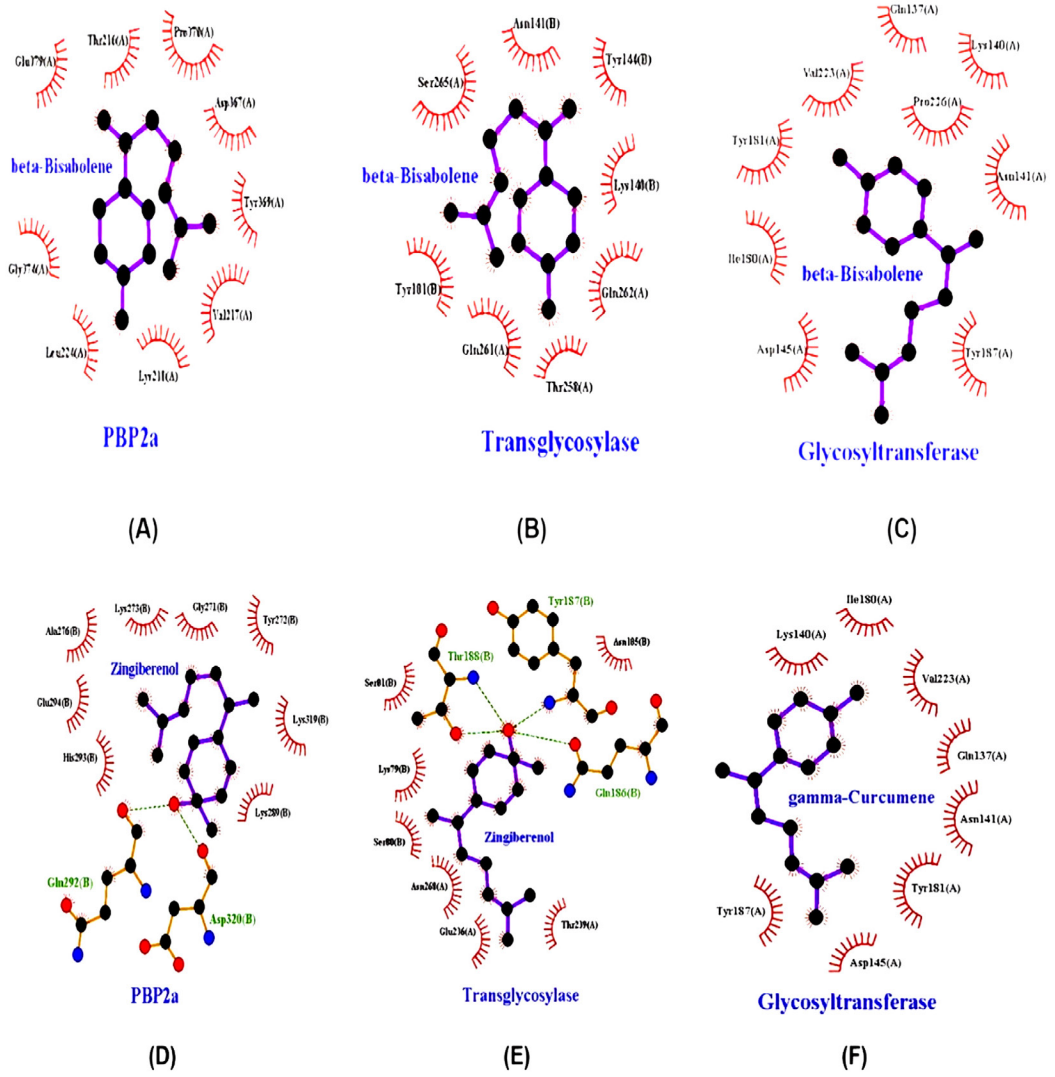

Figure 2. 2D visualization of molecular interactions of ligands with targets. (A) PBAP2a_beta-bisabolene (CAS); (B) Transglycosylase_beta-bisabolene (CAS); (C) Glycosyltransferase_beta-bisabolene (CAS); (D) PBAP2a_zingiberenol; (E) Transglycosylase_zingiberenol; (F) Glycosyltransferase_gamma-curcumene

and is a unique marker with anti-MRSA activity. Both red ginger rhizomes and avocado leaves have potential uses as herbal components or standardized herbal therapies, according to the results of this study.

