Computational and *in vitro* analyses of the antibacterial effect of the ethanolic extract of *Pluchea indica* L. leaves

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Abstract. The most common gram-negative, *Escherichia coli*, and gram-positive bacteria, *Bacillus* spp., have evolved different mechanisms that have caused the emergence of multi-drug resistance. As a result, drugs that block the bacterial growth cycle are needed. Here, *in silico* and *in vitro* studies were performed to assess compounds in the *Pluchea indica* leaf extract, a medicinal plant, that can inhibit bacterial proteins. Briefly, *P. indica* leaves were extracted using ethanol. The crude extract was then subjected to gas chromatography-mass spectrometry for metabolite screening. Molecular docking simulations with rhomboid protease (R^{pro}) (Protein data bank ID number: 3ZMI from *E. coli* and filamenting temperature-sensitive mutant Z (FtsZ) protein data bank ID number: 2VAM from *Bacillus subtilis* were performed. Moreover, the well diffusion method was used to confirm the antibacterial activity of *P. indica* leaf extract. A total of 10 compounds were identified in the *P. indica* extract and used for computational analysis. Based on drug-likeness prediction, *P. indica* compounds may be drug-like molecules. Binding affinity tests indicated that 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol and 11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol had the most negative values. Accordingly, these compounds may be potential ligands that bind to bacterial proteins. The root mean square fluctuation values was <2 Å, indicating stable fluctuation binding for the ligand-protein complex. According to *in vitro* antibacterial assays, a high concentration (50%) of the *P. indica* extract markedly inhibited *E. coli* and *B. subtilis*, with inhibitory zone diameters of 31.86±1.63 and 21.09±0.09 mm, respectively. Overall, the compounds in the *P. indica* leaf extract were identified as functional inhibitors of *E. coli* and *B. subtilis* proteins via *in silico* analysis. This may facilitate development of antibacterial agents.

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Introduction

Humans are surrounded by microorganisms, such as bacteria, fungi, protozoa, parasites and viruses (1,2). Commensal bacterial flora, such as *Escherichia coli*, also reside in the human digestive system; however, the spread of this bacteria to other body parts, including the bloodstream, induces pathogenicity (3,4), which ultimately causes pathogenic infections.

Pathogenic microorganisms invade living organisms with rapid self-division and acquire adaptability in new environments, including the human body, and cause contagious diseases. Antibiotics have been used worldwide to cure bacterial infections (2,5). However, bacteria can evade antibiotics via mutations, leading to resistance (6).

Commercial antibiotics are a major risk factor for antimicrobial resistance (AMR) due to overused and other reason was microorganism mutations has taken place spontaneously (7). Other associated external factors contribute to AMR, such as overcrowded living conditions and the consumption of livestock treated with antibiotics (3,4). Most external infections that occur due to *Bacillus* spp. are known as food poisoning (8).

The development of new antibacterial agents can address the AMR crisis (5). However, antimicrobial consumption must first be monitored as prolonged use may trigger the occurrence of AMR (9). The discovery of novel antibacterial agents

from natural products, particularly plants, must be sustained. Many reports on plant-derived compounds serve as a basis for new drug development (1,10). Notably, the use of whole herbs induces an enhanced effect compared with single compounds; for example the combination compounds of *Ziziphus jujuba* polysaccharide and ginger 6-gingerol has synergistic effect on antioxidant and anticancer, than their dose alone (11,12).

Pluchea indica (L.) Less from the Asteraceae family is a native Indonesian plant also found in India and Thailand (13,14). *P. indica* exerts beneficial effects, including antidiabetic (14), antifungal (15), anti-*Mycobacterium tuberculosis* (16), antimicrobial (17) and wound healing (18) effects. In the present study, a computational pharmacology network analysis was performed with molecular docking of *P. indica* compounds as potential antibacterial agents against *E. coli* and *B. subtilis*. The present study aimed to highlight promising compounds in the ethanolic extract of *P. indica* that can be explored in drug discovery.

Materials and methods

Plant collection and identification. Dry *P. indica* was obtained from the Medicinal Plant Garden (Surabaya, Indonesia) and validated at the Plant Systematic Laboratory (Universitas Airlangga, Surabaya, Indonesia). A voucher specimen was deposited at the Plant Systematic Laboratory (no. PI0126012024).

Extraction. The leaves of *P. indica* were air-dried, ground into a powder (20 mesh size) and macerated in absolute ethanol (Pro Analysis; Merck KGaA) at a ratio of 1:10. The maceration process was performed at room temperature (28±2˚C) for 24 h. The extracted products were filtered using a filter paper, evaporated using a rotary evaporator at 60˚C, weighed to determine the yield and stored at 4˚C, as previously described (19).

Compound profiling via gas chromatography-mass spectrophotometry (GC-MS). Compound profiles of ethanolic extracts of *P. indica* leaves were determined using GC-MS. GC-MS analysis was performed using an Agilent GC-MSD (Agilent Technologies Deutschland GmbH; cat. no. 19091S-433UI) equipped with a capillary column (30.00 m x 250.00 μm x 0.25 μm) and a mass detector in electron impact mode with full scan (50,550 atomic mass unit). Helium was used as the carrier gas at a flow rate of 3 ml/min (total flow rate, 14 ml/min). The injector temperature was 280˚C and the oven temperature ranged from 60 to 250˚C. Peaks in the chromatograms were identified using the mass spectra. Chemicals were identified by comparing mass spectra to those in a Standard Reference Database (version 02. L, National Institute of Standards and Technology). Components with quality scores >80% were selected. The relative proportion of each component was estimated from the overall peak area in the chromatograph, as previously described (20).

