JIPK. Volume 17 No 3 October 2025
Sinta 1 (Decree No: 158/E/KPT/2021)
e-ISSN:2528-0759; p-ISSN:2085-5842
Available online at https://e-journal.unair.ac.id/JIPK

JIPK
(JURNAL ILMIAH PERIKANAN DAN KELAUTAN)
Scientific Journal of Fisheries and Marine

Research Article

In vitro and *In silico* Antibacterial Activity of *Centella asiatica* Leaves Bioactive Compounds Against Fish Pathogenic Bacteria

Septyan Andriyanto [1,4*], Maftuch [1], Sri Andayani [1], Nunak Nafiqoh [2], Lila Gardenia [2], Hessy Novita [2], and Muhammad Nursid [3]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Malang, 65145. Indonesia
[2]Research Centre of Veterinary Science, National Research and Innovation Agency (BRIN), Cibinong, Bogor, 16915. Indonesia
[3]Research Center for Marine and Land Water Bioindustry, National Research and Innovation Agency, Lombok, 83352. Indonesia
[4]Research Center for Conservation of Marine and Inland Water Resources, National Research and Innovation Agency, Cibinong, Bogor, 16915. Indonesia

OPEN ACCESS

ARTICLE INFO

Received: April 24, 2025
Accepted: June 19, 2025
Published: July 16, 2025
Available online: Sept 27, 2025

*) Corresponding author:
E-mail: septian09@student.ub.ac.id

Keywords:
GC-MS
Molecular docking
DNA gyrase
Crude Extracts
MIC

Abstract

Antimicrobial agents are crucial for managing bacterial infections in fish cultures. *Centella asiatica* is a medicinal plant recognised for its diverse bioactive compounds with important antibacterial properties. The present study aimed to investigate the antibacterial activity of *C. asiatica* leaves bioactive compounds on fish pathogenic bacteria using an *In vitro* and *In silico* approach. The maceration method was used to extract bioactive compounds from *C. asiatica* leaves and was identified using Gas Chromatography-Mass Spectrometry (GC-MS). *In vitro* analysis of antibacterial activity was evaluated using the minimum inhibitory concentration method. While *In silico* molecular docking is applied alongside assessing Lipinski's rules of five, as well as absorption, distribution, metabolism, excretion, and toxicity properties. The result of the GC-MS examination of the *C. asiatica* leaf extracts identified 53 bioactive compounds. *In vitro* studies showed antibacterial efficacy of leaf extracts against fish pathogenic bacteria (*Streptococcus agalactiae*, *Bacillus subtilis*, and *Staphylococcus aureus*) with minimum inhibitory concentration values of 12,5 mg/ml. In silico molecular docking analysis showed that several bioactive compounds have the potential to be DNA gyrase inhibitors. Compound 13-Hexyloxacyclotridec-10-en-2-one has the highest inhibition with binding energy of −7,4 Kcal/mol compared to ciprofloxacin as drug standard with a binding energy value −7,3 Kcal/mol. The following compound is gamma.-Muurolene (−6,7 Kcal/mol), Copaene (−6,6 Kcal/mol) and Humulene (−6,6 Kcal/mol). These results suggest that bioactive compounds of *C. asiatica* leaves extracts hold promise as potential antibacterial agents for treating fish pathogenic bacteria infections.

Cite this as: Andriyanto, S., Maftuch, M., Andayani, S., Nafiqoh, N., Gardenia, L., Novita, H., & Nursid, M. (2025). *In vitro* and *In silico* antibacterial activity of *Centella asiatica* leaves extracts against fish pathogenic bacteria. *Jurnal Ilmiah Perikanan dan Kelautan*, 17(3):591-607. https://doi.org/10.20473/jipk.v17i3.72072

1. Introduction

Pathogenic bacteria increasingly impact fish culture, as higher population densities can exacerbate disease outbreaks. Effective antimicrobial agents are essential for addressing bacterial infections in fish culture frameworks. Recent studies show that traditional medicinal plant extracts, particularly *Centella asiatica*, may help manage bacterial pathogens in aquaculture (Si *et al*., 2023; Jenitha, 2023).

Centella asiatica, also known as Pegagan in Indonesia, is a notable medicinal plant known for its many bioactive components demonstrating remarkable antibacterial activities. This distinctive herb, part of the Apiaceae family, has been traditionally utilized in several civilizations, especially in Asia, to treat numerous diseases. The medicinal effectiveness of *C. asiatica* is largely attributed to its wide range of bioactive substances, notably terpenoids, saponins, flavonoids, tannins, alkaloid, and steroids, which play a significant role in enhancing its bioactivity, particularly its antibacterial properties (Liu *et al*., 2020; Magaña *et al*., 2020; Yusof *et al*., 2020; Akkol *et al*., 2021; Mohapatra *et al*., 2021). This plant contains several important triterpenes, including asiaticoside, madecassoside, asiatic acid, and madecassic acid, all of which have recognized health benefits, especially their antibacterial effects (Sun *et al*., 2020; Tripathy *et al*., 2022; Wei *et al*., 2023; Wang *et al*., 2024b).

Previous studies reported strong antibacterial effects of *C. asiatica* extracts against common fish pathogens like *Vibrio harveyi* and *Aeromonas hydrophila*, which cause significant economic losses in aquaculture (Rukisah *et al*., 2019). Leaf extracts and endophytic fungi associated with *C. asiatica* have been shown strong antimicrobial activity in aquaculture and can suppress the growth of fish and shellfish pathogenic bacteria (Shankar and Sathiavelu, 2024). *Centella asiatica* extracts are beneficial not only for pathogen control but also for improving biosecurity in aquaculture systems. This will reduce the use of conventional antibiotics, leading to increased antimicrobial resistance (Bondad-Reantaso *et al*., 2023). *Streptococcus agalactiae*, *Bacillus subtilis*, and *Staphylococcus aureus* are aquatic bacterial pathogens that significantly impact the ecosystem. These pathogens can be found in a variety of fish species and shrimp. They persist in both freshwater and marine ecosystems, potentially leading to infection outbreaks (Wang *et al*., 2020; Zelellw *et al*., 2021; Chen *et al*., 2023).

The present study focused on investigation of the antimicrobial activity of *C. asiatica* leaves bioactive compounds on pathogenic bacteria by *in vitro* and *in silico* approach. This study offers a novel approach to address antibiotic resistance in fish pathogenic bacteria, including *S. agalactiae*, *S. aureus* and *B. subtilis*. The use of *in silico* methods to assess the affinity of these compounds for DNA gyrase offers important insights into their potential as antimicrobial agents. This study investigates antibacterial activity by *in vitro* and continued with molecular docking of bioactive compounds from *C. asiatica*, as potential inhibitors of DNA gyrase in pathogenic bacteria which is still rarely practiced today, and only a few data have been published.

2. Materials and Methods

2.1 Materials

2.1.1 The equipments

The main equipment and tools used in this research included: vacuum rotary evaporator (Buchi, Swiss), spectrophotometer (Thermoscientific, USA), bacterial incubator (Memmert, Germany), micropipettes (Eppendorf, Germany), microtips (Axygen, USA), microtubes (Axygen, USA), laboratory glassware (Pyrex, USA), Separating funnel (Schott Duran, Germany), Petridish (SPL Life Sciences, South Korea), and 96well plate (Biologix, USA).

2.1.2 The materials

The plant material from *Centella asiatica* was obtained in Tegal Waru, Ciampea, Bogor Regency, West Java, Indonesia (6°34′19″S 106°41′58″E). The leaves used in the study were old leaves. Other materials used in this study were distilled water, ethanol (Merck, USA), n-hexane (Merck, USA), tryptic soy agar (Merck, USA), mueller hinton broth (MHB) (Himedia, India), NaCl (Oxoid, United Kingdom), and Phosphat Buffer Saline (Himedia, India).

2.1.3 Ethical approval

Ethical approval was not required for this study as no experimental animals were involved.

