

Integrative GC–MS Metabolite Profiling and Structure-Based Molecular Docking for Identification of Antibacterial Leads from *Calotropis gigantea* and *Nyctanthes arbor-tristis*

Suparna C Chakraborty

chakraborty.suparna79@gmail.com

Dr. Babasaheb Ambedkar Marathwada University

Rohini Kulkarni

Dr. Babasaheb Ambedkar Marathwada University

Research Article

Keywords: *Calotropis gigantea*, *Nyctanthes arbor-tristis*, Phytochemicals, GC–MS metabolite profiling, ADMET analysis, Molecular docking

Posted Date: September 1st, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10795237/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The rapid emergence of multidrug-resistant bacterial pathogens has accelerated the search for novel antibacterial agents from medicinal plants. This study investigated the antibacterial potential of *Calotropis gigantea* and *Nyctanthes arbor-tristis* leaf extracts using an integrated approach involving gas chromatography-mass spectrometry (GC-MS), in vitro antibacterial assays, ADMET prediction, and molecular docking against *Cutibacterium acnes*, *Vibrio vulnificus*, *Aeromonas hydrophila* subsp. *hydrophila*, and *Escherichia coli*. GC-MS profiling identified diverse bioactive metabolites, including diethyl phthalate in *C. gigantea* and several amine derivatives in *N. arbor-tristis*. Antibacterial evaluation revealed that *N. arbor-tristis* exhibited superior activity, with minimum inhibitory concentrations (MICs) of 6.25–25 µg/mL and minimum bactericidal concentrations (MBCs) of 12.5–50 µg/mL. Selected compounds, namely α-tocopherol-β-D-mannoside, phytol, and linolenic acid, demonstrated favourable drug-likeness and ADMET characteristics, although some compounds showed relatively high lipophilicity. Molecular docking revealed that α-tocopherol-β-D-mannoside displayed the strongest binding affinity toward the *V. vulnificus* target protein with a binding energy of – 9.9 kcal/mol, supported by multiple hydrogen-bonding and hydrophobic interactions. Overall, the findings indicate that *N. arbor-tristis* is a promising source of antibacterial phytochemicals and identify α-tocopherol-β-D-mannoside as a potential lead candidate for developing plant-derived therapeutics against multidrug-resistant bacterial pathogens.

Introduction

The rise of antibiotic-resistant microbes has fuelled the quest for new antibacterial drugs that can combat such resistant bacteria. Medicinal plants are some of the most valuable sources because they contain numerous phytochemical compounds that interact with several bacterial targets, thus serving as an excellent platform for discovering new antibacterials (Sibuh et al. 2021; Mandal & Mandal, 2024). Combining phytochemical analysis with computation-based techniques can greatly accelerate the process of finding biologically active substances (Abhinand et al. 2023). Traditional medicinal plants like *Calotropis gigantea* and *Nyctanthes arbor-tristis* have garnered much attention owing to their ethnomedicinal value and numerous pharmacological applications (Sah & Verma 2012; Singh et al. 2014; Aggarwal et al. 2025). The former medicinal plant, *C. gigantea*, commonly called the giant milkweed, has long been recognized for its medicinal properties and efficacy as wound healer and treatment for infections and inflammations. This plant has an array of phytochemical compounds, namely, terpenoids, flavonoids, phenols, sterols, and fatty acids (Sharma et al. 2016; Madhavan et al. 2020). Laboratory experiments have shown that the extracts from *C. gigantea* possess antibacterial properties against microbial pathogens (Bhandari et al. 2022). GC–MS analysis has been used to determine the active compounds responsible for these biological activities (Rajamohan et al. 2014; Sangeetha et al. 2020). Moreover, molecular docking studies have suggested that these phytochemicals effectively interact with bacterial targets, which makes them valuable antibacterial candidates (Beg et al. 2020).