The selection of these proteins was based on their potential in MRSA physiology in producing virulence factors and resistance to antimicrobial agents. PPB2A is a peptidoglycan transpeptidase that works together with the PBP2 transglycosylase domain from *S. aureus*, which accelerates cell wall production in the presence of β-lactam antibiotics, thereby allowing the bacteria to survive and develop. Transglycosylase is an important cleavage enzyme involved in the peptidoglycan turnover of Gram-negative bacteria.[48] This enzyme belongs to the glycoside hydrolase family, catalyzing the non-hydrolytic cleavage of the glycosidic linkage between MurNAc and GlcNAc in peptidoglycan, producing muropeptide 1,6-anhydromuramyl disaccharide.[49] Furthermore, glycosyltransferase is a component of cell wall biosynthetic enzymes that has been studied to play an important role in the final phase of bacterial peptidoglycan synthesis.[50] Glycosyltransferases are responsible for the elongation of glycan strands using lipid-linked disaccharides-pentapeptides as substrates. A group of bifunctional high molecular

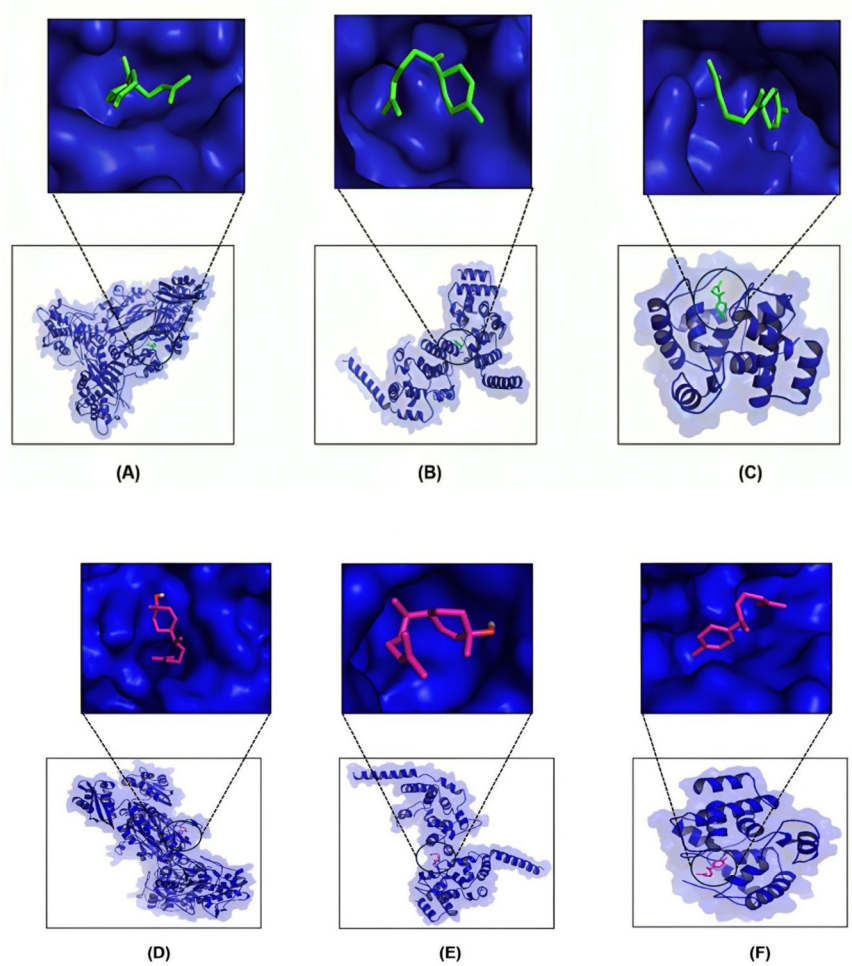

Figure 3. 3D structure resulting from docking of the ligand with the target. Ligands from avocado leaves extracts (green) and red ginger rhizome extracts (magenta). (A) PBAP2a_beta-bisabolene (CAS); (B) Transglycosylase_beta-bisabolene (CAS); (C) Glycosyltransferase_beta-bisabolene (CAS); (D) PBAP2a_zingiberenol; (E) Transglycosylase_zingiberenol; (F) Glycosyltransferase_gamma-curcumene