In silico pathway analysis of antimicrobial compounds

Sample retrieval. The following compounds were collected via GC-MS: Limonene oxide, cis-, ethyltetramethylcyclopentadiene; hexadecanoic acid, methyl ester; 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol; 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol; 8,11-octadecadienoic acid, methyl ester; 9,12,15-octadecatrienoic acid, methyl ester; phytol; silane, [(methylsilyl) methyl](silylmethyl)- and n-hexadecanoic acid. PubChem database (pubchem.ncbi.nlm.nih.gov/) was used to retrieve the compound ID number (CID), simplified molecular input line entry system (SMILE) Canonical and 3D files in sdf format for the ligands (21). The Protein Data Bank (PDB) file was input in Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) database (rcsb.org/) for target preparation, which involved *B. subtilis*-Filamenting temperature-sensitive mutant Z (FtsZ) (Z ring) and *E. coli*-Rhomboid protease (R^{pro}). Water molecules and native ligands on targets were removed using PyMOL software v.2.5.2 (Schrödinger, Inc.) under an academic license (21,22).

Drug-likeness prediction. Compounds were assessed as drug-like molecules by referring to drug-likeness rules, such as Lipinski (23), Ghose (24), Veber (25), Egan (26) and Muegge (27). Bioavailability score was used to identify the ability of candidate drug molecules to circulate in the body. Drug-likeness analysis was performed using SwissADME (swissadme.ch/) (20,28).

Ligand-protein docking. The binding activity of the ligand to the target and the interaction pattern were identified using molecular docking. Docking was performed to determine the antibacterial potential and inhibitor mechanism between *P. indica* compounds and targets. Of note, the grid covered the entire surface of the target. In addition, the docking grid consisted of FtsZ: Center(Å) X:28.189 Y: -7.627 Z: -4.629 dimensions (Å) X: 26.235 Y: 21.066 Z: 29.239 & R^{pro}: Center (Å) X:15.407 Y: -11.974 Z: 43.963 dimensions (Å) X: 28.085 Y: 21.013 Z: 38.783. The docking simulation was performed using PyRx 0.9.9 software (Scripps Research) under an academic license (29,30).

Chemical interaction. The molecular interactions between the molecular complexes from docking simulation were identified using Discovery Studio Visualizer™ v.16.1 (Dassault Systèmes SE). Weak bond interactions, such as van der Waals, hydrogen, hydrophobic, π-alkyl, and electrostatic interactions, were formed in the ligand-protein complex. These interactions contribute to initiation of an inhibitory response of the ligand toward specific target domains (31).

Molecular dynamic simulation. Molecular stability analysis or docking validation was conducted using molecular dynamics simulations in CABS-flex-2 (biocomp.chem.uw.edu.pl/CABSflex2). Molecular stability was displayed using a root mean square fluctuation (RMSF) graph. To achieve molecular stability, the protein-ligand complex must have RMSF value <3 Å (32,33).

Antimicrobial activity assessment

Media and inoculum preparation. Two bacterial strains, *B. subtilis* (cat. no. 1248) was purchased from Thailand Institute of Scientific and Technology Research and *E. coli* (cat. no. 25922) was purchased from The American Type Culture Collection, were tested using antimicrobial assays. Solid and liquid media were used to conduct antimicrobial experiments and maintain bacterial cultures. Nutrient agar (NA; Merck, Germany) was used as the solid medium to test

Table I. GC-MS analysis of *Pluchea indica* ethanolic extract.

Peak	RT	Compound	Molecular formula	Molecular weight, g/mol	Chromatogram peak area, %
1	31.468	Limonene oxide, cis-	$C_{10}H_{16}O$	152.23	2.34
2	32.670	Ethyltetramethylcyclopentadiene	$C_{11}H_{18}$	150.26	1.32
3	35.541	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	3.88
4	37.221	10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol	$C_{15}H_{24}O$	220.35	1.83
5	37.313	11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol	$C_{15}H_{24}O$	220.35	4.70
6	40.399	8,11-Octadecadienoic acid, methylester	$C_{19}H_{34}O_2$	294.50	2.44
7	41.527	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.50	3.82
8	42.213	Phytol	$C_{20}H_{40}O$	296.53	9.33
9	45.147	Silane, [(methylsilyl)methyl](silylmethyl)-	$C_3H_7Si_3$	127.34	3.36
10	46.857	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	27.36

RT, retention time.

antibacterial activity. NA was used in the reaction tubes to maintain the microbial culture. Nutrient broth (NB, Merck, Germany) was employed as liquid media for bacterial strain subcultures and precultures. The bacterial strains were pre-cultured in culture bottles containing sterile NB in a 37˚C incubator for 24 h, then diluted to 10% cultures before inoculation. The absorbance of the 10% bacterial culture was adjusted with sterile water using a spectrophotometer to meet the 0.5 McFarland standard (19).

Well diffusion assay. Well diffusion assay was performed to determine the antimicrobial activity based on diameter of the inhibitory zone (DIZ) on the surface of NA. A total of 10 ml NA was placed in a sterile Petri dish and allowed to harden. Thereafter, 30 ml NA was added. Following cooling (25±2˚C) of the second layer, 1 ml bacterial culture was added to the NA and the second layer was allowed to completely solidify. A total of four wells was created on the second layer of the film for samples and controls. Extracts diluted in 10% DMSO (250 and 500 mg/ml) served as the samples, 10% DMSO served as the negative control and chloramphenicol (1000 μg/ml) served as the positive control. Each well received 10 μl diluted samples; four replicates were prepared. The plates were incubated at 37˚C for 24 h. DIZ was measured using Vernier calipers and the mean was calculated and compared with that of the positive control, as previously described (20). The percentage of inhibition (PI) was calculated as follows: PI (%)=mean DIZ of the extract/DIZ of positive control x 100% (1). The antimicrobial data were obtained from three independent experiments and are expressed as the mean ± standard deviation.

