Iranian Journal of Microbiology
Volume 16 Number 2 (April 2024) 208-218

ORIGINAL ARTICLE

Antibacterial activity of Shallots (*Allium xwakegi* Araki.) cultivars in Palu Valley against *Salmonella* Typhi ATCC 27870 through *in vitro* and in silico evaluation

Sitti Rahmah Sari[1], Riuh Wardhani[1,2], Faiqah Umar[1,3], Dirayah Rauf Husain[1*], Ade Chandra Iwansyah[4]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, South Sulawesi, Indonesia
[2]Department of Animal and Dairy Science, College of Agriculture and Life Sciences, Chungnam National University, Daejeon, Republic of Korea
[3]Medical State Laboratory, Ministry of Health, Makassar, Indonesia
[4]Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta, Indonesia

Received: September 2023**, Accepted:** February 2024

ABSTRACT

Background and Objectives: Shallots, recognized for their minimal toxicity, cost-effectiveness, and widespread availability, are increasingly considered a viable source of biological activity. This study evaluates the antibacterial efficacy of a specific shallot cultivar from Palu Valley, Indonesia, against *Salmonella typhi*, the pathogen responsible for typhoid fever.
Materials and Methods: Utilizing thin-layer chromatography (TLC-bioautography) and gas chromatography-mass spectroscopy (GC-MS), the study identifies active compounds in shallot ethanol extract and employs molecular docking to assess interactions between receptors and ligands.
Results: Findings indicate significant antibacterial activity, with a notable inhibition zone diameter of 31.5 mm at spot Rf 0.28 in TLC bioautography and an optimum concentration of 2% yielding an average clear zone diameter of 28.27 mm in the agar diffusion test. GC-MS analysis reveals 41 compounds, predominantly dodecanoic acid and 1,2,3-propanetriyl ester. Additionally, molecular docking reveals the lowest binding affinity (-7.3 kcal/mol) for Ergost-8-En-3-Ol, 14-Methyl-, (3.Beta,5.Alpha.) against DNA gyrase.
Conclusion: This study confirms Palu Valley shallot extract's potent antibacterial effect against *Salmonella typhi,* highlighting its therapeutic potential.

Keywords: Shallots; Antibacterial; *Salmonella* Typhi; *Docking simulation*

INTRODUCTION

Indonesia is renowned for its rich biodiversity, which significantly contributes to its traditional medical practices (1). Among the diverse plants, the shallot (*Allium xwakegi* Araki.) emerges as a plant of considerable interest. This hybrid is the result of crossing *Allium fistulosum* L. with *Allium ascalonicum* L., and it exhibits remarkable potential (2). In Central Sulawesi, the Palu Valley is distinguished for

*Corresponding author: Dirayah Rauf Husain, DEA, Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, South Sulawesi, Indonesia. Tel: +6285255333644 Fax: +62411-585188 Email: dirayahrh@unhas.ac.id

Copyright © 2024 The Authors. Published by Tehran University of Medical Sciences.
This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0/). Noncommercial uses of the work are permitted, provided the original work is properly cited.

its cultivation of shallot cultivars, which are a central element of the region's economy. These cultivars are integral to the production of the region's famed fried shallot industry, a unique feature of Palu's culinary culture. However, the investigation into the bioactive compounds present in these Palu Valley shallot cultivars is notably lacking. This study highlights a significant research gap that warrants further exploration.

Shallots are increasingly recognized for their multifaceted pharmacological potential, encompassing a wide spectrum of therapeutic applications such as antimicrobial, antioxidant, analgesic, anti-inflammatory, antidiabetic, hypolipidemic, antihypertensive, and immunoprotective actions (3). These health-promoting effects are largely attributed to the rich array of bioactive constituents found in shallots, including organosulfur compounds, phenolic compounds, polysaccharides, and steroids (4). Among these, phenolic compounds are noted for their ability to denature bacterial cell proteins, thereby exerting antibacterial effects (5). Similarly, steroids have been implicated as antibacterial agents, with their mechanism of action associated with disrupting lipid membranes in bacteria, leading to liposome leakage and cellular compromise (6).

Phytochemicals from agricultural plants might assist as an effective option for antimicrobials. This relates to the several issues of pharmaceuticals toxicity and antibiotic resistance (7). In the treatment of bacterial infections, broad-spectrum antibiotics are occasionally necessary, however they can often result in enhanced toxicity for the patient. Resistance has always emerged alongside the discovery of new antibiotic drugs. Resistant strains can spread if infection control procedures are ignored. The lack of alternative discoveries to substitute ineffective antimicrobials is a challenge in preserving the efficacy of current antimicrobials (8). Subsequently, there is an increasing demand for alternative antimicrobial agents that are non-toxic, affordable, easily accessible, and efficient in treating infectious diseases caused by bacteria and other pathogens (9).

Meanwhile, despite its long presence in human history, systemic infection caused by *S.* Typhi still a major life-threatening health problem, especially in developing countries (10). Therefore, the purpose of this study is focused on analyzing *in vitro* and in silico the antibacterial activity of com- pounds in shallot cultivars of Palu Valley on *S.* Typhi bacterial cultures.

MATERIALS AND METHODS

Extraction. Shallots were purchased from the Traditional Market in Palu, Central Sulawesi, Indonesia. The shallot bulbs were cut into small pieces and dried for seven days. They were then macerated using 96% ethanol (Sigma-Aldrich) for five days. Furthermore, this step was repeated three times so that the macerate was obtained. The macerate was concentrated using a rotary evaporator (Rotavapor R-300) (11).

Thin layer chromatography (TLC) test. TLC was performed following with some modifications (12). The G60F254 silica plate was cut to a size of 1 ×10 cm. The plate was then activated in an oven at 100°C. The sample of shallot ethanol extract was smeared on the bottom edge and dried. Furthermore, the TLC plate was inserted into a chamber containing chloroform and acetone (2:8) mobile phases that have been saturated. The stains formed on the plate were then sprayed with spotting reagents and observed directly or under UV with a wavelength of 366 nm. The color spots were identified by spraying the reagents aluminum chloride ($AlCl_3$), iron (III) chloride ($FeCl_3$), Dragendorff, and Lieberman Burchard.