Other closely related species like *C. procera* reinforce the pharmacological importance of the present genus through their antibacterial and antioxidant activity, metabolite profiling using GC-MS, and docking studies (Naser et al. 2019; Al-Rowaily et al. 2020). Recent integrated techniques employing metabolomics and computational tools have discovered bioactive compounds having biological potential, indicating the presence of similar structural moieties within all *Calotropis* species (Rani et al. 2019; Rodríguez-Macías et al. 2025; Nigussie et al. 2026). Another medicinal plant of equal importance includes *N. arbor-tristis*, commonly referred to as parijat or night-flowering jasmine. Traditional uses include fever, infections, inflammation and have shown the presence of phytochemicals such as iridoids, flavonoids, glycosides, and phenolics (Sah & Verma 2012; Mishra et al. 2022). Studies have validated the antibacterial activity of *N. arbor-tristis* against both gram-positive and gram-negative bacteria (Gahtori et al. 2024; Sattar et al. 2024). Metabolite profiling through GC-MS and analysis of its essential oil have revealed several active constituents (Karthick et al. 2019). Computation-based analysis has proven effective interactions of phytochemicals against microbes and inflammation, through docking and simulation studies (Ahmad et al. 2023; Sharma & Shourie 2025). Further explorations centered around antiviral and antifungal mechanisms additionally emphasize the remarkable binding affinity and pharmaceutical qualities of the studied compounds (Albiheyri et al. 2024; Govindasamy et al. 2025; Akhtar et al. 2026). Therefore, these substances are suitable for structure-based antibacterial drug design research (Sarkar & Singh 2022). Bacteria that have been selected for protein-ligand interaction studies in this project are chosen due to their clinical significance, pathogenicity, and previous laboratory findings that indicate their sensitivity to phytochemicals. Bacterial pathogens such as *E. coli*, *A. hydrophila*, and *V. vulnificus* can be responsible for a variety of infections, which involve the gastrointestinal system, wounds, and systemic manifestations. Besides, some bacteria like *C. acnes* can cause cutaneous ailments. Lastly, the availability of structural information for bacterial proteins allows for accurate computer modelling of the interactions between a candidate ligand and a target enzyme, thus allowing the discovery of new antibacterial agents (Sibuh et al. 2021; Abhinand et al. 2023).

The application of GC–MS together with molecular docking has been proven to be an efficient method for the discovery of bioactive compounds from medicinal plants. The use of GC–MS allows for quick identification of phytochemicals that are either volatile or semi-volatile, offering a chemical basis for the selection of these phytochemicals (Madhavan et al. 2020). On the other hand, molecular docking predicts the interaction of these phytochemicals with bacterial targets, thereby assisting in the identification of potentially useful antibacterial compounds (Sibuh et al. 2021; Abhinand et al. 2023). In the current study, all the computational approaches, such as docking analysis, and data processing have been performed through the Google Colab platform, which is a cloud-based platform, facilitating bioinformatics/cheminformatics workflow (Ravikumar et al. 2026). The use of this platform ensures the efficient processing of phytochemicals without necessitating any expensive computational hardware (Kim et al. 2025; Fedai et al. 2025). There has been extensive research on *C. gigantea* and *N. arbor-tristis*; however, the comparative analysis involving both GC–MS analysis and molecular docking has not been carried out widely, as the literature consists primarily of independent phytochemical/biological analysis (Gahtori et al.

2024; Jamal et al. 2025). Thus, the current study attempts to use an integrative approach to compare these two plants and identify novel antibacterial agents from them in order to develop plant-based drugs for AMR (Sibuh et al. 2021; Mandal & Mandal 2024).

Materials and Methods

Plant Material Collection and Extraction

Leaves of *C. gigantea* and *N. arbor-tristis* were picked, cleaned with water, shade-dried, and made into powder form. Each of the powders of *C. gigantea* and *N. arbor-tristis* was then Soxhlet extracted using 95% ethanol for 6–8 hours. The extract was reduced by pressure with the help of a rotary evaporator and kept in the refrigerator at 4°C. The extraction process followed standardized procedures used in phytochemical analysis (Dhivya and Manimegalai 2013; Madhavan et al. 2020).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of *Calotropis* and *Nyctanthes* extracts were evaluated using the broth micro-dilution technique according to CLSI guidelines (CLSI 2018). The collected bacteria from were cultured in Mueller-Hinton broth (MHB) to a 0.5 McFarland standard (1.5×10^8 CFU/mL). The extracts were solubilized in 5% DMSO to prepare stock solutions (1000 µg/mL) that were further diluted to concentrations ranging between 0.1 and 100 µg/mL. Both 100 µL of the extract solution and 100 µL of bacteria were added to each well. Following a 24-hour incubation period at 37°C, the MIC was established based on the lowest concentration where no growth was visually observed and corroborated by OD600 readings. To determine the MBC, 100 µL of the culture was transferred into MHB agar plates from all turbid wells and incubated for 24 hours.

GC–MS Analysis of Phytochemicals

GC-MS technique was conducted to determine the presence of phytoconstituents in the plant extracts (Sharma et al. 2016; Madhavan et al. 2020). The test was carried out using Agilent 7890A GC-MS instrument with DB-5MS capillary column. Helium gas was used for the carrier gas whereas the MS detector was run in the mode of electron ionization (70 eV). The identity of different compounds was determined based on spectra comparison from the NIST library.

Selection of Bioactive Compounds

The bioactive compounds that were chosen from the GC–MS data, which consisted of prominent peaks, were chosen based on their biological activities and relevance, as well as what has already been documented for *C. gigantea* and *N. arbor-tristis*. Compounds such as phytol, fatty acids, amines, and tocopherols were considered because of their known ability to possess antimicrobial and antioxidant activities, as well as disrupting membranes, as demonstrated in previous literature (Mishra et al. 2022; Gahtori et al. 2024; Sharma and Shourie 2025; Ahmad et al. 2023).