2.2 Methods

2.2.1 Identification of bioactive compounds

The extraction procedure involved submerging 300 g of dried plant material in 1500 mL of a 70% ethanol solution. The precipitate was then separated from the filtrate. The filtrate was further concentrated with a rotary evaporator set at a temperature

between 40-45ºC until a concentrated extract was obtained (Biradar and Rachetti, 2013). The GC-MS method was employed for the qualitative and quantitative characterization of *C. asiatica* leaves extract (Magaña *et al*., 2020). Bioactive compound characterization was conducted in the Integrated Advanced Chemistry Laboratory, Serpong-BRIN, using Gas Chromatography-Mass Spectrometry (GC-MS).

mg/mL in MHB medium. A 96-microwell plate was utilized, where test tubes were filled with 160 µL of MHB. Subsequently, 20 µL of crude extracts and fraction solutions at varying concentrations were added, followed by inoculation with 60 µL of bacterial isolates at a density of 10^8 cfu/mL. The mixture was then incubated for 24 hours. The concentration of crude extracts that most effectively inhibits the

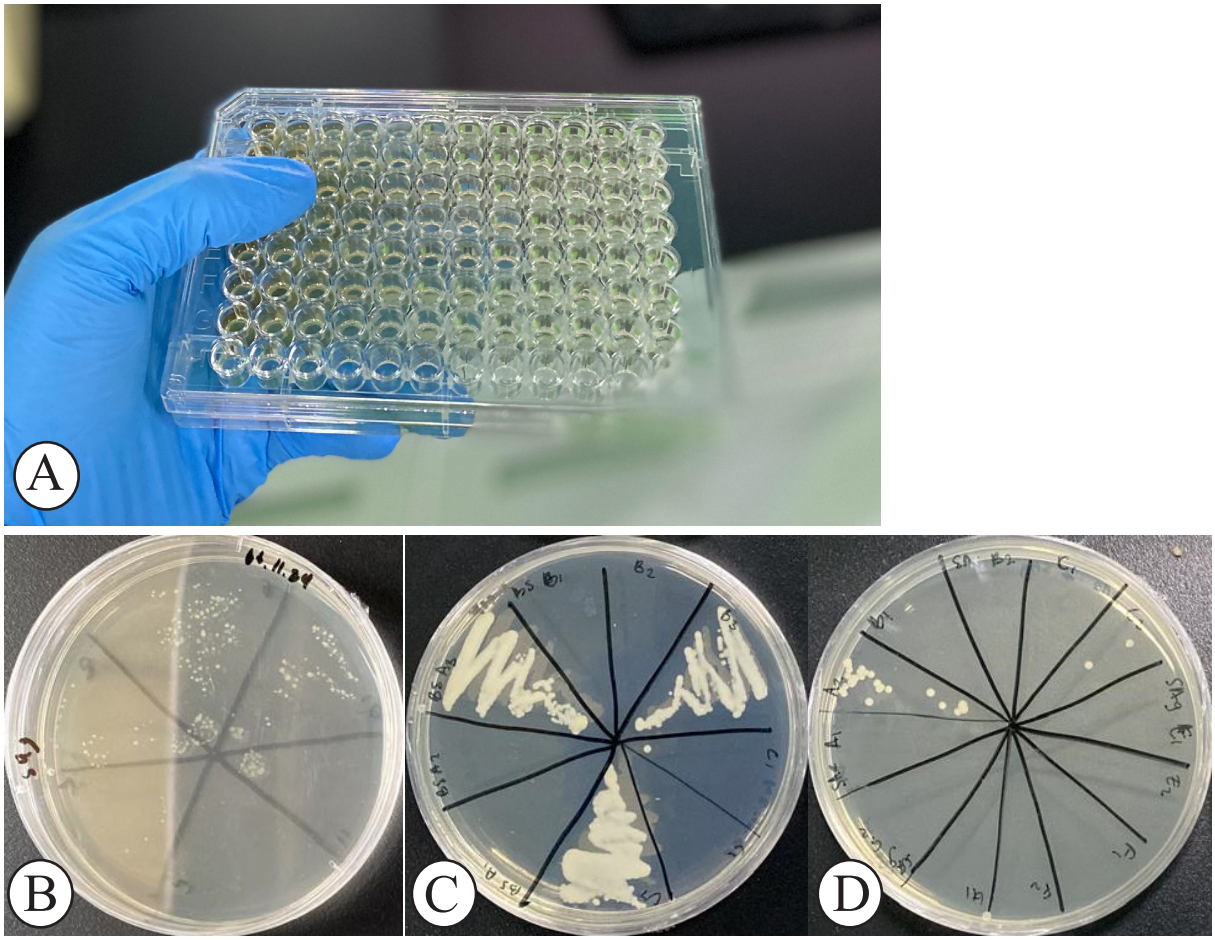


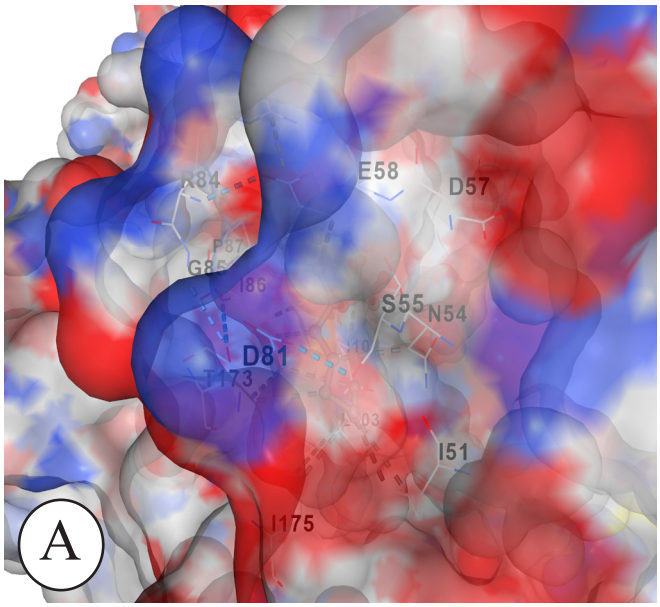
Figure 1. The minimum inhibitory concentration assay. A. Agar well diffusion; B. *S. agalactiae* isolate; C. *B. substilis* isolate; D. *S. aureus* isolate.