TLC-Bioautography test. This test was carried out by preparing a nutrient agar medium (Merck) containing 0.1 mL of *S.* Typhi bacterial suspension (ATCC 27870) that was equal to 0.5 McFarland (1.5×10^8 CFU/mL). As much as 10 mL of the medium was then poured into a Petri dish and allowed to solidify. The eluted TLC plate was then affixed to the surface of the medium for 60 minutes so that the compounds on the plate diffused, and the plate was then removed. Last, it was then incubated at 37ºC for 24 hours (13).

Antibacterial test. The antibacterial activity test was carried out by the agar diffusion assay (cylinder plate method) (14). In this case, shallot extract was made in five different concentrations of 2, 4, 6, 8, and 10%. This antibacterial testing was carried out on a solid Mueller Hinton agar medium (Himedia) as a base layer. After the base layer is solid, a medium containing 0.1 mL of bacterial suspension (0.5 McFarland) is mixed into a petri dish as the second layer. The second layer was aseptically holed. Shallot extract with each concentration was added to the hole in as much as 200 µL. Finally, the medium was incu-

bated for 24 hours at 37°C (5).

Compound analysis by GCMS. The analysis of shallot compounds was conducted using a GC-MS instrument. Initially, 0.2 g of extract was combined with 5 mL of ethanol. This mixture underwent extraction in a sonicator for 20 minutes, maintaining a temperature of 40°C. Subsequently, the resultant extract was transferred into a GC-MS vial. The GC-MS settings were adjusted to a pressure of 76.9 kPa and an injector temperature of 250°C, operating in splitless mode with a ratio of 1:10. The flow rate was set at 14 mL/min. Furthermore, ion source and interface temperatures were maintained at 200°C and 280°C, respectively, with solvent cut times set for 3 minutes in the 400-700 m/z range. The column used was an SH-Rxi-5Sil MS, measuring 30 meters in length and 0.25 mm in inner diameter. The initial column temperature was set to 70°C and held for 2 minutes. This was followed by a temperature increase to 200°C at a rate of 100°C/min, leading to a final temperature of 280°C. The final stage had a hold time of 9 minutes and a rate of 50°C/min. The total duration of the sample analysis was 36 minutes. The chromatogram data obtained from this process was subsequently analyzed using the NIST 17 and Wiley 9 libraries (15).

In silico test. In silico technique utilizes computer simulations to predict and study biological, chemical, and pharmacological activities, assisting in areas such as drug discovery and genomics. In this study, molecular docking was performed to predict ligand (shallot componds) and macromoleculs (target protein) interaction. Chemical compounds as ligands were selected from GC-MS results of shallot ethanol extract, 3D structures, simplified molecular-input line-entry system (SMILES) ligands, and ID numbers were taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Protein macromolecules were obtained from the Protein Data Bank (www.rcsb.org/). Protein structures were processed using PyMOL v1.7.4.5 to remove non-protein molecules. The target proteins of this study were penicillin-binding protein (PBP) (PDB code: 1hd8), DNA gyrase (PDB code: 5l3j), and dihydropteroate synthase (DHPS) (PDB code: 1ajz). Molecular docking was conducted with the Vina Wizard feature integrated into PyRx 0.8. Interactions between the target ligands and proteins were visualized and analyzed using Discovery Studio v21.1.0.20298 (16).

RESULTS

Extraction. The determination of the shallot cultivar in Palu Valley was carried out by identifying plants morphologically. Based on the observation, this cultivar have the characteristics of round, slightly oval bulbs, pale red and whitish color, a length of 2.5-3.0 cm, and a diameter of 1.5-2.5 cm (Fig. 1).

Fig. 1. Shallot (*Allium xwakegi* Araki.) Cultivar of Palu Valley.

The extraction of shallots was done using the maceration technique. This technique involves soaking the shallot simply in a closed container with 96% ethanol solvent and leaving it at room temperature for 5 days with frequent stirring. This process was intended to break down and destroy plant cell walls to release soluble phytochemicals (17). After 3 days, the mixture was filtered. The result obtained was a reddish-brown ethanol extract, which had a distinctive odor and was soluble in ethanol. From 500 grams of shallots, 100 grams of ethanol extract weight were produced.

Thin layer chromatography (TLC) results. The chemical components were tested in the ethanol extract of shallot with the separation of active compounds by TLC using acetone: chloroform (8:2) eluent. Each chemical compound was then moved at a certain rate and the rate of movement was expressed as a retention factor (Rf). The retention factor is the ratio of the distance traveled by the mobile phase from the starting line to the end to the distance of the movement of chemical compounds that have been separated.

The results of TLC analysis on Shallot's ethanol extract, as presented in Table 1, reveal the presence of various phytochemicals. Distinct spots were observed with the Dragendorff reagent indicating the presence

Table 1. TLC Results of Shallot's Ethanol Extract

Reagents	Rf	Description
Dragendorff	0,80	-
	0,63	+Alkaloid
Liebermann- Burchard	0,81	+Steroids
	0,78	+Steroids
	0,28	+Steroids
AAluminum Chloride	0,75	+Flavonoids
Iron (III) Chloride	0,83	+Phenol
	0,78	-

of alkaloids with Rf values of 0.80 and 0.63. Additionally, the use of Liebermann-Burchard and Aluminum Chloride reagents identified steroids and flavonoids with Rf values ranging from 0.28 to 0.81. The Iron (III) Chloride reagent further indicated the presence of phenolic compounds with an Rf of 0.83. These findings provide a quantitative measure of the compound distribution, denoting a rich phytochemical profile in the shallot extract. The variability in Rf values suggests a diversity of compound polarities, reflecting the complex nature of the extract's composition.