Results

Extraction product and compound identification. The ethanolic extraction of *P. indica* dried leaves yielded ~17.79% crude extract from 88.8 g raw material (dried leaves). A total of 12 compounds, was identified via GC-MS. (Table I). Hexadecanoic acid methyl ester, 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol, phytol, silane and n-hexadecanoic acid had a quality score >80% and higher peak area than other compounds identified via GC-MS (Fig. 1).

Computational analysis using molecular docking. The compounds were confirmed using CID, with information on canonical SMILE and 2-dimentional (2D) structures from the PubChem (Table II). Two targets, FtsZ from *B. subtilis* and R^{pro} protein from *E. coli*, were employed (Fig. 2). Before compound processing with the target via molecular docking, all compounds were checked for drug-likeness to assess similarity to an existing drug. Rules, such as Lipinski's Rule of Five (Ro5), Ghose, Veber, Egan and Muegge, were also applied. Notably, all compounds in the *P. indica* extract were predicted to be drug-like molecules with a bioavailability score of 0.55, except n-hexadecanoic acid (Table III).

Interactions of targets from bacterial proteins and ligands from all *P. indica* compounds were subjected to computational analysis using molecular docking. *P. indica* compounds with the most negative binding affinities were 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol and 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol (both-6.0 kcal/mol) on FtsZ (PDB ID: 2VAM) for *B. subtilis* and 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.beta.-ol on R^{pro} (PDB ID: 3ZMI) for *E. coli* (-7.8 kcal/mol; Table IV). Molecular interaction between targeted proteins and the pocket-binding domain of ligands from *P. indica* was performed and visualized as 3D structure. Compounds with the most negative binding affinities were 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol and 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol (Fig. 2). The analysis also revealed that the ligands formed weak bonds, such as van der Waals and π-alkyl bonds for 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.β.-ol, on FtsZ-*B. subtilis and* R^{pro}-*E. coli*. For 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol, the weak bonds were van der Waals, hydrogen and alkyl bonds in FtsZ-*B. subtilis* (Fig. 3; Table V).

To determine the stability of protein-ligand binding, docking validation was performed via molecular dynamics

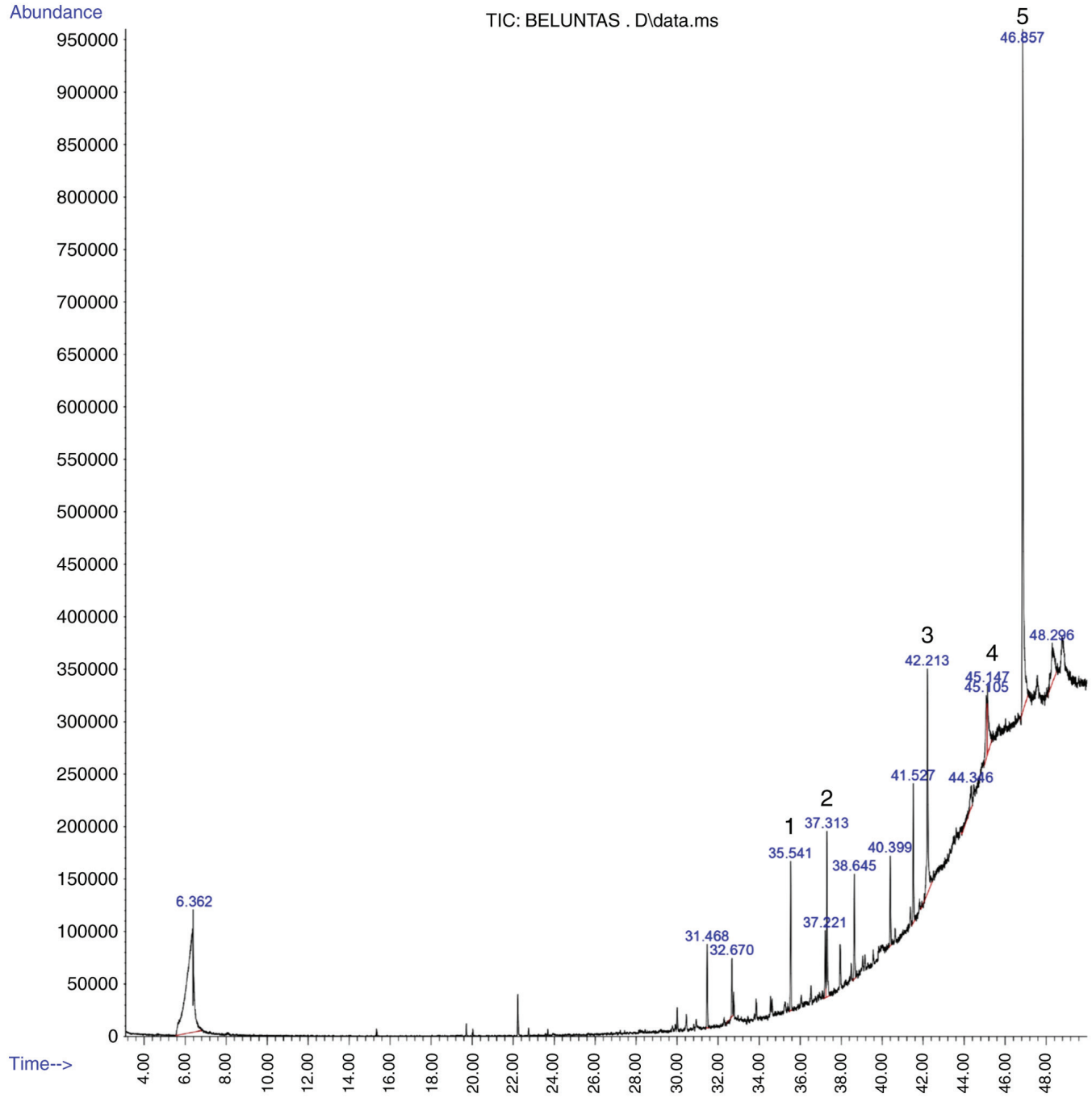
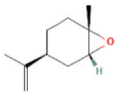
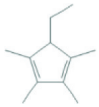
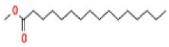
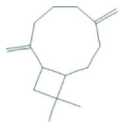
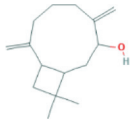
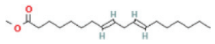
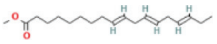