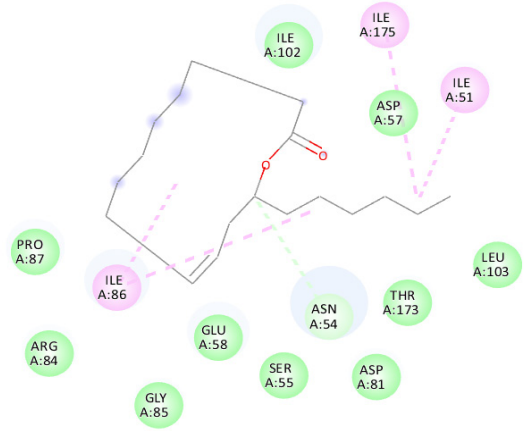
2.2.2 In vitro analysis

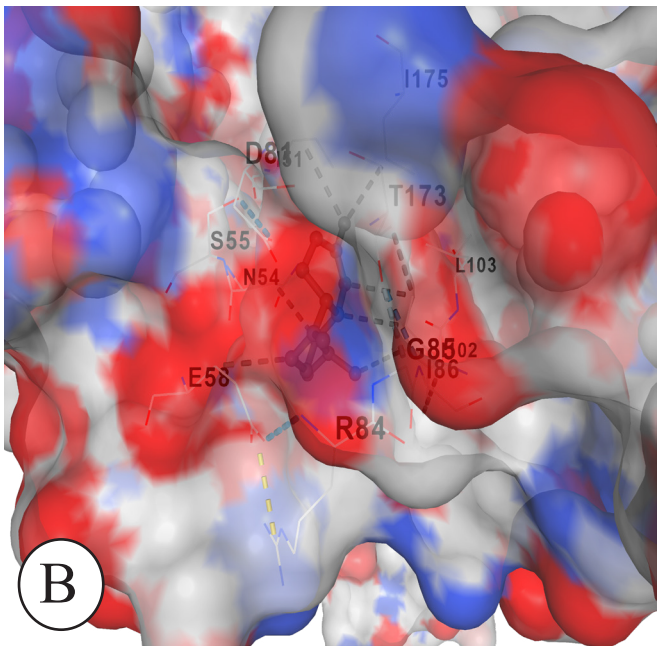
Pathogenic bacterial isolates *Bacillus subtilis* (Inacc B1210) and *Staphylococcus aureus* (Inacc B4) were obtained from the Indonesian Culture Collection Laboratory (InaCC), Cibinong, BRIN. Meanwhile, *Streptococcus agalactiae* was isolated from infected fish. *In vitro* crude extracts antibacterial activity was evaluated using modified minimum inhibitory concentration (MIC) methods. The MIC was determined using the serial two-fold dilution method (Choudhury *et al*., 2024). Experiments involved preparing a solution of crude extracts (50 mg) at concentrations of 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.390, 0.195, 0.098, 0.049, and 0.024 growth of selected bacteria is indicated by the precise visual assessment of the turbidity of the test tube. Bacterial growth was observed visually, and the MIC value was established as the lowest concentration capable of halting bacterial growth, marked by a transition in color from yellow to pink. In the MIC test, Oxytetracycline antibiotic was used as the control.

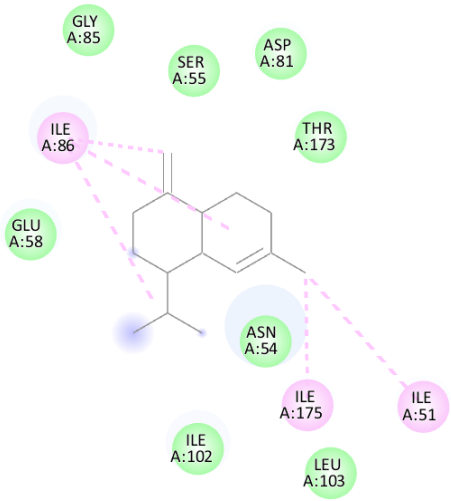
2.2.3 In silico analysis

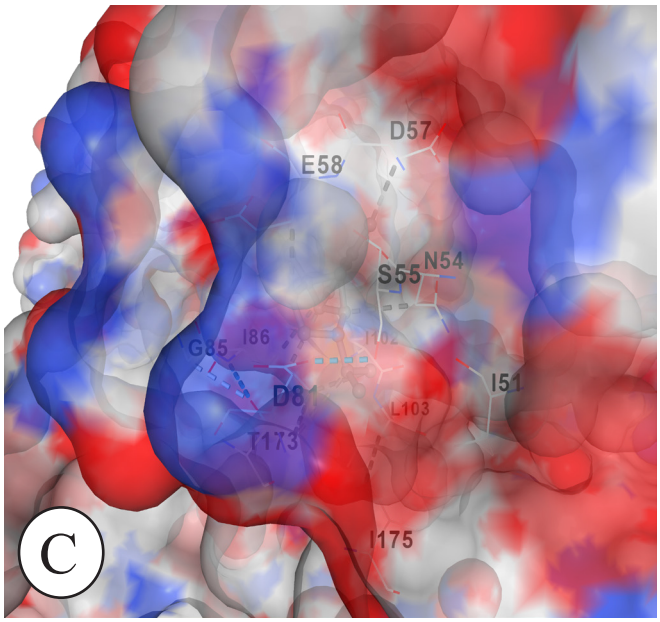
The PubChem database was used to obtain the details of the bioactive compounds, including their Lipinski's rules of five (Ro5) and ADME/T (Absorption, Distribution, Metabolism, Excretion,

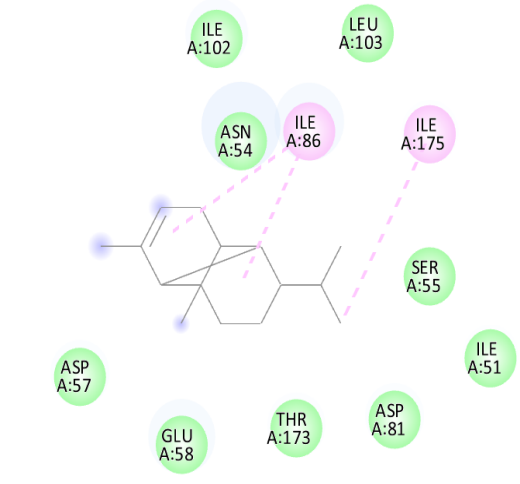

R84
E58
D57
P87
G85
I86
S55
N54
D81
T173
I51
I175
A


ILE A:102
ILE A:175
ILE A:51
ASP A:57
PRO A:87
ILE A:86
LEU A:103
THR A:173
ASN A:54
GLU A:58
ARG A:84
SER A:55
ASP A:81
GLY A:85
Interactions
van der Waals
Alkyl
Carbon Hydrogen Bond


I175
D81
T173
S55
N54
L103
G85
I86
E58
R84
B


GLY A:85
SER A:55
ASP A:81
ILE A:86
THR A:173
GLU A:58
ASN A:54
ILE A:175
ILE A:51
ILE A:102
LEU A:103
Interactions
van der Waals
Alkyl


D57
E58
S55
N54
G85
I86
D81
L103
I51
T173
I175
C


ILE A:102
LEU A:103
ASN A:54
ILE A:86
ILE A:175
SER A:55
ILE A:51
ASP A:57
GLU A:58
THR A:173
ASP A:81
Interactions
van der Waals
Alkyl