Antibacterial activity by TLC- Bioautography. The chromatography plate from the TLC test was followed by a bioautography TLC test (Fig. 2). The aim was to determine the potential antibacterial activity of shallot ethanol extract against *S.* Typhi bacteria at the specific spot. The compounds with antibacterial potential were transferred from the chromatogram plate to the agar medium. In this case, the positive results are indicated by the presence of a clear zone on the surface of the agar former spot on the chromatogram that has been affixed before. The test results can be seen in (Fig. 2).

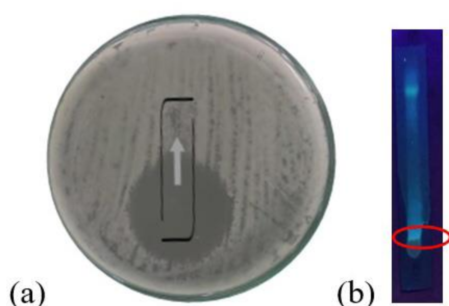

Fig. 2. (a) Bioautography TLC results, inhibition zone diameter of 31.5 mm (b) Spot point (uv 366nm) after spraying Liebermann-Burchard reagent.

Antibacterial test. The shallot ethanol extract sample was subjected to an antibacterial test by diffusion method against *S.* Typhi bacteria. In this case, the positive results were indicated by the presence of a clear zone around the hole on the surface of MHA media. The test results can be seen in Fig. 3 and Table 2.

Gas chromatography-mass spectroscopy (GC-MS) results. In order to determine the content of the compound present in the shallot ethanol extract, an analysis was carried out through GC-MS screening in Fig. 4 and Table 3.

In silico test. The molecular docking between the ligand and the target protein used the Vina wizard in the PyRx program. The results of the ΔG^o binding affinity value of the ligand and target protein are presented in the Table 4 and Fig. 5.

DISCUSSION

Ethanol extract samples were subjected to phytochemical and antibacterial tests against *S.* Typhi. The results of TLC showed that there was a total of eight spots of compounds that could be seen on the chromatogram plate with different Rf values. These results indicated that the ethanol extract of shallots contains alkaloid, flavonoid, phenol, and steroid groups.

The TLC results for Shallot's Ethanol Extract align with established literature, confirming the presence of various bioactive compounds. Flavonoids, including quercetin and its derivatives, were previously identified in shallot peels and bulbs, highlighting their antioxidant potential (18, 19). The significant phenolic content observed corresponds with findings indicating shallots' rich polyphenolic profile and higher phenolic and diallyl disulfide contents compared to garlic (20, 21). Additionally, shallots have been noted for their high levels of glycosidic flavonoids, which contribute to their antioxidant, antibacterial, and anti-inflammatory properties (22). The correlation of these compounds with health benefits, such as cancer prevention and potent antioxidant activity, underscores the therapeutic relevance of the phytochemicals detected in the current study (23, 24).

Furthermore, the TLC-bioautography test results of shallot ethanol extract showed inhibitory activity

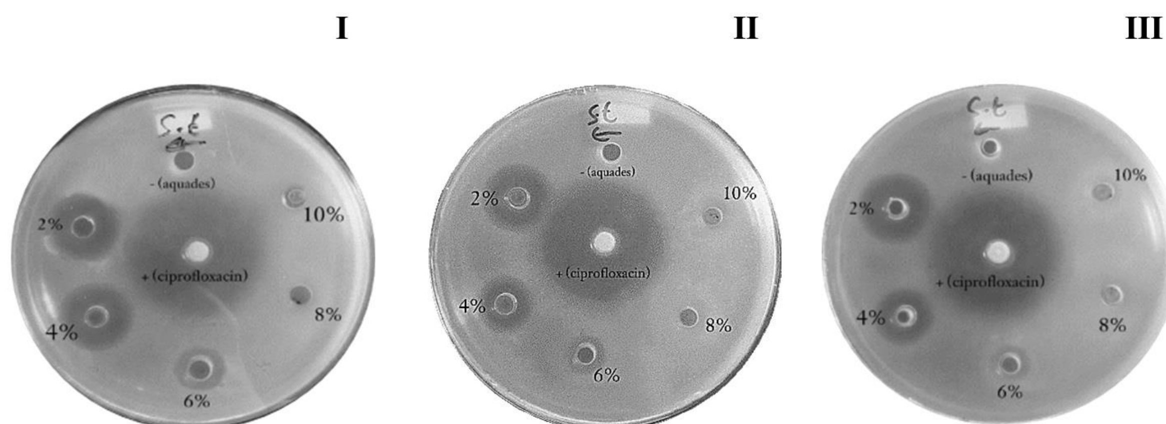

Fig. 3. Antibacterial Test Results of Shallot Ethanol Extract against *S.* Typhi

Table 2. Antibacterial test results of ethanol extract of Shallot

Replicate	Positive Control (ciprofloxacin)	Negative Control (aquades)	Inhibition Zone Diameter (mm)				
			2%	4%	6%	8%	10%
I	50,65	0	28,05	26	14,1	0	0
II	49,8	0	28,65	21,53	13,43	0	0
II	51,1	0	28,1	20,48	12,9	0	0
Average	50,2	0	28,27	22,67	13,48	0	0