Table 5. Molecular docking results of avocado leaves extract and red ginger rhizome compounds against PBP2a, transglycosylase and glycosyltransferase receptors in MRSA

Source	CID	Compounds	Binding Affinity (kcal/mol)		
			PBP2a	Trans-glycosylase	Glyco-syltransferase
Avocado leaves extracts	92776	Zingiberene (CAS)	-5.6	-5.4	-5.4
	5281516	E,E-alpha-farnese	-5.5	-5.0	-5.4
	10104370	beta-bisabolene (CAS)	-5.7	-5.7	-5.9
	519764	beta-sesquiphellandrene (CAS)	-5.5	-5.5	-5.2
	10446	Neophytadiene	-5.4	-4.5	-4.1
	3931	9,12-octadecadienoic acid, methyl ester, (E,E)- (CAS)	-5.2	-4.6	-5.3
Red ginger extracts	8843	2,6-Octadienal, 3,7-dimethyl-, (Z)-	-5.2	-5.0	-4.9
	637566	Geraniol	-5.0	-5.0	-5.0
	12304273	gamma-curcumene	-6.0	-5.3	-5.9
	13213649	Zingiberenol	-6.2	-6.0	-5.5
	5356544	d-Nerolidol	-5.8	-5.2	-5.0

weight penicillin-binding proteins possessing glycosyltransferase activity has been identified in *S. aureus.*[51]

The drug-likeness prediction results in this study certainly have a greater number of compounds that have the potential to be medicinal compounds when compared to similar studies. *Garcinia atroviridis* phytochemical compounds were screened *in silico* as anti-Dengue Virus (DENV) agents based on drug similarities, only six of the 24 compounds met the criteria, including dodecanoic acid, atroviridin, naringenin, kaempherol, quercetin, and gentisein.[52] Similar research also revealed *in silico* studies of herbal extracts (basil, thyme, rosemary, and eucalyptus) on their inhibition of β-lactamase of *S. aureus* which showed that all the chemical compounds used met the Lipinski Rule's of Five criteria of not finding negative results in ADMET analysis.[53]

Related studies have reported on the use of computational techniques to predict the toxicity of several traditional Chinese medicine (TCM) formulations and most of the studies are concerned with the prediction of hepatotoxicity. This may be related to the fact that hepatotoxicity data are more widely available in public databases than other toxicity categories. However, here we add predictions of the toxicity of ligand compounds to their possible carcinogenic properties and LD_{50}. There are also several other toxicities that still need to be discussed, including cardiotoxicity, hemolytic toxicity, and nephrotoxicity.[54-56]

The results of the molecular docking simulation show that the compound beta-bisabolene (CAS) from avocado leaves extract has the most negative binding affinity for the three targets, then from red ginger extract, zingiberenol, has the most negative binding affinity for PBP2a and transglycosylase, and gamma-curcumene on glycosyltransferase (Table 5). The lowest or most negative binding affinity is needed to support the stability of interactions during cellular processes and has activity as an inhibitor on target receptors.[57] However, inhibition of this compound is still needed through *in vitro* and *in vivo* assays in future research. Potential compounds as antibiotic candidates from the extracts of avocado leaves and ginger rhizomes which act as target inhibitors consist of beta-bisabolene (CAS), zingiberenol, and gamma-curcumene. The molecular complex resulting from docking of the ligand-protein complex with the most negative binding affinity is displayed through the structure transparent surfaces, cartoons, and sticks (Figure 3).