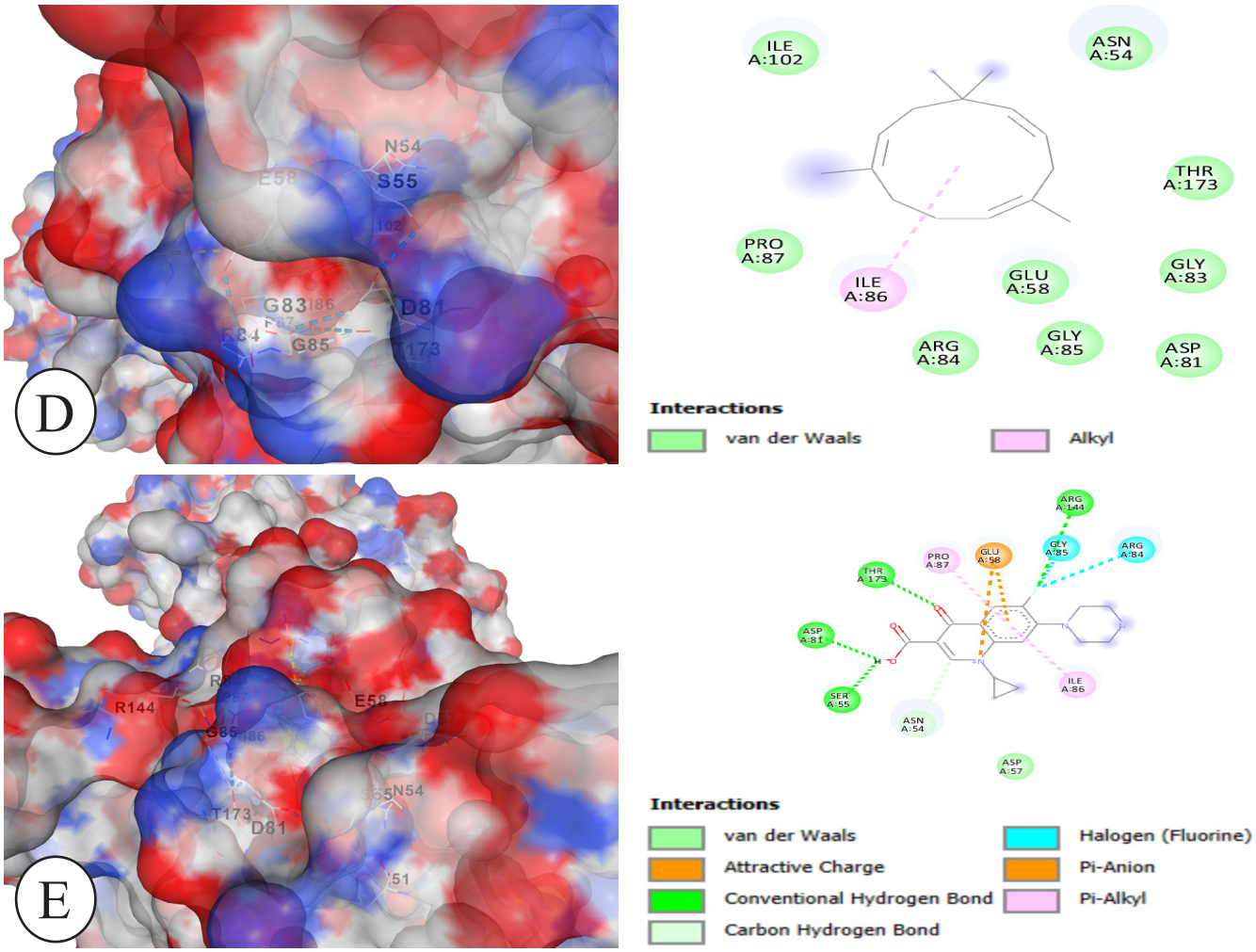


Figure 2. 3D and 2D interactions of four potential ligands and ciprofloxacin at the DNA gyrase site residues during molecular docking. A. 13-Hexyloxacyclotridec-10-en-2-one; B. γ-muurolene; C. copaene; D. humulene; E. ciprofloxacin.

and Toxicity) properties. *In silico* method applying molecular docking, alongside the assessment of Ro5 and ADME/T properties. By analyzing the chemical properties of compounds, Lipinski's Ro5 serves as a method to predict their oral bioavailability. Lipinski's criteria suggest that a compound is more likely to be an effective oral drug if it possesses the following characteristics: (1) a molecular weight (MW) not exceeding 500 Da, (2) a partition coefficient (logP) less than or equal to 5, (3) no more than 5 hydrogen bond donors (HBD), and (4) no more than 10 hydrogen bond acceptors (HBA) (Frau *et al*., 2018; Kumari and Kumar, 2023). The median lethal dosage (LD_{50}) values were determined through an assessment of the toxicity class utilizing ProTox-II.

The *in silico* analysis material used three-dimensional structures of all ligands of bioactive compounds derived in .sdf file format from the reputable National Center for Biotechnology Information (NCBI) PubChem database (https://pubchem.ncbi.nlm.nih.gov/). At CB-Dock (https://cadd.labshare.cn/cb-dock2/index.php), molecular docking studies were conducted. This involved identifying binding sites, assessing their dimensions and central coordinates, and adjusting the docking box size based on the ligands in the query. AutoDock Vina performed molecular docking based on three-dimensional structures of specific proteins and analyzed the mechanisms of action of bioactive compounds (Eberhardt *et al*., 2021; Xu *et al*., 2021). The proteins analyzed comprised DNA gyrase from *S. aureus* (PDB ID: 6tck). We assessed the typical inhibitor orientation in crystal structures. The highly credible Protein Data Bank was referenced for the protein X-ray structures. Binding postures and interaction diagrams were generated using BIOVIA Discovery Studio Visualizer 24.1.0.0 (https://www.3ds.com/products/biovia/discovery-studio/visualization).

Table 1. Bioactive compounds identified in the leaves extract of *Centella asiatica* and their Lipinski properties

No.	Compound Name	Area %	MW	HBA	HBD	TPSA	Log P (iLogP)	LD_{50}	Toxicity Class
Terpenes									
1	Copaene	1.31	204.35	0	0	0	3.40	3700	5
2	Caryophyllene	0.78	204.35	0	0	0	3.40	5300	5
3	cis-.beta.-Farnesene	0.65	204.35	0	0	0	3.86	5000	5
4	Humulene	0.52	204.35	0	0	0	3.29	3650	5
5	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	0.60	204.35	0	0	0	3.18	5300	5
6	.gamma.-Muurolene	0.49	204.35	0	0	0	3.39	4400	5
7	Caryophyllene oxide	1.68	220.35	1	0	12.53	3.15	5000	5
8	2-Pentadecanone, 6,10,14-trimethyl-	2.07	268.48	1	0	17.07	4.39	5000	5
9	Phytol	1.62	296.53	1	1	20.23	4.85	5000	5
Fatty Acids									
10	Heptanal	1.21	114.19	1	0	17.07	2.01	5000	5
11	Hexanoic acid	0.71	116.16	2	1	37.3	1.57	93	3
12	Heptanoic acid	0.73	130.18	2	1	37.3	1.79	900	4
13	Hexadecanoic acid, methyl ester	1.86	270.45	2	0	26.3	4.41	5000	5
14	n-Hexadecanoic acid	16.97	256.42	2	1	37.3	3.85	900	4
15	Ethyl 9-tetradecenoate	0.35	254.41	2	0	26.3	4.31	5000	5
16	Hexadecanoic acid, ethyl ester	9.59	284.48	2	0	26.3	4.65	5000	5
17	9,12-Octadecadienoic acid, methyl ester	0.82	294.47	2	0	26.3	0	20000	6

No.	Compound Name	Area %	MW	HBA	HBD	TPSA	Log P (iLogP)	LD_{50}	Toxicity Class
18	9-Octadecenoic acid (Z)-, methyl ester	1.42	296.49	2	0	26.3	4.63	3000	5
19	9,12-Octadecadienoic acid (Z,Z)-	4.95	280.45	2	1	37.3	0	10000	6
20	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	9.35	278.43	2	1	37.3	0	10000	6
21	Linoleic acid ethyl ester	4.21	308.5	2	0	26.3	0	20000	6
22	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	4.78	306.48	2	0	26.3	0	20000	6
Ricinoleic Acids									
23	Ricinoleic acid	2.60	298.46	3	2	57.53	3.86	11800	2
24	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	0.70	312.49	3	1	46.53	4.41	3000	5
Acyclic Acids									
25	2-Propenamide	0.65	71.08	1	1	43.09	0.68	107	3
Ketones									
26	13-Hexyloxacyclotridec-10-en-2-one	1.76	280.45	2	0	26.3	4.03	34900	6
Amines									
27	Benzyl alcohol, p-hydroxy-.alpha.-[(methylamino)methyl]-	0.68	167.2	3	3	52.49	1.52	4450	5
Nitrosamines									
28	Ethanamine, N-ethyl-N-nitroso-	0.56	102.14	2	0	32.67	1.99	200	3
Pyrones									
29	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.64	144.12	4	2	66.76	1.19	595	4

No.	Compound Name	Area %	MW	HBA	HBD	TPSA	Log P (iLogP)	LD_{50}	Toxicity Class
Lactones									
30	2(4H)-Benzofura-none, 5,6,7,7a-tetrahy-dro-4,4,7a-trimethyl-	0.69	180.24	2	0	26.3	2.29	34	2
Alkenes									
31	Neophytadiene	1.62	278.52	0	0	0	5.05	5050	6
Benzofurans									
32	Loliolide	0.77	196.24	3	1	46.53	2.01	34	2
Aldehydes									
33	Benzeneacetaldehyde	1.43	120.15	1	0	17.07	1.33	1550	4
Carbohydrates									
34	Erythritol	0.94	122.12	4	4	80.92	0.94	23000	6
Monosaccharide									
35	dl-Threitol	0.65	122.12	4	4	80.92	0.61	23000	6
Epoxide									
36	(1R,3E,7E,11R)-1,5,5,8-Te-tramethyl-12-oxabicyc-lo[9.1.0]dodeca-3,7-diene	1.22	220.35	1	0	12.53	3.18	5000	5
Others									
37	.alpha.-D-Mannopyranoside, methyl 3,6-anhydro-	0.32	176.17	5	2	68.15	0	648	3
38	Acetic acid, hydroxy-, ethyl ester	1.97	104.1	3	1	46.53	1.36	2000	4
39	Oxime-, methoxy-phenyl-_	2.95	151.16	3	1	41.82	1.69	2000	4
40	Tetraacetyl-d-xylonic nitrile	0.32	343.29	10	0	146.06	1.86	7000	6