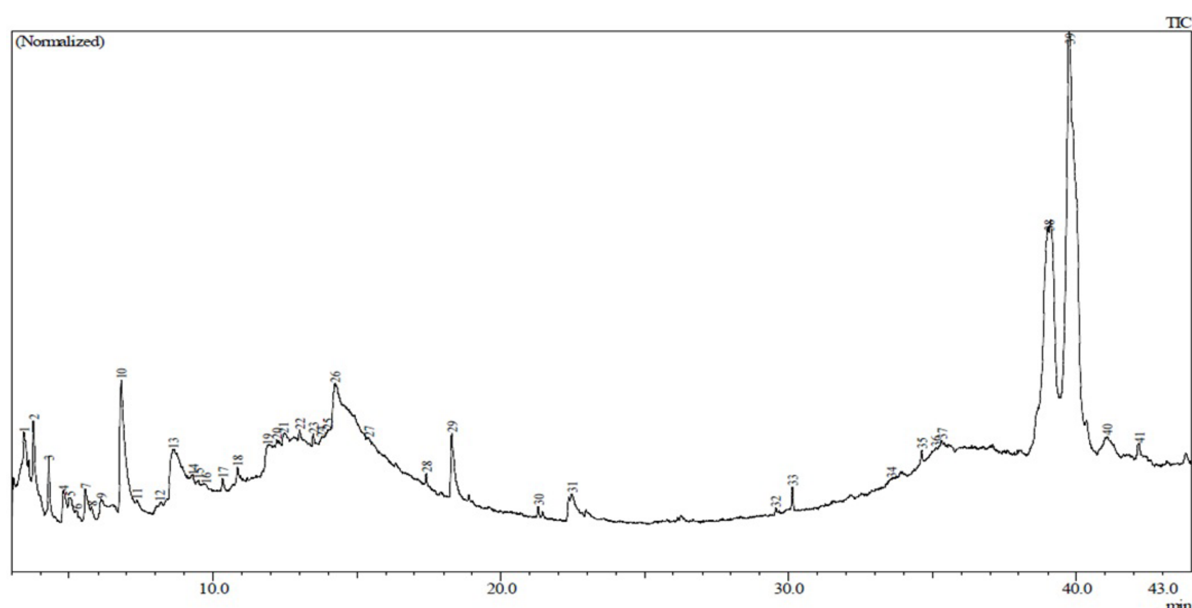

Fig. 4. Chromatogram of Shallot Ethanol Extract

against *S.* Typhi. The zone was found on the compound spot with an Rf of 0.28. Based on the TLC spot marker test, it is known that the Rf of 0.28 detected the presence of steroid compounds. The mechanism of action of steroids as antibacterials is related to lipid membranes and sensitivity to steroid components that cause leakage in bacterial liposomes. Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing decreased membrane integrity and altered cell membrane morphology that causes cell fragility and lysis (6). These results are supported by several previous

Table 3. Phytochemical Components Identified in Shallot's Ethanol Extract by GC-MS

(Chromatogram Code) **Compound**	**Retention Time**	**Area (%)**	**Chemical formula**
Fatty Aldehydes			
[27]3-Isopropyl-4-Methyl-1-Decen-4-Ol	15.425	0,89	$C_{14}H_{28}O$
[31]Cis-9-Hexadecenal	22.468	1,22	$C_{16}H_{30}O$
Alkana			
[12]Nonane, 5-Methyl-	8.158	0,25	$C_{10}H_{22}$
[32]Octacosane	29.560	0,09	$C_{28}H_{58}$
Alkaloid			
[20]Xanthosin (CAS)	12.225	0,70	$C_{10}H_{12}N_4O_6$
[1]Oxime-, Methoxy-Phenyl-	3.427	2,92	$C_8H_9NO_2$
Aril Aldehyde			
[13]5-Hydroxymethylfurfural	8.613	4,95	$C_6H_6O_3$
Benzoic acid			
[33]1,2-Benzenedicarboxylic Acid	30.134	0,26	$C_{24}H_{38}O_4$
Fatty Acids			
[6]Succinic Acid, Di(But-2-En-1-Yl) Ester	5.258	0,29	$C_{12}H_{18}O_4$
[18]3(2H)-Furanone, 2-Hexyl-5-Methyl-	10.850	0,51	$C_{11}H_{18}O_2$
[24]9,10-Secochola-5,7,10(19)-Trien-24-Al,3-Hydroxy-,(3.Beta.,5Z,7E)-	13.758	0,44	$C_{24}H_{36}O_2$
[30]10,13-Octadecadienoic Acid, Methyl Ester	21.306	0,12	$C_{19}H_{34}O_2$
[29]N-Hexadecanoic Acid	18.308	1,76	$C_{16}H_{32}O_2$
[28]Hexadecanoic Acid, Methyl Ester	17.417	0,23	$C_{17}H_{34}O_2$
Azole			
([16]1,2,3-Thiadiazole-4-Carboxylic Acid, Hydrazide	9.758	0,28	$C_3H_4N_4OS$
Benzene			
[5]Benzeneacetaldehyde (CAS)	5.042	0,79	C_8H_8O
[34]Benzene, 1,1'-Oxybis[(1,1-Dimethylbutyl)- (CAS)	33.575	0,18	$C_{24}H_{34}O$
[37]4-Nitrophenyl Laurate	35.320	1,10	$C_{18}H_{27}NO_4$
[14]2-Methoxy-4-Vinylphenol	9.308	0,61	$C_9H_{10}O_2$
Eater			
[11]2-Acetyl-Octanoic Acid, Ethyl Ester	7.358	0,08	$C_{12}H_{22}O_3$
Phenol			
[17]Benzene, 1-Chloro-4-Methoxy-	10.331	0,20	$C_7H_7C_1O$
Furan			
[7]2,5-Dimethylfuran-3,4(2H,5H)-Dione	5.551	0,92	$C_6H_8O_3$
[8]2,5-Anhydro-1,6-Dideoxyhexo-3,4-Diulose	5.792	0,24	$C_6H_8O_3$
[3]2,4-Dihydroxy-2,5-Dimethyl-3(2H)-Furan-3-One	4.288	0,79	$C_6H_8O_4$
[23]3(2H)-Furanone, 5-Methyl-2-Octyl-	13.490	0,17	$C_{13}H_{22}O_2$
Glycerol			
[38]Dodecanoic Acid, 1,2,3-Propanetriyl Ester (CAS)	39.046	21,73	$C_{39}H_{74}O_6$
[39]Dodecanoic Acid, 1,2,3-Propanetriyl Ester	39.776	33,53	$C_{39}H_{74}O_6$
[40]Eicosanoic Acid, 2-[(1-Oxohexadecyl)Oxy]-1-[[(1-Oxohexadecyl)Oxy]Methyl]Ethyl Ester	41.076	1,64	$C_{55}H_{106}O_6$
Saturated hydrocarbons			
[22]1-Cyclopentyleicosane	13.014	0,25	$C_{25}H_{50}$
Ketone			
[2]1,2-Cyclopentanedione	3761	2,05	$C_5H_6O_2$
[25](S)-1-(1-Cyclopentenyl)-Propanol	13.992	0,52	$C_8H_{14}O$
Lactones			

Table 3. Continuing...