The compound beta-bisabolene is commonly found in essential oils of medicinal plants with natural antimicrobial and antioxidant activity. Apart from the avocado leaves extracts in this study, the compound beta-bisabolene can also be found in carrots, lemons, cubes, oranges and oregano and is generally used as a natural flavoring in beverage products.[58] The Zingiberenol compound was reported to be found in the GC-MS results of Chinese ginger essential oil extract at RT

29.409 and 29.830.[59] The zingiberenol compound significantly inhibited the effects of nitric oxide production in RAW 264.7 macrophages induced with LPS, indicating the immunomodulatory activity of this extract.[60] The compound curcumene was reportedly identified in the essential oil of the rhizome of *Curcuma longa*, *C. aeruginosa*, and *C. longa*. In addition, *in vitro* and *in silico* testing of this compound showed anti-dengue fever activity by inhibiting DENV-2 NS2B-NS3.[61] This report may be the first to report the compounds beta-bisabolene (avocado leaves) and zingiberenol and gamma-curcumene (red ginger rhizomes) in inhibiting the virulence factors of MRSA *in silico*.

CONCLUSION

The total phytoconstituents obtained from the extracts of avocado leaves and red ginger rhizome were 43 types of compounds. Prediction of bioactivity results show in our study show that the compound 6,11-hexadecadien-1-ol from avocado leaves extracts has computationally hepatotoxic properties. There are at least three compounds, namely beta-bisabolene, from avocado leaves extract, zingiberenol and gamma-curcumene, from red ginger rhizome extracts which are able to bind to the active site of MRSA resistance-related proteins (PBAP2a, transglycosylase and glycosyltransferase) with lower binding affinity values than inhibitors. By observing the *in silico* data and the potential active compounds contained in avocado leaves and red ginger rhizome extracts, a promising antibacterial agent could possibly be obtained from these traditional plants to be utilized against MRSA. The mechanism of action played by each compound is through inhibition of three proteins related to antibiotic resistance controlled by MRSA. Further researches using *in vitro* and *in vivo* approaches are very important and recommended to ensure the synergistic effect of these two extracts against MRSA infections.

ACKNOWLEDGMENTS

The authors would like to thank the Institute for Research and Community Service (LPPM) at Universitas Dhyana Pura for supporting the implementation of this research. The authors also thank all those who have helped carry out the research, such as students, workers at the Science and Health Laboratory, Universitas Dhyana Pura, and ASCAdemia who have provided proofreading services for this manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved the final manuscript for publication.

FUNDING

This research was funded by the Institute for Research and Community Service (LPPM) Universitas Dhyana Pura through the Higher Education Excellence Research Scheme Research Funding Grant Program in 2022 with Contract Number: 02/UNDHIRA-LPPM/Lit./2022.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Aguilar GR, Swetschinski LR, Weaver ND, et al. The burden of antimicrobial resistance in the Americas in 2019: A cross-country systematic analysis. *Lancet Reg Health*. 2023;25:1-16. doi: 10.1016/j.lana.2023.100561
2. Vos T, Lim SS, Abbafati C, et al. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* 2020;396(10258):1204-1222. doi: 10.1016/S0140-6736(20)30925-9
3. Naylor NR, Atun R, Zhu N, et al. Estimating the burden of antimicrobial resistance:a systematic literature review. *Antimicrob Resist Infect Control.* 2018;7(1):58. doi: 10.1186/s13756-018-0336-y
4. Varela MF, Stephen J, Lekshmi M, et al. Bacterial Resistance to Antimicrobial Agents. *Antibiotics.* 2021;10(5):593. doi: 10.3390/antibiotics10050593
5. Ghosh D, Veeraraghavan B, Elangovan R, Vivekanandan P. Antibiotic Resistance and Epigenetics:More to It than Meets the Eye. *Antimicrob Agents Chemother.*