No.	Compound Name	Area %	MW	HBA	HBD	TPSA	Log P (iLogP)	LD_{50}	Toxicity Class
41	2,4-Hexanedione, 5,5-dimethyl-1-phenyl-	0.73	218.29	2	0	34.14	2.34	4000	5
42	.beta.-Alanine, TMS derivative	2.49	161.27	3	1	52.32	2.1	2280	5
43	Hydrazinecarboximidothioic acid, ethyl ester	0.78	119.19	1	2	89.7	0.76	815	4
44	11,11-Dimethyl-4,8-dimethylenebicyclo[7.2.0]undecan-3-ol	1.54	220.35	1	1	20.23	3.04	3900	5
45	2-Propenoic acid, pentadecyl ester	1.06	282.5	2	0	26.3	4.89	5000	5
46	.alpha.-Methyl-3,4-(methylenedioxy) phenethylamine hydrochloride	0.91	215.68	3	1	44.48	0	13	2
47	Oxazepam, 2TMS derivative	0.42	431.08	3	0	41.9	4.18	1148	4
48	Decane, 3,8-dimethyl-	0.97	170.33	0	0	0	3.59	750	3
49	1-Octadecanesulphonyl chloride	1.42	353	2	0	42.52	4.84	2100	5
50	2-(2',4',4',6',6',8',8'-Heptamethyltetrasiloxan- 2'-yloxy)-2,4,4,6,6,8,8,10,10-nonamethylcyclopentasiloxane	0.35	653.32	10	0	92.3	6.33	1540	4
51	Palmitic Acid, TMS derivative	0.82	328.61	2	0	26.3	5.33	2280	4
52	Hexasiloxane, tetradecamethyl-	0.60	458.99	5	0	46.15	5.76	1540	4
53	Octadecanoic acid, 2-methyl-, methyl ester	0.89	312.53	2	0	26.3	5.38	5000	5

Description: MW (molecular weight-g/mol), HBA (hydrogen bond acceptors), HBD (hydrogen bond donors), TPSA (topological polar surface area), LD50 (lethal dose-mg/kg).

Table 2. Molecular docking results analysis of potential bioactive compounds identified from *C. asiatica* and ciprofloxacin (drug standard) against DNA Gyrase (PDB id: 6tck)

No.	Compound Name	Compound ID	DNA Gyrase	
			Binding Energy	Cavity Volume
1	13-Hexyloxacyclotridec-10-en-2-one	6536948	−7.4	560
2	.gamma.-Muurolene	6432308	−6.7	384
3	Copaene	12303902	−6.6	560
4	Humulene	5281520	−6.6	560
5	Caryophyllene	5281515	−6.5	384
6	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	5367460	−6.4	560
7	2,4-Hexanedione, 5,5-dimethyl-1-phenyl-	581252	−6.3	384
8	cis-.beta.-Farnesene	5317319	−6.2	384
9	1-Octadecanesulphonyl chloride	66281	−6.1	560
10	Phytol	5280435	−6.1	560
11	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	5280934	−6.1	560
12	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	6429274	−6.0	384
13	Caryophyllene oxide	1742210	−5.9	560
14	Benzyl alcohol, p-hydroxy-.alpha.-[(methylamino) methyl]-	7172	−5.9	560
15	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	10704181	−5.7	560
16	9,12-Octadecadienoic acid, methyl ester	5284421	−5.7	560

No.	Compound Name	Compound ID	DNA Gyrase	
			Binding Energy	Cavity Volume
17	2-Pentadecanone, 6,10,14-trimethyl-	10408	−5.6	560
18	9,12-Octadecadienoic acid (Z,Z)-	5280450	−5.6	384
19	Oxime-, methoxy-phenyl-_	9602988	−5.5	384
20	Hexadecanoic acid, ethyl ester	12366	−5.5	384
21	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	5354133	−5.4	560
22	Ethyl 9-tetradecenoate	12054546	−5.3	560
23	2-Propenoic acid, pentadecyl ester	543579	−5.2	560
24	Hexadecanoic acid, methyl ester	8181	−5.1	560
25	Linoleic acid ethyl ester	5282184	−5.0	384
26	9-Octadecenoic acid (Z)-, methyl ester	5364509	−4.9	384
27	Erythritol	222285	−4.4	560
28	dl-Threitol	8998	−4.4	384
29	Heptanal	8130	−4.3	560
30	Acetic acid, hydroxy-, ethyl ester	12184	−4.1	560
31	.beta.-Alanine, TMS derivative	554627	−4.0	560
	Ciprofloxacin (drug standard)		−7.3	560

3. Results and Discussion

3.1 Results

3.1.1 Gas chromatography analysis

The GC-MS results of the *C. asiatica* leaves extracts yielded 53 bioactive compounds (Table 1). The compounds of *C. asiatica* leaves extract include terpenes, fatty acids, ricinoleic acids, acyclic acids, ketones, amines, nitrosamines, pyrones, lactones, alkenes, benzofurans, aldehydes, carbohydrates, monosaccharides, epoxides, and various other component classes.

3.1.2 Antibacterial activity of C. asiatica

In vitro analysis shows that the minimum inhibitory concentration is the lowest concentration of antimicrobial agents capable of inhibiting the growth of harmful microorganisms.

The MIC of crude extract was evaluated using the agar well diffusion assay (Figure 1). The MIC values of *C. asiatica* leaves extracts against *S. agalactiae*, *B. subtilis*, and *S. aureus* were 12.5 mg/mL. The results showed a positive antibacterial effect of *C. asiatica* extract against Gram-positive bacteria.

In silico Ro5 and ADME/T analysis identified 31 potential drug-candidate compounds with antibacterial properties (Table 2). The 31 active compounds derived from the leaves extract of *C. asiatica* demonstrated molecular weights between 104.1 Da and 353 Da. The toxicity assessment of 31 compounds indicated an LD_{50} toxicity range of 2,000 to 34,900 mg/kg.

Figure 2 illustrates the comparison of binding patterns and molecular interactions of the evaluated compounds with the highest binding energies against the drug standard ciprofloxacin, recognized as a DNA gyrase inhibitor. The four highest-ranking ligands for DNA Gyrase, determined by Vina score, are 13-Hexyloxacyclotridec-10-en-2-one (−7.4 kcal/mol), γ-Muurolene (−6.7 kcal/mol), Copaene (−6.6 kcal/mol), and Humulene (−6.6 kcal/mol).

3.2 Discussion

3.2.1 Gas chromatography identification

GC-MS analysis of *C. asiatica* leaves extract in accordance with previous studies by Micheli *et al.* (2022), Jenitha (2023), and Taleghani *et al.* (2024) found the metabolites of *C. asiatica*, which include triterpenoids, phenolics, flavonoids, phenylpropanoids, acyclic acids, ketones, and amines. The findings align with those of Yang *et al.* (2023), Pillai *et al.* (2024), and Rafi *et al.* (2024), which indicate that *C. asiatica* comprises numerous bioactive components, such as terpenoids, flavonoids, saponins, tannins, amino acids, fatty acids, alkaloids, steroids, and and other categories. According to Sieberi *et al.* (2020) and Taghizadeh and Jalili (2024) that *C. asiatica* bioactive compounds, especially triterpenoids, flavonoids, and phenolic compounds, play an important role as antibacterial agents. Likewise, Pham *et al.* (2020) and Menon *et al.* (2023) mentioned that the antibacterial properties of phytochemical compounds contained in *C. asiatica* are applied to a variety of pathogenic microbial organisms.

3.2.2 In vitro and in silico antibacterial activity

The antibacterial activity of *C. asiatica* leaves extracts against three pathogenic bacteria (*S. agalactiae*, *B. subtilis*, and *S. aureus*) was determined. In the antibacterial activity, the extract concentration is significant in preventing the growth of pathogenic bacteria. The MIC values against *S. agalactiae*, *B. subtilis* and *S. aureus* were 12.5 mg/mL. The results are in accordance with those reported by Zhang *et al.* (2020), Qurrotuaini *et al.* (2022), and Kathirvel *et al.* (2025), *C. asiatica* leaves extracts exhibit activity against *Acinetobacter calcoaceticus anitratus*, *Bacillus cereus*, *Enterococcus avium*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus agalactiae* with a MIC ranging from 1.25 to 25 mg/mL.