Figure 1. Gas chromatography-mass spectrometry chromatogram of *Pluchea indica* ethanolic extract. 1, hexadecanoic acid, methyl ester; 2, 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol; 3, phytol; 4, silane; 5, n-hexadecanoic acid.

simulation by referring to the RMSF value. The RMSF values from binding site on ligand interaction domains were <2 Å, indicating stability. The RMSF value was 0.608 Å for the 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol_FtsZ complex, 0.157 Å for the 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol_FtsZ complex, and 1.095 Å for the 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol_R^{pro} complex (Table V). The molecular interactions were due to van der Waals (Asn25, Asp187, Gly22, Gly21, Arg143, Gly104, Gly107, Met105, Glu139, Asn166, Thr133) and π-alkyl bonds (Phe183, Pro135) in the 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.β-ol_FtsZ complex; van der Waals bonds (Ser201, Asn154, Phe153, His150, Gly240, Ala239) and π-alkyl bonds (Val204, Trp157, His254, Trp236, Tyr205, Met149) in the 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol_FtsZ complex and van der Waals (Glu34, Asp199, Val35, Thr203, Gln36, Ile298, Asn301, and Gln195), hydrogen (Asn299 and Glu300) and alkyl bonds (Val297) in the 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.β-ol_R^{pro} complex (Fig. 3). Fig. 4 shows the structural fluctuations and RMSF graph of the target protein.

In vitro antibacterial activity based on well diffusion assay. The antibacterial activity of *P. indica* extract was determined using the well diffusion method. Notably, 50% *P. indica*

Table II. Compounds extracted from *Pluchea indica*.

Compound	PubChem CID no.	Compound structure	2D structure
Limonene oxide, cis-	6452061	$CC(=C)C_1CCC_2(C(C_1)O_2)C$	
Ethyltetramethylcyclopentadiene	585272	$CCC_1C(=C(C(=C_1C)C)C)C$	
Hexadecanoic acid, methyl ester	8181	$CCCCCCCCCCCCCCCC(=O)OC$	
10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.β.-ol	577397	$CC_1(CC2C_1CCC(=C)CCCC_2=C)C$	
11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0_undecan-3-ol	91715484	$CC_1(CC_2C_1CC(C(=C)CCCC_2=C)O)C$	
8,11-Octadecadienoic acid, methyl ester	5319737	$CCCCCCC=CCC=CCCCCCCC(=O)OC$	
9,12,15-Octadecatrienoic acid, methyl ester	5367462	$CCC=CCC=CCC=CCCCCCCCC(=O)OC$	
Phytol	5280435	$CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C$	
Silane, [(methylsilyl)methyl](silylmethyl)-	6329174	$C(Si)C(Si)C(Si)$	
n-Hexadecanoic acid	985	$CCCCCCCCCCCCCCCC(=O)O$	

CID, compound ID number.