[26]3-Deoxy-D-Mannoic Lactone	14.238	8,95	$C_6H_{10}O_5$
Monosaccharides			
[21]D-Allose	12.442	1,53	$C_6H_{12}O_6$
Nitro			
[19]1,3-Propanediol, 2-(Hydroxymethyl)-2-Nitro-	11.908	1,56	$C_4H_9NO_5$
Organosulfonat			
[4]Sulfurous Acid, Bis(2-Methylpropyl) Ester	4.797	0,98	$C_8H_{18}O_3S$
Pyran			
[10]4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl	6809	4,75	$C_6H_8O_4$
Steroid			
[41]Ergost-8-En-3-Ol, 14-Methyl-, (3.Beta.,5.Alpha.)	42.182	0,74	$C_{29}H_{50}O$
Terpenoid			
[9]N-(3-Butenyl)-N-Methylcyclohexanamine	6115	0,38	$C_{11}H_{21}N$
[35]2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-Hexamethyl-, (All-E)	34.627	0,26	$C_{30}H_{50}$
Organic Trisulfide			
[15](E)-1-Allyl-3-(Prop-1-En-1-Yl)Trisulfane	9.492	0,43	$C_6H_{10}S_3$
Trialkylheterosilanes			
[36]N-Nonadecanoic Acid, Pentamethyldisilyl Ester	35.108	0,05	$C_{24}H_{52}O_2Si_2$

Table 4. ΔGº Binding Affinity Value of Compound Ligands in Shallots

Ligand	Type	ΔGº Binding Afinity (kcal/mol)		
		5l3j	1ajz	1hd8
Ergost-8-En-3-Ol, 14-Methyl-, (3.Beta.,5.Alpha.)	Steroid	-7,3	-6,9	-7,5
Dodecanoic acid, 1,2,3-propanetriyl ester (CAS)	Gliserol	-5,7	-4,8	-4,2
3-Deoxy-d-mannoic lactone	Lakton	-5,5	-6,0	-5,5
5-Hydroxymethylfurfural	Aril Aldehid	-4,9	-4,8	-5,0
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Piran	-5,0	-5,2	-5,4
Oxime-, methoxy-phenyl	Alkaloid	-6,1	-5,2	-6,1

studies, which report that in general, shallots can inhibit or kill *S.* Typhi pathogenic bacteria (25-26).

The inhibition test, which demonstrates that the ideal concentration is 2% with an average clear zone diameter of 28.27 mm, supports the results of the TLC test. According to the previous study (17), the shallot crude extract contains a complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids, and flavonoids, that can have potential as antibiotics. In line with other research (27, 28), these compounds were found in Allium species and were found to have strong antimicrobial properties against bacteria, fungi, and viruses that infect humans. Based on the test results, it can be concluded that phytochemical compounds may have antimicrobial effects. For example, it has been shown. that saponins and flavonoids in Allium species can inhibit several types of bacteria (29). In addition, allium also has antimicrobial phenolic compounds (3). Meanwhile, alkaloids and steroids indicate that the plant can be used as an antibacterial agent (30, 31). In this study, the concentration of shallot extract showed varying antibacterial activity against pathogens. Furthermore, according to another previous study (32), the antimicrobial efficacy of plants depends on their chemical structure, active components, and concentration.

A total of 41 compounds were detected in the ethanol extract. Dodecanoic acid, 1,2,3-Propanetriyl Ester (55.26%), 3-Deoxy-D-Mannoic Lactone (8.95%), 5-Hydroxymethylfurfural (4.95%), 4H-Pyran-4-One, 2,3-Dihydro-3,5-Dihydroxy-6-Methyl- (4.75%), and Oxime-, Methoxy-Phenyl- (2.92%) were the most prevalent compounds. Lipids, especially the