The results indicate that *C. asiatica* has a significant inhibitory effect on the growth of Gram-positive bacteria within 24 hours. Sieberi *et al.* (2020) reported that ethanol and Dichloro methane (DCM) extracts of *C. asiatica* inhibit the growth of Gram-positive and Gram-negative bacteria. While Menon *et al.* (2023) suggested that *in vitro* studies have also shown a significant reduction in the number of colonies of pathogenic bacteria after treatment with *C. asiatica* extract.

The antibacterial mechanism of bioactive compounds in *C. asiatica* functions synergistically within bacterial cells by inhibiting nucleic acid synthesis, which is thought to involve the loss of bacterial membrane integrity. This results in increased permeability and subsequent cell death and influences the bacterial metabolic system (Wong and Ramli, 2021; Maitra *et al.*, 2022; Qurrotuaini *et al.*, 2022; Wei *et al.*, 2023). Another study found that the antibacterial mechanism is related to the inhibition of quorum-sensing activity that prevents communication between bacteria in biofilm formation as well as in increased pathogenicity (Sieberi *et al.*, 2020; Taghizadeh and Jalili, 2024).

The results of the *in silico* analysis showed that 31 bioactive compounds passed the Lipinski Ro5 and ADME/T test. Zafar *et al.* (2020) and Nguyen *et al.* (2023) reported that Lipinski Ro5 indicate that a molecular weight of under 500 Da implies potential for cellular membrane penetration. Both compounds exhibited HBA, HBD, and iLog P values of less than 10, less than 5, and less than 5, respectively, while the TPSA value was less than or equal to 140 Å. LD_{50} value indicates reduced chemical toxicity to the tested organism. Determining toxicity levels using computer-based tools such as ProTox-II and Swiss ADME in molecular docking can facilitate the classification of bioactive compounds based on their toxicity in accordance with standard drug criteria (Lane *et al.*, 2023; Li *et al.*, 2024; Ghanem *et al.*, 2024). Abishad *et al.* (2021) and Wu *et al.* (2021) suggested that enhancing the safety of these

drugs prior to market introduction requires a focus on ADME and toxicity-related factors. The SWISS ADME online program was employed to assess the drug-likeness of phytocompounds. The molecular factors associated with rule violations and the acquisition of bioactive compounds are detailed in the table of Lipinski parameters.

The results of *in vitro* studies demonstrated the antibacterial potential of *C. asiatica* leaves extracts, so further *in silico* evaluations were carried out to identify the compounds that could significantly inhibit DNA gyrase, a critical enzyme in bacterial cell development. In the docking investigations of the DNA gyrase binding site, 13-Hexyloxacyclotridec-10-en-2-one had the greatest binding energy of −7.4 kcal/mol surpassing drug standard ciprofloxacin with binding energy −7.3 kcal/mol. Based on previous research, Selvarajan *et al.* (2023) reported that 13−Hexyloxacyclotridec−10−en−2−one exhibits wide antibacterial activity against numerous pathogenic bacteria. Singh *et al.* (2023) also stated that 13−Hexyloxacyclotridec−10−en−2−one efficiently suppresses the development of bacteria such as *Staphylococcus aureus* and *Escherichia coli*. As a broad-spectrum antibiotic in the fluoroquinolone group, ciprofloxacin is widely utilized to treat a range of bacterial infections, including those caused by both Gram-positive and Gram-negative bacteria. Research reveals that ciprofloxacin exerts its bactericidal action by binding to the bacterial enzymes DNA gyrase and topoisomerase IV, which prevents DNA replication from occurring (Hussein *et al.*, 2022; Grigor'eva *et al.*, 2023). Furthermore, the approach illustrated how *C. asiatica* demonstrates its antibacterial activity by disrupting bacterial DNA processing. The bioactive compounds of *C. asiatica* can elicit apoptosis through mechanisms involving DNA synthesis (Jenitha, 2023). This observation underscores a crucial connection to the role of DNA gyrase, a crucial enzyme in bacterial DNA replication. Inhibition of DNA gyrase obstructs bacterial replication, hence averting infection. Moreover, studies reveal that *C. asiatica* extracts, with their considerable antibacterial activity at minimal concentrations, hold great potential as agents that inhibit bacterial proliferation (Agneeswari *et al.*, 2019).

It appeared beneficial for performing molecular docking studies that align *in silico* and *in vitro.* results depending on the findings of the *in vitro* inquiry. This study employed molecular docking analysis via the CB-Dock server and GC-MS analysis to evaluate the interactions between the bioactive compounds in *C. asiatica* leaves extracts and the target protein DNA gyrase. Eberhardt *et al.* (2021) and Wang *et al.* (2024a) suggested that docking studies are employed in drug development to forecast the interactions between ligands and receptors, as well as to rank compounds according to binding energies or fitness scores. While Liu *et al.* (2022) and Zheng *et al.* (2024) stated that the CB-Dock methodology consists of three phases: first, assessing the curvature of the protein surface; second, clustering to pinpoint active site cavities; and third, performing docking with AutoDock Vina.

The rise of bacterial resistance to existing treatment agents has prompted the development of new antimicrobial drugs aimed at selectively inhibiting evolving bacterial targets that face ongoing challenges. This study demonstrates that molecular docking analysis shows 13−Hexyloxacyclotridec−10−en−2−one possesses greater selectivity for the DNA gyrase binding site than ciprofloxacin, the standard medication. The compound 13-Hexyloxacyclotridec-10-en-2-one may offer a solid starting point for developing novel chemical entities that exhibit potent antibacterial effects. The results suggest that bioactive compounds derived from *C. asiatica* leaves extracts could function as effective antibacterial agents against fish pathogenic bacteria.

4. Conclusion

The bioactive compounds of *Centella asiatica* leaves extracts were analyzed via GC-MS, encompassing terpenes, fatty acids, ricinoleic acids, acyclic acids, ketones, amines, nitrosamines, pyrones, lactones, alkenes, benzofurans, aldehydes, carbohydrates, monosaccharides, epoxides, and other compounds classes. The leaves extract demonstrated antibacterial effectiveness against fish pathogenic bacteria (*Streptococcus agalactiae*, *Bacillus subtilis*, and *Staphylococcus aureus*) with MIC values of 12.5 mg/mL. Through *in silico* analysis, 31 compounds met the criteria of five drug-likeness features. Furthermore, molecular docking investigations showed that 13-Hexyloxacyclotridec-10-en-2-one had the most antibacterial activity. The results demonstrated that bioactive compounds from *Centella asiatica* leaves extracts have the potential as antibacterial agents.

Acknowledgement

The authors would like to thank Research Centre of Veterinary Science, the Research Organization for Health-BRIN for support this research.

Authors' Contributions

All authors have contributed to the final manuscript. Each author's contribution is as follows, SA; designed the experiment, collected data, and writing-original manuscript. NN, LG, HN; data analysis and editing. MM, SA, MN; writing-review and critical revision of the article.

Conflict of Interest

The authors declare that there is no conflict of interest.

Declaration of Artificial Intelligence (AI)

The author(s) affirm that no artificial intelligence (AI) tools, services, or technologies were employed in the creation, editing, or refinement of this manuscript. All content presented is the result of the independent intellectual efforts of the author(s), ensuring originality and integrity.

Funding Information

This research was supported by the Research Organization for Health-the National Research and Innovation Agency.

References

Abishad, P., Niveditha, P., Unni, V., Vergis, J., Kurkure, N. V., Chaudhari, S., & Barbuddhe S. B. (2021). In silico molecular docking and in vitro antimicrobial efficacy of phytochemicals against multi-drug-resistant enteroaggregative *Escherichia coli* and non-typhoidal *Salmonella* spp. *Gut Pathogens*, 13(46):1-11.