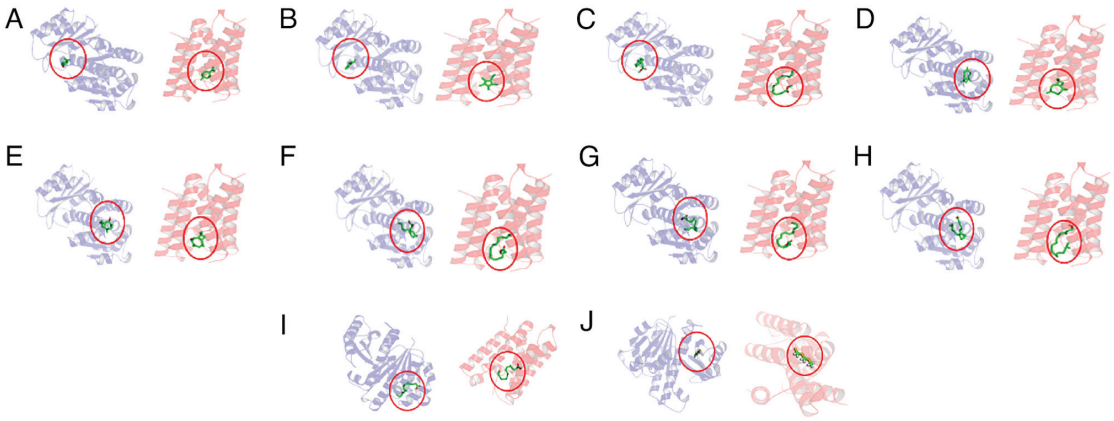


Figure 2. Molecular visualization of compounds from *Pluchea Indica* and targets. (A) Limonene oxide, cis-. (B) Ethyltetramethylcyclopentadiene. (C) Hexadecanoic acid, methyl ester. (D) 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol. (E) 11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol. (F) 8,11-Octadecadienoic acid, methyl ester. (G) 9,12,15-Octadecatrienoic acid, methyl ester. (H) Phytol. (I) n-Hexadecanoic acid. (J) Silane, [(methylsilyl)methyl](silylmethyl)-. Green, compound; circle, *Pluchea indica;* blue, FtsZ-*Bacillus subtilis;* red, R^{pro}-*Escherichia coli*. FtsZ, filamenting temperature-sensitive mutant Z; R^{pro}, Rhomboid protease.

extract exhibited the strongest inhibitory activity against *E. coli* growth (DIZ, 31.86±1.63 mm), with PI >80% that of the positive control (chloramphenicol). Moreover, 25% extract exhibited strong inhibitory activity (DIZ, 21.29±1.02 mm), with PI >~50% (Table VI). The 50% extract caused a larger DIZ than the 25% extract against *B. subtilis*, with PI >40% at

Table III. *Pluchea indica* compounds predicted as drug-like molecules.

Compound	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability score
Limonene oxide, cis-	Pass	Fail	Pass	Pass	Fail	0.55
Ethyltetramethylcyclopentadiene	Pass	Fail	Pass	Pass	Fail	0.55
Hexadecanoic acid, methyl ester	Pass	Fail	Fail	Pass	Fail	0.55
10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol	Pass	Pass	Pass	Pass	Fail	0.55
11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol	Pass	Pass	Pass	Pass	Fail	0.55
8,11-Octadecadienoic acid, methyl ester	Pass	Fail	Fail	Fail	Fail	0.55
9,12,15-Octadecatrienoic acid, methyl ester	Pass	Fail	Fail	Pass	Fail	0.55
Phytol	Pass	Fail	Fail	Fail	Fail	0.55
Silane, [(methylsilyl)methyl](silylmethyl)-	Pass	Fail	Pass	Pass	Fail	0.55
n-Hexadecanoic acid	Pass	Pass	Fail	Pass	Fail	0.85

Table IV. Binding affinity from the docking analysis.

Compound	Binding affinity, kcal/mol	
	Bacillus subtilis (FtsZ PDB ID: 2VAM)	*Escherichia coli* (R^{pro} PDB ID: 3ZMI)
Limonene oxide, cis-	-5.8	-6.8
Ethyltetramethylcyclopentadiene	-5.4	-6.3
Hexadecanoic acid, methyl ester	-4.9	-5.8
10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol	-6.0	-7.8
11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol	-6.0	-7.2
8,11-Octadecadienoic acid, methyl ester	-4.8	-5.6
9,12,15-Octadecatrienoic acid, methyl ester	-4.7	-6.1
Phytol	-4.7	-6.1
n-Hexadecanoic acid	-5.0	-5.5
Silane, [(methylsilyl)methyl](silylmethyl)-	-4.5	-5.0

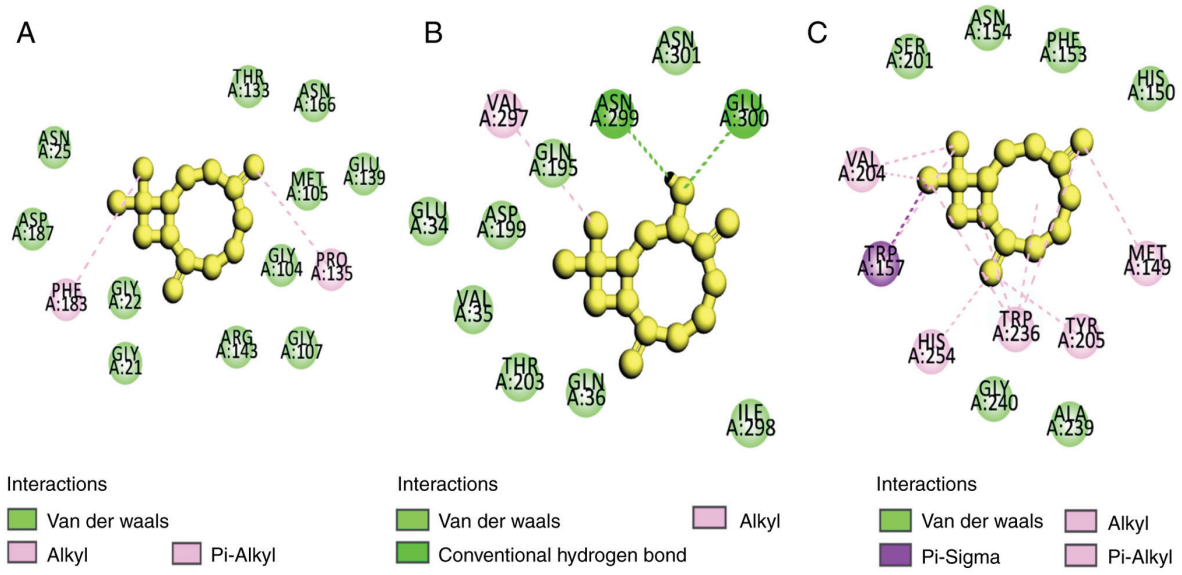


Figure 3. Molecular interaction of ligand (yellow line) and target domain (circle). (A) 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol_FtsZ. (B) 11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol_FtsZ. (C) 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol_R^{pro}. FtsZ, filamenting temperature-sensitive mutant Z; R^{pro}, Rhomboid protease.