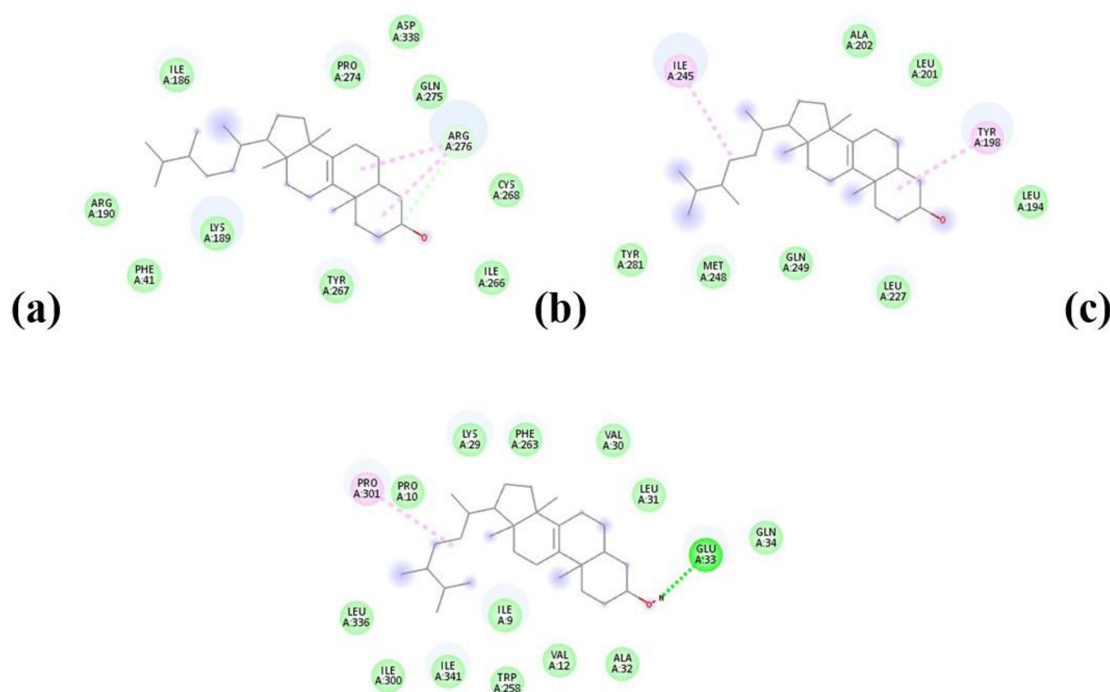

Fig. 5. Interaction of Ergost-8-En-3-Ol,14-Methyl-,(3.Beta.,5.Alpha.) with the receptors gyr B (5l3j) (a) DHPS (1ajz) (b) and PBP (1hd8) (c)

fatty acid class, are compounds whose derivatives are widely detected. The results of this study are the main characteristics of the compounds present in shallots. It is known that lipids are generally a constituent of all plant cells, functioning as membrane components, metabolite storage products, and as a source of energy (33). Bulbs of allium species are widely known to contain oily substances that characterize shallots, including allicin, flavanoids, and their derivatives. These volatile lipid compounds have characteristic odors derived from organic di- and trisulfides and thioaldehydes (34-36). The research related to the composition of shallots is still being explored. This is due to differences in varieties, climate, geography, soil fertility, and cultivation practices, which play an important role in the proportion of compounds in shallots (28).

Antimicrobials made from lipid derivatives exert their effects on different cellular targets in different ways. Generally, the main target is the cell membrane and several parts inside it, such as enzymes and proteins. Fatty acids can disrupt the integrity of cell membranes in various ways, which can lead to the leakage of important cellular metabolites and even cell lysis (18). The inhibitory activity of bacteria on lipid derivatives such as steroids and glycerol, specifically in onion extract, has not been widely reported. However, several studies (37-39), reported that essential oil from shallots can effectively inhibit the activity of pathogenic bacteria.

Molecular docking is a structure-based drug design method that simulates molecular interactions and predicts binding modes and affinities between receptors and ligands (40). The main goal of molecular docking is to obtain an optimized docking conformer for the two interacting molecules to achieve free energy reduction in the whole system. Molecular docking produces the energy value (ΔG^o binding affinity) for forming a bond between the ligand and the target protein (receptor). The smaller the bond energy value shown, the more stable the ligand-receptor bond. Stable bonds indicate that the activity of the results of ligand and receptor interactions is getting bigger (41). A total of five compounds with a high percent area and one steroid compound detected were selected from the GCMS test results to be used as molecular ligands for docking. The receptors used are proteins that are critical targets of antimicrobial agents. Receptor selection is very important as a differentiator from eukaryotic cells to allow selective toxicity. In this study, two types of receptors were used, namely, PBP, which plays an important role in

cell wall formation. DHPS, which contributes to the folate biosynthesis pathway; and DNA gyrase, which is responsible for nucleic acid synthesis (42-44).

Penicillin-binding proteins (PBPs) are essential in bacterial cell wall synthesis, catalyzing both the polymerization of glycan strands and the cross-linking of these chains, alongside hydrolyzing specific peptide bonds (45). The synthesis of peptidoglycan, a crucial cell wall component, is vital for bacterial survival, and compounds targeting PBPs can disrupt this process, leading to bacterial lysis. Additionally, the inhibition of dihydropteroate synthase (DHPS) impairs the folic acid metabolic pathway, crucial for DNA and amino acid synthesis, thereby hindering pathogenic bacterial growth (46). Similarly, targeting topoisomerase II, or DNA gyrase, interferes with DNA replication in pathogenic bacteria, further inhibiting their growth (47). These mechanisms highlight the complex interplay of biochemical pathways in bacterial inhibition and their potential as antibacterial therapy targets.

The molecular docking study revealed that ergost-8-En-3-Ol,14-Methyl-, (3.Beta.,5.Alpha.) exhibited the lowest binding affinity, suggesting it as the most potent inhibitor among the tested compounds from the steroid class. This finding aligns with the results obtained from the TLC-bioautography test, confirming its inhibitory efficacy. Additionally, five other prevalent compounds in shallots demonstrated significant inhibitory potential against *S*. Typhi bacteria. Notably, compounds belonging to the Oxime- and methoxy-phenyl categories were particularly effective in this regard.

Overall, the broad-spectrum efficacy of shallot extracts can be traced back to their rich phytochemical composition. Saponins, flavonoids, and a myriad of other bioactive compounds within shallots have been identified as key antimicrobial agents (18, 19). Such comparisons not only highlight the unique medicinal properties of shallots but also offer a comparative advantage in their utilization over other related species. The significance of shallot extracts extends beyond mere antibacterial action, encompassing wound healing, cytotoxicity against harmful cells, and biofilm inhibition (48, 49). These multifaceted potential positions shallot extracts as a versatile candidate for medical and healthcare applications. The current study's results not only corroborate these documented antibacterial properties but also add a molecular dimension through docking tests, suggesting a targeted mechanism of action at the molecular level, which could pave the way for novel antimicrobial drug development.