Agneeswari, S., Santhanam, A., & Jenishini, J. (2019). Characterization and antimicrobial activity of *Centella asiatica*. *International Journal of Engineering and Advanced Technology*, 9(6):125-131.

Akkol, K. E., **Çankaya**, T. I., Karatoprak, S. G., Carpar, E., Sobarzo-Sánchez, E., & Capasso, R. (2021). Natural compounds as medical strategies in the prevention and treatment of psychiatric disorders seen in neurological diseases. *Frontiers in Pharmacology*, 12(669638):1-27.

Biradar, S. R., & Rachetti, B. D. (2013). Extraction of some secondary metabolites & thin layer chromatography from different parts of *Centella asiatica* L. (Urb). *American Journal of Life Sciences*, 1(6):243-247.

Bondad-Reantaso, M. G., MacKinnon, B., Karunasagar, I., Fridman, S., Alday-Sanz, V., Brun, E., Groumellec, M. L., Li, A., Surachetpong, W., Karunasagar, I., Hao, B., Dall'Occo, A., Urbani, R., & Caputo, A. (2023). Review of alternatives to antibiotic use in aquaculture. *Reviews in Aquaculture*, 15(4):1421-1451.

Chen, X. W., Wu, J. H., Liu, Y. L., Munang'andu, H. M., & Peng, B. (2023). Fructose promotes Ampicillin killing of antibiotic-resistant *Streptococcus agalactiae*. *Virulence*, 14(1):1-16.

Choudhury, D., Alanbari, R., Saveliev, P., Sokurenko, E., Fuzi, M., & Tchesnokova, V. (2024). Clonal and resistance profiles of fluoroquinolone-resistant uropathogenic *Escherichia coli* in countries with different practices of antibiotic prescription. *Frontiers in Microbiology*, 15(1446818):1-11.

Eberhardt, J., Santos-Martins, D., Tillack, A. F., & Forli, S. (2021). AutoDock Vina 1.2. 0: New docking methods, expanded force field, and python bindings. *Journal of Chemical Information and Modeling*, 61(8):3891-3898.

Frau, J., Flores-Holgúın, N., & Glossman-Mitnik, D. (2018). Chemical reactivity properties, PKA values, ages inhibitor abilities and bioactivity scores of the mirabamides A-H peptides of marine origin studied by means of conceptual DFT. *Marine Drugs*, 16(302):1-19.

Ghanem, H., Khaoua, O., Ouahab, A., Benbellat, N., & Haba, H. (2024). In silico pharmacodynamics, antineoplastic activity and molecular docking of two phytochemicals isolated from *Thymelaea microphylla*. *Letters in Drug Design & Discovery*, 21(13):2644-2660.

Grigor'eva, A. E., Bardasheva, A. V., Ryabova, E. S., Tupitsyna, A. V., Zadvornykh, D. A., Koroleva, L. S., Silnikov, V. N., Tikunova, N. V., & Ryabchikova E. I. (2023). Changes in the ultrastructure of *Staphylococcus aureus* cells make it possible to identify and analyze the injuring effects of ciprofloxacin, polycationic amphiphile and their hybrid. *Microorganisms*, 11(2192):1-11.

Hussein, M. E., Mohamed, O. G., El-Fishawy, A. M., El-Askary, H. I., El-Senousy, A. S., El-Beih, A. A., Nossier, E. S., Naglah, A. M., Almehizia, A. A., Tripathi, A., & Hamed, A. A. (2022). Identification of antibacterial metabolites from endophytic fungus *Aspergillus fumigatus*, isolated from *Albizia lucidior* leaves (Fabaceae), utilizing metabolomic and molecular docking techniques. *Molecules*, 27(1117):1-21.

Jenitha, K. (2023). Antimicrobial activity and phytochemical analysis of extract of *Centella asiatica*. *International Journal of Zoological Investigations*, 9(2):160-168.

Kathirvel, A., Ramalingam, S., Harini, S. T., Ranjith, N., Kumar, G. S., Lalithambigai, K., Atchudan, R., Habila, M. A., Aljuwayid, A. M., & Yun, H. K. (2025). Eco-friendly synthesis of zirconium dioxide nanoparticles from *Toddalia asiatica*: Applications in dye degradation, antioxidant and antibacterial activity. *Nanomaterials*, 15(84):1-18.

Kumari, S., & Kumar, P. (2023). Design and computational analysis of an mmp9 inhibitor in hypoxia-induced glioblastoma multiforme. *ACS Omega*, 8(11):10565-10590.

Lane, T. R., Harris, J., Urbina, F., & Ekins S. (2023). Comparing LD_{50}/LC_{50} machine learning models for multiple species. *ACS Chemical Health & Safety,* 30(2):83-97.

Li, M., Han, R., Li, J., Wu, W., & Gu J. (2024). Research progress in acute oral toxicity testing methods. *International Journal of Biology and Life Sciences*, 6(1):19-22.

Liu, Y. T., Chuang, Y. C., Lo, Y. S., Lin, C. C., His, Y. T., Hsieh, M. J., & Chen M. K. (2020). Asiatic acid, extracted from *Centella asiatica* and induces apoptosis pathway through the phosphorylation p38 mitogen-activated protein kinase in cisplatin-resistant nasopharyngeal carcinoma cells. *Biomolecules*, 10(184):1-13.

Liu, Y., Yang, X., Gan, J., Chen, S., Xiao, Z. X., & Cao Y. (2022). CB-Dock2: Improved protein–ligand blind docking by integrating cavity detection, docking and homologous template fitting. *Nucleic Acids Research*, 50(W1):W159-W164.

Magaña, A. A., Wright, K., Vaswani, A., Caruso, M., Reed, R. L., Bailey, C. F., Nguyen, T., Gray, N. E., Soumyanath, A., Quinn, J., Stevens, J. F., & Maier C. S. (2020). Integration of mass spectral fingerprinting analysis with precursor ion (MS1) quantification for the characterisation of botanical extracts: application to extracts of *Centella asiatica* (L.) urban. *Phytochemical Analysis*, 31(6):722-738.

Maitra, P., Basak, P., Okamoto, K., Miyoshi, S., Dutta, S., & Bhattacharya, S. (2022). Asiatic acid inhibits intracellular *Shigella flexneri* growth by inducing antimicrobial peptide gene expression. *Journal of Applied Microbiology*, 134(2):1-12.

Menon, S., Mishra, A., Kathirvel, M., Biju, C., Prudhvi, P., & Rao, G. E. (2023). Phytochemical analysis and in vitro evaluation of antibacterial and antidermatophytic activity of *Centella asiatica* (L.) urban extracts. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, 15(3):571-578.

Micheli, L., Toti, A., Lucarini, E., Ferrara, V., Ciampi, C., Olivero, G., Pittaluga, A., Mattoli, L., Pelucchini, C., Burico, M., Lucci, J., Carrino, D., Pacini, A., Pallanti, S., Mannelli, L. D. C., & Ghelardini, C. (2022). Efficacy of a vegetal mixture composed of *Zingiber officinale*, *Echinacea purpurea*, and *Centella asiatica* in a mouse model of neuroinflammation: *In vivo* and *ex vivo* analysis. *Frontiers in Nutrition*, 9 (887378):1-17.

Mohapatra, P., Ray, A., Sandeep, I. S., Parida, R., & Mohanty, S. (2021). Genetic and biochemical stability of in vitro raised and conventionally propagated *Centella asiatica*-A valuable medicinal herb. *South African Journal of Botany*, 140(2021):444-453.

Nguyen, V. B., Wang, S. L., Phan, T. Q., Pham, T. H. T., Huang, H. T., Liaw, C. C., & Nguyen, A. D. (2023). Screening and elucidation of chemical structures of novel mammalian α-Glucosidase inhibitors targeting anti-diabetes drug from herbals used by E De ethnic tribe in Vietnam. *Pharmaceuticals*, 16(756):1-20.