Table V. Molecular interaction and dynamic analysis of antimicrobial candidate compounds

Compound	Target	Ligand interaction domain	RMSF, Å		
			Binding site	Mean value of amino acid residues	
10,10-Dimethyl-2,6-dimethylenebicyclo (7.2.0)undecan-5.β.-ol	FtsZ PDB ID: 2VAM (*B. subtilis*)	van der Waals: Asn25, Asp187, Gly22, Gly21, Arg143, Gly104, Gly107, Met105, Glu139, Asn166, Thr133 π-alkyl: Phe183, Pro135	van der Waals: 0.517, 0.423, 0.390, 0.198, 0.730, 0.292, 1.251, 0.760, 1.295, 0.513, 0.281 π-alkyl: 0.688, 0.564	0.608	
	R^{pro} PDB ID: 3ZMI (*E. coli*)	van der Waals: Ser201, Asn154, Phe153, His150, Gly240, Ala239 π-alkyl: Val204, Trp157, His254, Trp236, Tyr205, Met149	van der Waals: 0.142, 0.384, 0.238, 1.014, 0.726, 0.897 π-alkyl: 0.726, 0.352, 0.343, 0.623, 0.116, 0.640	0.157	
11,11-Dimethyl-4,8-dimethylenebicyclo (7.2.0)undecan-3-ol	FtsZ PDB ID: 2VAM (*B. subtilis*)	van der Waals: Glu34, Asp199, Val35, Thr203, Gln36, Ile298, Asn301, Gln195 Hydrogen: Asn299, Glu300 Alkyl: Val297	van der Waals: 1.323, 1.180, 0.912, 1.462, 1.107, 0.707, 2.226, 0.099 Hydrogen: 0.964, 1.615 Alkyl: 0.449	1.095	

B. subtilis, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; FtsZ, filamenting temperature-sensitive mutant Z; R^{pro}, Rhomboid protease.

both concentrations compared with that of the positive control (Fig. 5). Therefore, the higher the concentration of the extract, the greater the antibacterial activity.

Discussion

To the best of our knowledge, novel drugs from plant-derived compounds have not been developed recently. The field of ethnopharmacology, which involves use traditional medicinal plants, can be applied in modern medical practice as therapeutic agent (34,35). *P. indica* was used as an antibacterial agent in the present study. Various factors, including solvents, can affect the proportion of bioactive compounds in an extract (36,37). In the present study, *P. indica* was extracted with ethanol, a universal solvent, to obtain bioactive compounds with antibacterial properties. Ethanol extract of *P. indica* is an antibacterial agent against *B. cereus*, *E. coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Salmonella typhimurium* (38).

In the present study, *E. coli* was employed as a representative gram-negative bacterium that commonly causes infection when it occupies the gastrointestinal and urinary systems, leading to individuals becoming immunocompromised (1,39). Gram-positive bacteria, including *Bacillus* spp., are commonly detected in the blood, stool and respiratory systems of infected patients (8). One of the 29 strains of *B. subtilis* was previously identified as MDR, with high resistance to norfloxacin (40).

Plant-derived compounds have gained popularity in drug development owing to minimal side effects on human health (41). Phytochemicals in *P. indica* extract have been demonstrated to exhibit notable wound-healing activity (18) and anti-venom potential (42). Recently, to accelerate drug discovery, conventional methods, such as high-throughput screening (HTS) and virtual HTS, have been developed (16). HTS frequently produces bulky hydrophobic metabolites that are poorly suited to chemical alterations (16). Thus, *in silico* docking studies are a bioinformatics tool to define the binding form and binding affinity score (43).

The principal mechanism of docking involves identifying plant metabolites as candidates for drug improvement via binding to the protein target of a cell (16,43,44). In the present study, computational analysis using docking simulations required bacterial protein targets. Several subcellular protein targets are present in *B. subtilis* and *E. coli*, including Murein cluster e-B protein (MreB), MreC, The partitioning motor protein (ParM), Z-associated protein D (ZapD) and FtsZ (45). MreB and MreC localize in a helical pattern along the longitudinal axis of the entire cell (46,47), ParM is present in intercellular filaments along the cell length (48) and ZapD is focally localized to the mid-cell of the septum in an FtsZ-dependent manner (49).

FtsZ protein plays a crucial role in bacterial division and is arranged in a large protein complex in the middle of dividing cells called divisomes (50). As the regulation and function of the divisome are dependent on FtsZ, this protein is the first to be recruited for division (51). Inhibition of FtsZ may be a promising approach to combat antibiotic resistance. FtsZ is conserved in most bacteria but absent in eukaryote cells. The structures of FtsZ protein are available in the PDB (ID numbers, such as PDB ID: 2VXY, 3VPA, 4DXD, 5H5G) and ID 2VAM, which identified the FtsZ protein from *B. subtilis*