2020;64(2):e02225-19. doi: 10.1128/AAC.02225-19
6. Bharadwaj A, Rastogi A, Pandey S, Gupta S, Sohal JS. Multidrug-Resistant Bacteria: Their Mechanism of Action and Prophylaxis. Kaushik S, editor. *BioMed ResInt*. 2022;2022:5419874. doi: 10.1155/2022/5419874
7. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol*. 2019;17(4):203-218. doi: 10.1038/s41579-018-0147-4
8. Cyr DD, Allen AS, Du G-J, et al. Evaluating genetic susceptibility to *Staphylococcus aureus* bacteremia in African Americans using admixture mapping. *Genes and Immunity.* 2017;18(2):95-99. doi: 10.1038/gene.2017.6
9. Falagas ME, Karageorgopoulos DE, Leptidis J, Korbila IP. MRSA in Africa:Filling the Global Map of Antimicrobial Resistance. *PLoS ONE*. 2013;8(7):1-12. doi: 10.1371/journal.pone.0068024
10. Mohamad FNA, Argimon S, Abdul SMN, et al. Diversity and Dissemination of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Genotypes in Southeast Asia. *Trop Med Infect Dis*. 2022;7(12):438. doi: 10.3390/tropicalmed7120438
11. Syahniar R, Rayhana R, Kharisma DS, Khatami M, Duarsa DBB. Methicillin-Resistant *Staphylococcus aureus* among Clinical Isolates in Indonesia: A Systematic Review. *Biomed Pharmacol J.* 2020;13(4):1871-1878. doi: 10.13005/bpj/2062
12. Santosaningsih D, Santoso S, Setijowati N, et al. Prevalence and characterisation of *Staphylococcus aureus* causing community acquired skin and soft tissue infections on Java and Bali, Indonesia. *Trop Med Int Health*. 2018;23(1):34-44. doi: 10.1111/tmi.13000
13. Rafif KA, Rehman S, Agus SS, et al. Detection of *mec*A gene and methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia. *F1000 Research*. 2022;11:722. doi: 10.12688/f1000research.122225.3
14. Chen H, Zhang J, He Y, et al. Exploring the Role of *Staphylococcus aureus* in Inflammatory Diseases. *Toxins*. 2022;14(7):464. doi: 10.3390/toxins14070464
15. Wong JWH, Ip M, Tang A, et al. Prevalence and risk factors of community-associated methicillin-resistant *Staphylococcus aureus* carriage in Asia-Pacific region from 2000 to 2016: A systematic review and meta-analysis. *Clin Epidemiol.* 2018;10:1489-1501. doi: 10.2147/CLEP.S160595
16. Hayati LN, Tyasningsih W, Praja RN, Chusniati S, Yunita MN, Wibawati PA. Isolation and Identification of *Staphylococcus aureus* in Dairy Milk of the Etawah Crossbred Goat with Subclinical Mastitis in Kalipuro Village, Banyuwangi. *Jurnal Medik Veteriner.* 2019;2(2):76. doi: 10.20473/jmv.vol2.iss2.2019.76-82
17. Tang Q, Tan P, Dai Z, et al. Hydrophobic modification improves the delivery of cell-penetrating peptides to eliminate intracellular pathogens in animals. *Acta Biomaterialia.* 2023;157:210-224. doi: 10.1016/j.actbio.2022.11.055
18. Ali T, Basit A, Karim AM, et al. Mutation-Based Antibiotic Resistance Mechanism in Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates. *Pharmaceuticals.* 2021;14(5):420. doi: 10.3390/ph14050420
19. Zou X, Cai S, Wang T, et al. Natural antibacterial agent-based nanoparticles for effective treatment of intracellular MRSA infection. *Acta Biomaterialia.* 2023;169:410-421. doi: 10.1016/j.actbio.2023.08.004
20. Song M, Liu Y, Li T, et al. Plant Natural Flavonoids Against Multidrug Resistant Pathogens. *Adv Sci.* 2021;8(15):1-11. doi: 10.1002/advs.202100749
21. Lee JH, Kim YG, Choi P, Ham J, Park JG, Lee J. Antibiofilm and Antivirulence Activities of 6-Gingerol and 6-Shogaol Against *Candida albicans* Due to Hyphal Inhibition. *Front Cell Infect Microbiol*. 2018;8:1-10. doi: 10.3389/fcimb.2018.00299
22. Zhou X, Seto SW, Chang D, et al. Synergistic Effects of Chinese Herbal Medicine: A Comprehensive Review of Methodology and Current Research. *Front Pharmacol.* 2016;7:201. doi: 10.3389/fphar.2016.00201
23. Mahfudh N, Mantali MF, Sulistyani N. Antioxidant and Antihyperlipidemic Effect of Purple Sweet Potato Leaf Extract (*Ipomoea batata*s L.) and Red Yeast Rice Combination in Hypercholesterol Rats. *Indonesian J Pharm*. 2022;33(1):93-99. doi: 10.22146/ijp.2115
24. Linda R. The inhibition of leaf extract *Moringa oleifera* on the formation biofilm bacteria *Enterococcus faecalis. Denta, Jurnal Kedokteran Gigi.* 2021;14(1):44-50. doi: 10.30649/denta.v14i1.7
25. Edet UO, Nwaokorie FO, Mbim EN, et al. Evaluation of *Annona muricata* extract against *Staphylococcus aureus* isolate and in-silico activity of bioactive compounds against Capsular protein (Cap5O). *BMC Complement Med Ther.* 2022;22(1):192. doi: 10.1186/s12906-022-03672-4
26. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. *Int J Mol Sci*. 2019;20(18):4331. doi: 10.3390/ijms20184331
27. Rafi M, Meitary N, Septaningsih DA, Bintang M. Phytochemical Profile and Antioxidant Activity of *Guazuma ulmifolia* Leaves Extracts Using Different Solvent Extraction. *Indonesian J Pharm*. 2020;31(3):171-180. doi: 10.22146/ijp.598
28. Pai A, Shenoy C. Physicochemical, Phytochemical, and GC-MS Analysis of Leaf and Fruit of *Pouteria campechiana* (Kunth) Baehni. *J Appl Biol Biotechnol.* 2020;8(04):90-97. doi: 10.7324/JABB.2020.80414
29. Kamalia AZ, Tunjung WAS. Efficacy of Different Solvents in the Extraction of Bioactive Compounds and Anticancer Activities of Thyme (*Thymus vulgaris* L.) Leaves and Twigs. *Indones J Pharm.* 2023;34(3):1-12. doi: 10.22146/ijp.5959
30. Watiniasih NL, Budiarsa IN, Antara ING, Wiradana PA. Propolis extract as a green bacterial corrosion inhibitor on three types of metals. *Biodiversitas J Biol Divers.* 2022;23(9):4852-4860. doi: 10.13057/biodiv/d230954
31. Kim S, Thiessen PA, Bolton EE, et al. PubChem Substance and Compound databases. *Nucleic Acids Res*. 2016;44(D1):D1202-D1213. doi: 10.1093/nar/gkv951
32. Lade H, Kim JS. Bacterial Targets of Antibiotics in Methicillin-Resistant *Staphylococcus aureus. Antibiotics*. 2021;10(4):1-29. doi: 10.3390/antibiotics10040398

33. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the Rule of 5 and drugability. *Adv Drug Deliv Rev*. 2016;101:89-98. doi: 10.1016/j.addr.2016.05.007
34. Sivasakthi P, Sabarathinam S, Vijayakumar TM. Network pharmacology and *in silico* pharmacokinetic prediction of Ozanimod in the management of ulcerative colitis:A computational study. *Health Sci Rep.* 2022;5(1):e473. doi: 10.1002/hsr2.473
35. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II:a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res.* 2018;46(W1):W257-W263. doi: 10.1093/nar/gky318
36. Rashidieh B, Etemadiafshar S, Memari G, et al. A molecular modeling based screening for potential inhibitors to alpha hemolysin from *Staphylococcus aureus*. *Bioinformation*. 2015;11(8):373-377. doi: 10.6026/97320630011373
37. Alnasser SM, Azam F, Alqarni MH, et al. Development and Evaluation of Novel Encapsulated Isoeugenol-Liposomal Gel Carrier System for Methicillin-Resistant *Staphylococcus aureus. Gels.* 2023;9(3):228. doi: 10.3390/gels9030228
38. Tabassum R, Kousar S, Mustafa G, Jamil A, Attique SA. *In Silico* Method for the Screening of Phytochemicals against Methicillin-Resistant *Staphylococcus Aureus*. Omri A, editor. *Biomed Res Int.* 2023;2023:5100400. doi: 10.1155/2023/5100400
39. Gomez-Aldapa CA, Rangel-Vargas E, Refugio Torres-Vitela M, et al. Antibacterial Activities of Hibiscus sabdariffa Extracts and Chemical Sanitizers Directly on Green Leaves Contaminated with Foodborne Pathogens. *J Food Prot.* 2018;38(1):209-217. doi: 10.4315/0362-028X.JFP-17-053
40. Khayyat SA, Roselin LS. 2018. Recent progress in photochemical reaction on main components of some essential oils. *J Saudi Chem Soc*. 2018;22(7):855-875. doi: 10.1016/j.jscs.2018.01.008
41. Kumar SP, Singh V, Ali M. Chemical Composition and Antimicrobial Activity of Fresh Rhizome Essential Oil of *Zingiber Officinale* Roscoe. *Pharmacogn J.* 2016;8(3):185-190. doi: 10.5530/pj.2016.3.3
42. Elmoslemany AM, El-Magd MA, Ghamry HI, Alshahrani MY, Zidan NS, Zedan AMG. Avocado Seeds Relieve Oxidative Stress-Dependent Nephrotoxicity but Enhance Immunosuppression Induced by Cyclosporine in Rats. *Antioxidants*. 2021;10(8):1194. doi: 10.3390/antiox10081194
43. El-Magd MA, Zedan AMG, Zidan NS, Sakran MI, Bahattab O, Oyouni AAA, Al-Amer OM, Alalawy AI, Elmoslemany AM. Avocado Seeds-Mediated Alleviation of Cyclosporine A-Induced Hepatotoxicity Involves the Inhibition of Oxidative Stress and Proapoptotic Endoplasmic Reticulum Stress. *Molecules* 2022;27(22): 7859. doi: 10.3390/molecules27227859
44. Dik DA, Marous DR, Fisher JF, Mobashery S. Lytic transglycosylases:concinnity in concision of the bacterial cell wall. *Crit Rev Biochem Mol Biol*. 2017;52(5):503-542. doi: 10.1080/10409238.2017.1337705
45. Scheurwater E, Reid CW, Clarke AJ. Lytic transglycosylases:Bacterial space-making autolysins. *Int J Biochem Cell Biol*. 2008;40(4):586-591. doi: 10.1016/j.biocel.2007.03.018