Pham, X. N., Nguyen, H. T., & Pham, N. T. (2020). Green synthesis and antibacterial activity of HAp@ Ag nanocomposite using *Centella asiatica* (L.) urban extract and eggshell. *International Journal of Biomateri-*

als, 2020(8841221):1-12.

Pillai, A. R. S., Bhosale, Y. K., & Roy, S. (2024). Extraction of bioactive compounds from *Centella asiatica* and enlightenment of its utilization into food packaging: A review. *International Journal of Food Science*, 2024(1249533):1-16.

Qurrotuaini, S., Wiqoyah, N., & Mustika, A. (2022). Antimicrobial activity of ethanol extract of *Centella asiatica* leaves on *Proteus mirabilis*, *Proteus vulgaris*, and *Yersinia enterocolitica* in vitro. *Molecular and Cellular Biomedical Sciences*, 6(3):135-140.

Rafi, M., Madya, M. M., Karomah, A. H., Septaningsih, D. A., Ridwan, T., Rohaeti, E., Aisyah, S., Idroes, R. (2024). LC-HRMS-based metabolomics for profiling the metabolites in different plant parts of *Centella asiatica*. *HAYATI Journal of Biosciences*, 31(6):1106-1115.

Rukisah, R., Maulianawati, D., & Cahyadi, J. (2019). In vitro antibacterial efficacy of leaves extract of *Centella asiatica* against *Vibrio harveyi* and *Aeromonas hydrophila*. *Indonesian Aquaculture Journal*, 14(2):69-74.

Selvarajan, V. S., Selvarajan, R., Pandiyan, J., & Abia, A. L. K. (2023). Unveiling the potency and harnessing the antibacterial activities of plant oils against foodborne pathogens. *Microbiology Research*, 14(3):1291-1300.

Shankar, S., & Sathiavelu, M. (2024). *Paradendryphiella arenariae* an endophytic fungus of *Centella asiatica* inhibits the bacterial pathogens of fish and shellfish. *Frontiers in Microbiology*, 15:1-17.

Si, Z., Ma, Z., Luo, F., Shi, J., Huang, J., Wang, Z., Wen, Y., & Han S. (2023). Establishment of SYBR green I real-time PCR for detection of *Streptococcus agalactiae* in aquaculture waters. *Journal of Applied Ichthyology*, 2023(9199300):1-9.

Sieberi, B. M., Omwenga, G. I., Wambua, R. K., Samoei, J. C., & Ngugi, M. P. (2020). Screening of the dichloromethane: Methanolic extract of *Centella asiatica* for antibacterial activities against *Salmonella typhi*, *Escherichia coli*, *Shigella sonnei*, *Bacillus subtilis*, and *Staphylococcus aureus*. *The Scientific World Journal*, 2020(6378712):1-8.

Singh, P., Sharma, A., Bordoloi, M., & Nandi, S. P. (2023). Antimicrobial, antioxidant, GC-MS analysis and molecular docking analysis of bioactive compounds of endophyte *Aspergillus flavus* from *Argemone mexicana*. *Journal of Microbiology, Biotechnology and Food Sciences*, 13(1):e9970.

Sun, B., Wu, L., Wu, Y., Zhang, C., Qin, L., Hayashi, M., Kudo, M., Gao, M., & Liu, T. (2020). Therapeutic potential of *Centella asiatica* and its triterpenes: A review. *Frontiers in Pharmacology*, 11:1-24.

Taghizadeh, M., & Jalili, S. (2024). Phytochemical content, antioxidant properties, and antibacterial activities of *Centella asiatica* L. *Natural Product Research*, 38(20):3693-3698.

Taleghani, A., Eghbali, S., Moghimi, R., & Mokaber-Esfahani M. (2024). *Crataegus pentagyna* willd. fruits, leaves and roots: Phytochemicals, antioxidant and antimicrobial potentials. *BMC Complementary Medicine and Therapies*, 24(126):1-20.

Tripathy, S., Verma, D. K., Thakur, M., Chakravorty, N., Singh, S., & Srivastav, P. P. (2022). Recent trends in extraction, identification and quantification methods of *Centella asiatica* phytochemicals with potential applications in food industry and therapeutic relevance: A review. *Food Bioscience*, 49(101864).

Wang, D., Li, J., Zhu, G., Zhao, K., Jiang, W., Li, H., Wang, W., Kumar, V., Dong, S., Zhu, W., & Tian, X. (2020). Mechanism of the potential therapeutic candidate Bacillus subtilis BSXE-1601 against shrimp pathogenic Vibrios and multifunctional metabolites biosynthetic capability of the strain as predicted by genome analysis. *Frontiers in Microbiology*, 11(581802):1-15.

Wang, R., Huang, H., Du, W., Xia, G., & Tan, Y. (2024a). Network pharmacology and *in vivo* validation to explore the potential active ingredients and mechanisms of Ziyin Buyang formula in diminished ovarian reserve. *Natural Product Communications*, 19(4):1-13.

Wang, X., Zhang, Y., Song, A., Wang, H., Wu, Y., Chang, W., Tian, B., Xu, J., Dai, H., Ma, Q., Wang, C., & Zhou, X. (2024b).

A printable hydrogel loaded with medicinal plant extract for promoting wound healing. *Advanced Healthcare Materials*, 13(8):e2303017.

Wei, C., Cui, P., & Liu, X. (2023). Antibacterial activity and mechanism of madecassic acid against *Staphylococcus aureus*. *Molecules*, 28(1895):1-17.

Wong, J. X., & Ramli, S. (2021). Antimicrobial activity of different types of *Centella asiatica* extracts against foodborne pathogens and food spoilage microorganisms. *LWT-Food Science and Technology*, 142(111026).

Wu, Z., Jiang, D., Wang, J., Hsieh, C. Y., Cao, D., & Hou T. (2021). Mining toxicity information from large amounts of toxicity data. *Journal of Medicinal Chemistry*, 64(10):6924-6936.

Xu, L., Zhang, J., Wang, Y., Zhang, Z., Wang, F., & Tang, X. (2021). Uncovering the mechanism of Ge-Gen-Qin-Lian decoction for treating ulcerative colitis based on network pharmacology and molecular docking verification. *Bioscience Reports*, 41(BSR20203565):1-25.

Yang, L., Marney, L., Magaña, A. A., Choi, J., Wright, K., Mcferrin, J., Gray, N. E., Soumyanath, A., Stevens, J. F., & Maier, C. S. (2023). Quantification of caffeoylquinic acids and triterpenes as targeted bioactive compounds of *Centella asiatica* in extracts and formulations by liquid chromatography mass spectrometry. *Journal of Chromatography Open*, 4(100091):1-10.

Yusof, N. N. M., Idris, K., Rahman, T. A. F. T. A., Adnan, L. A., & Aziz, N. A. A. (2020). Spectroscopy analysis of antimicrobial compound in *Centella asiatica* extract. *Malaysian Journal of Science Health & Technology*, 7(1):65-70.

Zafar, F., Gupta, A., Thangavel, K., Khatana, K., Sani, A. A., Ghosal, A., Tandon, P., & Nishat, N. (2020). Physicochemical and pharmacokinetic analysis of anacardic acid derivatives. *ACS Omega*, 5(11):6021-6030.

Zelellw, D. A., Dessie, G., Mengesha, E. W., Shiferaw, M. B., Merhaba, M. M., and Emishaw, S. (2021). A systemic review and meta-analysis of the leading pathogens causing neonatal sepsis in developing countries. *BioMed Research International,* 2021(6626983):1-20.

Zhang, Y., Yang, Z., & Cock I. E. (2020). *Centella asiatica* (l.) urban leaf extracts inhibit the growth of bacterial triggers of selected autoimmune inflammatory diseases and potentiate the activity of conventional antibiotics. *Pharmacognosy Communications*, 10(3):119-129.

Zheng, W., Lei, M., Yao, Y., Zhan, J., Zhang, Y., & Zhou Q. (2024). Mechanisms underlying the therapeutic effects of *Semen cuscutae* in treating recurrent spontaneous abortion based on network pharmacology and molecular docking. *Frontiers in Molecular Biosciences*, 11(1282100):1-13.