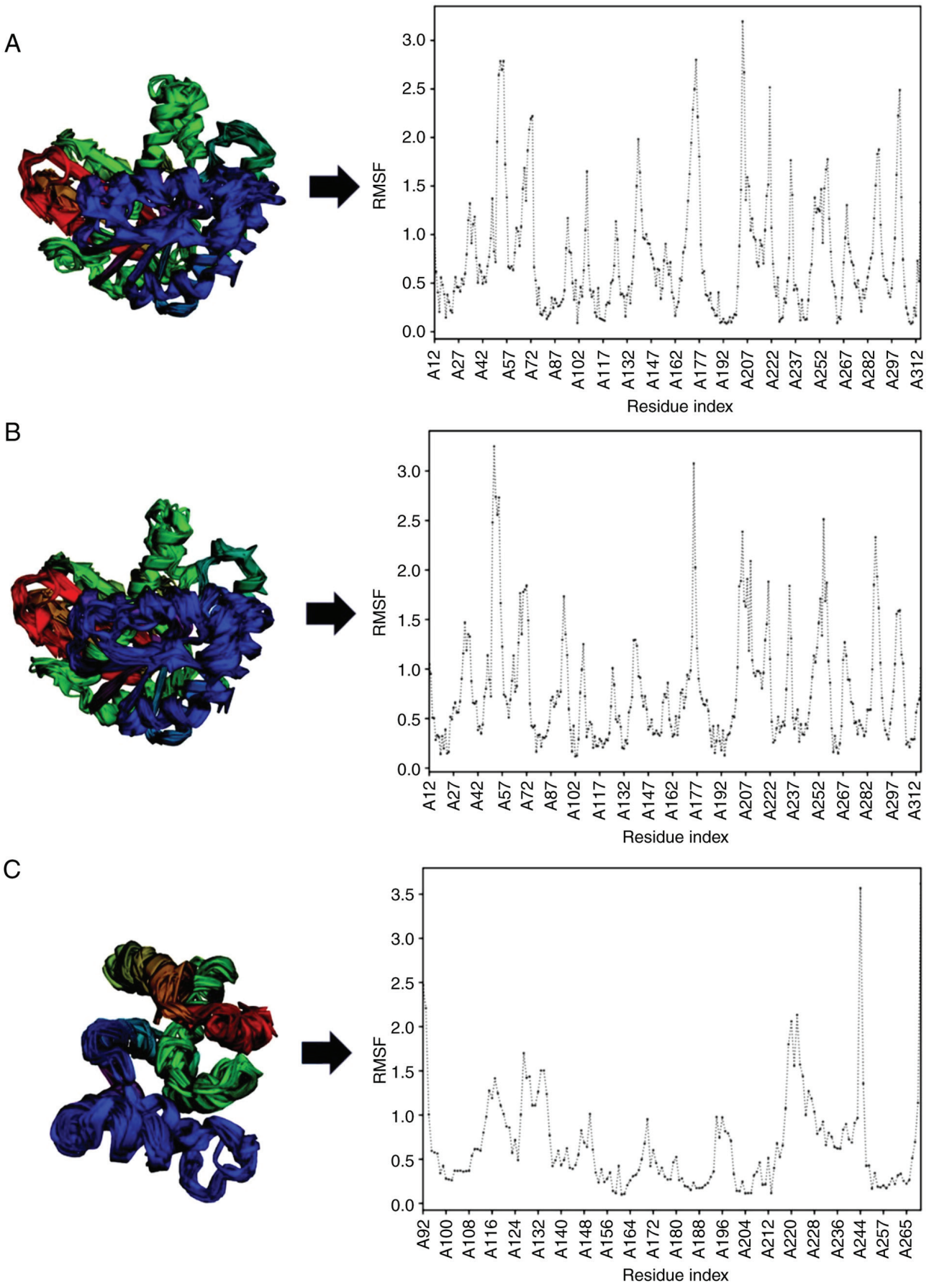


Figure 4. Molecular dynamic simulation plot of ligand-protein interaction stability and 3D protein fluctuation structure. (A) 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol_FtsZ; (B) 11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol_FtsZ. (C) 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol_R^{pro}. FtsZ, filamenting temperature-sensitive mutant Z; RMSF, Root mean square fluctuation; R^{pro}, Rhomboid protease.

Table VI. Well diffusion of *Pluchea indica* ethanolic extract.

	DIZ, mm		PI, %	
Group	*E. coli*	*B. subtilis*	*E. coli*	*B. subtilis*
25% extract	21.29±1.02	17.76±1.23	55.60	44.53
50% extract	31.86±1.63	21.09±0.09	82.13	50.74
Chloramphenicol	38.79±0.43	39.88±0.32	100.00	100.00

E. coli, Escherichia coli; B. subtilis, *Bacillus subtilis*; DIZ, diameter of the inhibitory zone; PI, percentage inhibition.

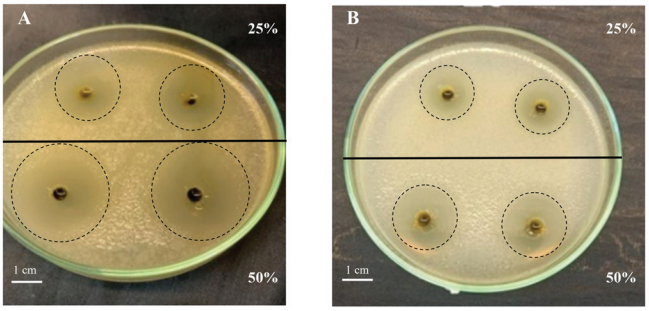


Figure 5. Antimicrobial activity of *Pluchea indica* ethanol extract against bacteria. Dashed line presented as inhibited growth zone. (A) *Escherichia coli*. (B) *Bacillus subtilis*.

as the protein target in this present study (51). R^{pro} is an intramembrane protease implicated in critical regulation of different cellular signaling processes (52,53). Thus, the inhibition of these bacterial proteins inhibits growth (52).