CONCLUSION

This study validates the antibacterial potential of shallot (Allium xwakegi Araki.) cultivars from Palu Valley against *Salmonella* Typhi, showcasing significant *in vitro* inhibition and identifying key compounds through GC-MS analysis. Molecular docking highlights Ergost-8-En-3-Ol 14-Methyl- (3.Beta.,5.Alpha.) with notable binding affinity, suggesting a targeted antibacterial mechanism. These findings underscore shallots' value as a natural antibacterial source, meriting further exploration for therapeutic applications. Future research should focus on isolating specific bioactive compounds, advancing our understanding of natural remedies against antibiotic-resistant pathogens.

REFERENCES

1. Saptana, Gunawan E, Perwita AD, Sukmaya SG, Darwis V, Ariningsih E, et al. The competitiveness analysis of shallot in Indonesia: A policy analysis matrix. *PLoS One* 2021; 16(9): e0256832.
2. Park SY, Kim HY. Effect of lyophilized chive (*Allium wakegi* Araki) supplementation to the frying batter mixture on quality attributes of fried chicken breast and tenderloin. *Food Chem* 2022; 13: 100216.
3. Teshika JD, Zakariyyah AM, Zaynab T, Zengin G, Rengasamy KR, Pandian SK, et al. Traditional and modern uses of onion bulb (*Allium cepa* L.): a systematic review. *Crit Rev Food Sci Nutr* 2019; 59(sup1): S39-S70.
4. Zhao X-X, Lin F-J, Li H, Li H-B, Wu D-T, Geng F, et al. Recent advances in bioactive compounds, health functions, and safety concerns of onion (*Allium cepa* L.). *Front Nutr* 2021; 8: 669805.
5. Nassarawa SS, Nayik GA, Gupta SD, Areche FO, Jagdale YD, Ansari MJ, et al. Chemical aspects of polyphenol-protein interactions and their antibacterial activity. *Crit Rev Food Sci Nutr* 2023; 63: 9482-9505.
6. Asmilia N, Abrar M, Fahrimal Y, Sutriana A, Husna Y. Potential of malacca leaf (*Phyllanthus emblica*) against *Salmonella* sp. *E3S Web Confer* 2020; 151: 1-5.
7. Price LB, Hungate BA, Koch BJ, Davis GS, Liu CM. Colonizing opportunistic pathogens (COPs): The

beasts in all of us. *PLoS Pathog* 2017; 13(8): e1006369.
8. Husain DR, Wardhani R. Antibacterial activity of endosymbiotic bacterial compound from Pheretima sp. Earthworms inhibit the growth of *Salmonella* Typhi and *Staphylococcus aureus: in vitro* and in silico approach. *Iran J Microbiol* 2021; 13: 537-543.
9. Breijyeh Z, Karaman R. Design and synthesis of novel antimicrobial agents. *Antibiotics (Basel)* 2023; 12: 628.
10. Buckle GC, Walker CL, Black RE. Typhoid fever and paratyphoid fever: systematic review to estimate global morbidity and mortality for 2010. *J Glob Health* 2012; 2: 010401.
11. Sae-Yun A, Ovatlarnporn C, Itharat A, Wiwattanapatapee R. Extraction of rotenone from Derris elliptica and Derris malaccensis by pressurized liquid extraction compared with maceration. *J Chromatogr A* 2006; 1125: 172-176.
12. Mohammad A, Bhawani SA. Silica thin-layer chromatographic studies of surfactants with mixed aqueous-organic eluents containing thiourea: simultaneous separation of co-existing cetyltrimethylammonium bromide, dodecyltrimethylammonium bromide, and polyoxyethylene (20) sorbitan monolaurate. *J Chromatogr Sci* 2008; 46: 298-303.
13. Harlita TD, Oedjijono, Asnani A. The antibacterial activity of dayak onion (Eleutherine palmifolia (L.) merr) towards pathogenic bacteria. *Trop Life Sci Res* 2018; 29: 39-52.
14. Kumar D, Kumar V, Jangra P, Singh S. Leucas cephalotes (Spreng): photochemical investigation and antimicrobial activity via cylinder-plate method or cup-plate method. *Int J Pharm Sci Res* 2016; 1: 28-32.
15. Insrasari SD, Arofah D, Kristamtini, Sudarmaji, Handoko DD. Volatile compounds profile of some Indonesian shallot varieties. *Earth Environ Sci* 2021; 746: 012009.
16. Husain DR, Wardhani R, Erviani AE. Antibacterial activity of bacteria isolated from earthworm (Pheretima sp.) gut against *Salmonella* Typhi and *Staphylococcus aureus: in vitro* experiments supported by computational docking. *Biodiversitas* 2022; 23: 1125-1131.
17. Azwanida N. A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med Aromat Plants* 2015; 4: 1000196.
18. Fattorusso E, Iorizzi M, Lanzotti V, Taglialatela-Scafati O. Chemical composition of shallot (*Allium ascalonicum* hort.). *J Agric Food Chem* 2002; 50: 5686-5690.
19. Ruksiriwanich W, Khantham C, Muangsanguan A, Chittasupho C, Rachtanapun P, Jantanasakulwong K, et al. Phytochemical constitution, anti-inflammation, anti-androgen, and hair growth-promoting potential of shallot (*Allium ascalonicum* L.) extract. *Plants (Basel)* 2022; 11: 1499.
20. Omoba OS, Olagunju AI, Akinrinlola FO, Oluwajuyitan TD. Shallot-enriched amaranth-based extruded snack influences blood glucose levels, hematological parameters, and carbohydrate degrading enzymes in streptozotocin-induced diabetic rats. *J Food Biochem* 2022; 46(11): e14098.
21. Jalal R, Bagheri SM, Moghimi A, Rasuli MB. Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance. *J Clin Biochem Nutr* 2007; 41: 218-223.
22. Hosseini FS, Falahati-Pour SK, Hajizadeh MR, Khoshdel A, Mirzaei MR, Ahmadirad H, et al. Persian shallot, Allium hirtifolium boiss, induced apoptosis in human hepatocellular carcinoma cells. *Cytotechnology* 2017; 69: 551-563.
23. Yang J, Meyers KJ, van der Heide J, Liu RH. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *J Agric Food Chem* 2004; 52: 6787-6793.
24. Ekowati J, Febriani K, Yaqin IN, Wulandari AA, Mulya IH, Nofianti KA, et al. Shallot skin rofiling, computational evaluation of physicochemical properties, ADMET, and molecular docking of its components against P2Y12 receptor. *J Basic Clin Physiol Pharmacol* 2021; 32: 429-437.
25. Bakht J, Khan S, Shafi M. Antimicrobial potentials of fresh *Allium cepa* against gram positive and gram negative bacteria and fungi. *Pak J Bot* 2013; 45: 1-6.
26. Hamza HJ. Antimicrobial activity of some plant extracts on microbial pathogens isolated from Hilla city hospitals, Iraq. *Med J Babylon* 2015; 12: 398-407.
27. Teshika JD, Zakariyyah AM, Zaynab T, Zengin G, Rengasamy KR, Pandian SK, et al. Traditional and modern uses of onion bulb (*Allium cepa* L.): a systematic review. *Crit Rev Food Sci Nutr* 2019; 59(sup1): S39-S70.
28. Matsuse K, Abdelrahman M, Ariyanti NA, Tsuji F, Hirata S, Nakajima, et al. Targeted metabolome profiling of Indonesian Shallots and Japanese long-day/short-day bulb onions. *Metabolites* 2022; 12: 1260.
29. Kyung KH. Antimicrobial properties of allium species. *Curr Opin Biotechnol* 2012; 23: 142-147.
30. Yan Y, Li X, Zhang C, Lv L, Gao B, Li M. Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics (Basel)* 2021; 10: 318.
31. Vollaro A, Esposito A, Antonaki E, Iula VD, D'Alonzo D, Guaragna A, et al. Steroid derivatives as potential antimicrobial agents against *Staphylococcus aureus* planktonic cells. *Microorganisms* 2020; 8: 468.
32. Bassolé IH, Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules* 2012; 17: 3989-4006.
33. Chandrasekaran M, Kannathasan K, Venkatesalu V. Antimicrobial activity of fatty acid methyl esters of