46. Wang QM, Peery RB, Johnson RB, Alborn WE, Yeh WK, Skatrud PL. Identification and Characterization of a Monofunctional Glycosyltransferase from *Staphylococcus aureus*. *J Bacteriol.* 2001;183(16):4779-4785. doi: 10.1128/JB.183.16.4779-4785.2001
47. Terrak M, Ghosh TK, Van HJ, et al. The catalytic, glycosyl transferase and acyl transferase modules of the cell wall peptidoglycan polymerizing penicillin binding protein 1b of *Escherichia coli. Mol Microbiol.* 1999;34(2):350-364. doi: 10.1046/j.1365-2958.1999.01612.x
48. Aini NS, Ansori ANM, Kharisma VD, et al. An *in Silico* Study: Phytochemical Compounds Screening of *Garcinia atroviridis* Griff. ex T. Anders as Anti-DENV. *J Pure App Microbiol*. 2023;17(4):2467-2478. doi: 10.22207/JPAM.17.4.45
49. Etminani F, Etminani A, Hasson SO, Judi HK, Akter S, Saki M. *In silico* study of inhibition effects of phytocompounds from four medicinal plants against the *Staphylococcus aureus* β-lactamase. *Inform Med Unlocked*. 2023;37:101186. doi: 10.1016/j.imu.2023.101186
50. Chang N, Gao J, Niu L, et al. Integrated artificial neural network analysis and functional cell based affinity mass spectrometry for screening a bifunctional activator of Ca^{2+} and β2AR in aconite. *J Pharm Biomed Anal*. 2020;190:113506. doi: 10.1016/j.jpba.2020.113506
51. Zheng S, Wang Y, Liu H, Chang W, Xu Y, Lin F. Prediction of Hemolytic Toxicity for Saponins by Machine-Learning. Methods. *Chem Res Toxicol*. 2019;32(6):1014-1026. doi: 10.1021/acs.chemrestox.8b00347
52. Sun Y, Shi S, Li Y, Wang Q. Development of quantitative structure-activity relationship models to predict potential nephrotoxic ingredients in traditional Chinese medicines. *Food Chem Toxicol.* 2019;128:163-170. doi: 10.1016/j.fct.2019.03.056
53. Botelho FD, dos Santos MC, Goncalves AdaS, et al. Ligand-Based Virtual Screening, Molecular Docking, Molecular Dynamics, and MM-PBSA Calculations towards the Identification of Potential Novel Ricin Inhibitors. *Toxin*s. 2020;12(12):746. doi: 10.3390/toxins12120746
54. Hata H, Phuoc TD, Marzouk SM, Kitao A. Binding free energy of protein/ligand complexes calculated using dissociation Parallel Cascade Selection Molecular Dynamics and Markov state model. *Biophys Physicobiol.* 2021;18:305-316. doi: 10.2142/biophysico.bppb-v18.037
55. Wahyuni DK, Wacharasindhu S, Bankeeree W, et al. Molecular simulation of compounds from n-hexane fraction of *Sonchus arvensis* L. leaves as SARS-CoV-2 antiviral through inhibitor activity targeting strategic viral protein. *J Pharm Pharmacogn Res*. 2022;10(6):1126-1138. doi: 10.56499/jppres22.1489_10.6.1126
56. Barton D, Chickos J. The vapor pressure and vaporization enthalpy of (-) β-Elemene and (-) β-Bisabolene by correlation gas chromatography. *J Chem Thermodyn.* 2020;148:106139. doi: 10.1016/j.jct.2020.106139
57. Wong FF, Abdullah MO, Hii YR, et al. A preliminary investigation of China Ginger and Kuching Local Ginger species: Oil extracts and synthesis towards potential