Computational approaches to drug discovery have led to the development of alternative tools to decrease costs and determine the effectiveness of potential drug candidates. Computational screening from ligand-based sources has been performed to identify potential compound inhibitors for drug repurposing (16,43). However, information such as CID, (SMILE) Canonical and 3D files about the candidate ligands must first be obtained. The present study revealed 10 compounds in the *P. indica* leaf extract using GC-MS screening.

Drug-likeness analysis can assist in increasing probability of a natural chemical progressing through clinical trials (54). The present study used Lipinski's Ro5, Ghose, Veber and Muegge. Ro5 assesses lipophilicity (LogP), molecular mass, hydrogen bonding and molar refractivity (55). Veber measures oral bioavailability based on molecular weight, topological surface area and hydrogen and rotatable bonds. Ghose evaluates drug likeness using LogP, refractivity and the number of atoms. Muegge evaluation predicts drug-likeness using drug databases and pharmacophore calculations (56,57). Two compounds, 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.beta.-ol and 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol, passed four (Lipinski, Ghose, Veber, and Egan) of these rules. In this analysis, a compound must pass at least one rule to proceed to the next step in the molecular docking and dynamic simulation. Implementation of drug-likeness rules improves physicochemical and pharmacokinetic profiles of active substances (58,59).

The Veber's rule-based bioavailability score, which indicates the capacity for absorption and circulation, determines the pharmacokinetic profile of drug-like molecules. Here, the best performance indicator of antibacterial drug effectiveness against target microorganisms, such as *E. coli* and *B. subtilis*, had a bioavailability score of 0.55 (60,61).

Ligands form interaction patterns or pocket-binding regions on weakly bonded targets. The binding affinity is the binding energy of the ligand during its interaction with the target. According to Gibbs' rule, a lower binding affinity value indicates increased ligand activity, which means the compound with the most negative value is the predicted ligand (62,63).

Candidate antimicrobial agents with inhibitory activity should have a lower (the most negative) binding affinity (64-66). Based on docking simulation, 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol and 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol from *P. indica* may be antimicrobial drugs with inhibitory activities against FtsZ and R^{pro} proteins. 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β.-ol is a terpenoid derivative found in the extracts of *Mammea siamensis* flower and young leaves (67); this compound is also one of the volatile compounds found in the *Artemisia argyi* fruit extract (68). 11,11-Dimethyl-4,8-dimethylenebicyclo(7.2.0) undecan-3-ol (bicyclo (7.2.0) undecan-3-ol) and 11,11-dimethyl-4,8-bis(methylene)-are volatile compounds in *Achillea millefolium* essential oil (69), propolis (70), and this compound in *Syzygium aromaticum* extract has potential as an antifungal and nematicidal (71). Notably, 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol is positively associated with antibacterial activity (72).

Further analysis revealed that these two ligands could form van der Waals, hydrogen, and π-alkyl bonds. Weak-bond interactions in protein inhibitors, serve a role in promoting biological responses such as disrupt the regulate enzyme, interfering the metabolic role and blocking or slowing enzymatic function. The existence of van der Waals, hydrogen, hydrophobic, π-alkyl, and electrostatic interactions can increase stability of ligand-protein interactions (73).

In the present study, docking validation was performed through molecular dynamics simulation of a ligand-protein complex. The molecular complexes, 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.-ol_FtsZ, 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol_FtsZ, and 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0)undecan-5.β-ol_R^{pro}, were identified based on their molecular stability. RMSF of molecular complexes with stable fluctuations is <3 Å (74,75).

In vitro, higher concentrations of the extract induced stronger antibacterial activity. Based on a previous study, this activity could be classified as very strong as DIZ was >15 mm (76). The compounds in the *P. indica* leaf extract exhibited strong antibacterial activity against gram-negative *E. coli*. The compounds predicted as potential antibacterial agents were 10,10-dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.β.-ol and 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol. However, the present study had some limitations. The *in vitro* experiments must be supplemented by additional analysis to confirm that the compounds have effective activity against the protein target. The *in silico* approach requires more extensive protein screening to ensure compounds affect bacterial cells. The present study excluded the non-proteins analysis and only focus on essential bacterial proteins, such as bacterial proteins that serve key role in division and growth (Rpro and FtsZ). The present study only compared the compounds in the extract and excluded the positive control in molecular docking analysis to reduce false positive and to improve reliability and efficiency as previously described (77,78).

Overall, 10 compounds were identified in the *P. indica* leaf extract. 10,10-Dimethyl-2,6-dimethylenebicyclo(7.2.0) undecan-5.β.-ol and 11,11-dimethyl-4,8-dimethylenebicyclo(7.2.0)undecan-3-ol formed a ligand-protein complex with FtsZ from *B. subtilis* and R^{pro} from *E. coli*. Based on *in vitro* experiments, the 50% *P. indica* extract had the strongest inhibitory effect on the growth of *E. coli* and *B. subtilis*. Therefore, these ligands from *P. indica* leaf extract may serve as candidate inhibitors of targeted proteins that contribute to pathogenicity in the bacterial life cycle.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

DKW, JJ, CR, SS, PP, SP and HP designed the study. CR, VDK, AJS and CTR performed experiments and analyzed data. DKW, VDK and AJS wrote and edited the manuscript. DKW, JJ, SS, PP, SP and HP confirm the authenticity of all the raw data and reviewed the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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