some members of chenopodiaceae. *Z Naturforsch C J Biosci* 2008; 63: 331-336.
34. Starkenmann C, Niclass Y, Troccaz M. Nonvolatile S-Alk(en) ylthio-L-cysteine derivatives in fresh Onion (*Allium cepa* L. Cultivar). *J Agric Food Chem* 2011; 59: 9457-9465.
35. Breu W. *Allium cepa* L. (Onion) Part 1: Chemistry and analysis. *Phytomedicine* 1996; 3: 293-306.
36. Desbois AP. Potential applications of antimicrobial fatty acids in medicine, agriculture and other industries. *Recent Pat Antiinfect Drug Discov* 2012; 7: 111-122.
37. Bag A, Chattopadhyay RR. Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. *PloS One* 2015; 10(7): e0131321.
38. Ye CL, Dai DH, Hu WL. Antimicrobial and antioxidant activities of the essential oil from onion (*Allium cepa* L.). *Food Control* 2013; 30: 4853.
39. Abdel-Salam AF, Shahenda ME, Jehan BA. Antimicrobial and antioxidant activities of red onion, garlic and leek in sausage. *Afr J Microbiol Res* 2014; 8: 2574-2582.
40. Guedes IA, de Magalhães CS, Dardenne LE. Receptor–ligand molecular docking. *Biophys Rev* 2014; 6: 75-87.
41. Agarwal S, Chadha D, Mehrotra R. Molecular modeling and spectroscopic studies of semustine binding with DNA and its comparison with lomustine–DNA adduct formation. *J Biomol Struct Dyn* 2015; 33: 1653-1668.
42. Morgan RE, Batot GO, Dement JM, Rao VA, Eadsforth TC, Hunter WN. Crystal structures of Burkholderia cenocepacia dihydropteroate synthase in the apo-form and complexed with the product 7, 8-dihydropteroate. *BMC Struct Biol* 2011; 11: 21.
43. Hevener KE, Yun M-K, Qi J, Kerr ID, Babaoglu K, Hurdle JG, et al. Structural studies of pterin-based inhibitors of dihydropteroate synthase. *J Med Chem* 2010; 53: 166-177.
44. Yogavel M, Nettleship JE, Sharma A, Harlos K, Jamwal A, Chaturvedi R, et al. Structure of 6-hydroxymethyl-7, 8-dihydropterin pyrophosphokinase–dihydropteroate synthase from Plasmodium vivax sheds light on drug resistance. *J Biol Chem* 2018; 293: 14962-14972.
45. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: Structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev* 2008; 32: 234-258.
46. Azam MA, Thathan J, Jubie S. Dual targeting DNA gyrase B (GyrB) and topoisomerse IV (ParE) inhibitors: a review. *Bioorg Chem* 2015; 62: 41-63.
47. Rajendram M, Hurley KA, Foss MH, Thornton KM, Moore JT, Shaw JT, et al. Gyramides prevent bacterial growth by inhibiting DNA gyrase and altering chromosome topology. *ACS Chem Biol* 2014; 9: 1312-1319.
48. Karunanidhi A, Ghaznavi-Rad E, Hamat RA, Pichika MR, Lung LTT, Mohd Fauzi F, et al. Antibacterial and antibiofilm activities of nonpolar extracts of Allium stipitatum Regel. against multidrug resistant bacteria. *Biomed Res Int* 2018; 2018: 9845075.
49. Aghdam SZ, Minaeian S, Karimi MS, Bafroee AST. The antibacterial effects of the mixture of silver nanoparticles with the shallot and nettle alcoholic extracts. *J Appl Biotechnol Rep* 2019; 6: 158-164.