greener insect repellent. *The Journal of Natural Pesticide Research.* 2023;6:100061. doi: 10.1016/j.napere.2023.100061

58. Hien NT, Cuc DT, Thuy NTT, et al. Labdane- type diterpenoids and sesquiterpenes from *Curcuma aromatica* and their nitric oxide inhibitory activity in lipopolysaccharide-stimulated RAW264.7 macrophages. *J Asian Nat Prod Res.* 2023;26(3):387-393. doi: 10.1080/10286020.2023.2220273
59. Jani NA, Maarof NI, Zahari MMFM, et al. Phytochemical profiling of the essential oils from three Curcuma species and their *in vitro* and *in silico* dengue protease inhibition activity. *Nat Prod Res.* 2023;38(6):926-932. doi: 10.1080/14786419.2023.2208256
60. Dibha A, Wahyuningsih S, Ansori A, et al. Utilization of Secondary Metabolites in Algae *Kappaphycus alvarezii* as a Breast Cancer Drug with a Computational Method. *Pharmacogn J*. 2022;14(3):536-543. doi: 10.5530/pj.2022.14.68
61. Mir WR, Bhat BA, Rather MA, et al. Molecular docking analysis and evaluation of the antimicrobial properties of the constituents of *Geranium wallichianum* D. Don ex Sweet from Kashmir Himalaya. *Sci Rep*. 2022;12(1):12547. doi: 10.1038/s41598-022-16102-9