Ligand Preparation and ADMET Analysis

These ligands have been obtained from the PubChem database using their compound IDs and subsequently subjected to energy minimization to produce optimized three-dimensional structures through Open Babel according to standard procedures (Beg et al. 2020; Sibuh et al. 2021). Properties like drug likeness and ADMET evaluation have been carried out using SwissADME and pkCSM (Pires et al. 2015).

Protein Preparation and Molecular Docking Analysis

Protein targets for bacteria were sourced from the Protein Data Bank, preprocessed through elimination of water molecules, addition of hydrogen atoms, and optimization before performing the docking process. The interaction between proteins and their ligands was simulated using structure-based computational modeling. In this study, the ColabFold method, which employs the AlphaFold2 algorithm, was employed for protein structure prediction, and multimer modeling and recycling optimization processes were used to improve the accuracy of predictions (Sibuh et al. 2021; Kim et al. 2025; Ohue 2023; Ravikumar et al. 2026). Binding affinities were quantified in units of kcal/mol, with lower binding energy indicating higher affinity of the ligands with the protein target.

Results

MIC and MBC

It is clear from the results that both the plant extracts were able to exhibit antibacterial properties against all the tested bacteria, and compared to *Calotropis*, the extract from *N. arbor-tristis* was more potent, as it's MIC and MBC (Fig. 1) values were relatively low. For *Calotropis*, the MIC values ranged between 25–100 µg/mL, while the MBC values ranged between 50–200 µg/mL. On the other hand, the MIC values of *N. arbor-tristis* ranged

between 6.25-25 µg/mL, and the MBC values ranged between 12.5–50 µg/mL. It can be said that *E. coli* was the most resistant bacteria among all, and *C. acnes* was the most sensitive bacteria.

Antibacterial effect

The study reveals that *Calotropis* as well as *Nyctanthes* were effective against all tested strains (Fig. 2) with *N. arbor-tristis* having greater zones of inhibition than *Calotropis*. The zones of inhibition of *N. arbor-tristis* were between 17 and 22 mm compared to that of *Calotropis*, which had lesser zones varying between 10 and 14 mm. Out of the three tested bacteria, *C. acnes* was more sensitive, while *E. coli* was the least sensitive.

GC–MS Metabolite Profiling of *Calotropis gigantea*

GC–MS analysis of *C. gigantea* leaf extract revealed a diverse phytochemical composition with compounds distributed across a wide retention time range (Table 1). The major compound identified was diethyl phthalate (61.11%), indicating its dominant presence in the extract. Other important compounds included 1-tetradecanamine, N,N-dimethyl- (8.57%), α-tocopherol-β-D-mannoside (5.73%), n-hexadecanoic acid (4.31%), and 9,12,15-octadecatrienoic acid (4.13%). Minor constituents such as phytol and neophytadiene were also detected. The extract was rich in fatty acids, terpenoids, and antioxidant compounds, suggesting significant pharmacological potential.

Table 1
GC-MS analysis of *Calotropis gigantea*

Retention Time (min)	Area %	Compound Name	Chemical Formula	Molecular Weight	PubChem CID
7.693	0.60	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150	332
9.396	0.87	Phenol, 4-ethenyl-2,6-dimethoxy-	C ₁₀ H ₁₂ O ₃	180	35960
9.557	61.11	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222	6781
10.201	8.57	1-Tetradecanamine, N,N-dimethyl-	C ₁₆ H ₃₅ N	241	8211
10.265	0.31	Tridecanal	C ₁₃ H ₂₆ O	198	25311
10.909	1.88	Neophytadiene	C ₂₀ H ₃₈	278	10446
11.291	0.55	Lidocaine	C ₁₄ H ₂₂ N ₂ O	234	3676
11.539	4.31	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	985
11.745	0.34	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, ethyl ester	C ₁₉ H ₃₀ O ₃	306	64871
12.301	0.85	Phytol	C ₂₀ H ₄₀ O	296	5280435
12.380	2.33	10E,12Z-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	5282801
12.411	4.13	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	278	5280934
12.497	0.83	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	5281
15.356	1.49	Tetracosamethyl-cyclododecasiloxane	C ₂₄ H ₇₂ O ₁₂ Si ₁₂	888	167767
17.519	1.54	Stigmast-5-en-3-ol, oleate	C ₄₇ H ₈₂ O ₂	678	20831071
17.630	5.73	.alpha.-Tocopherol-.beta.-D-mannoside	C ₃₅ H ₆₀ O ₇	592	597057

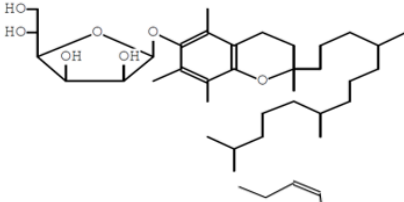
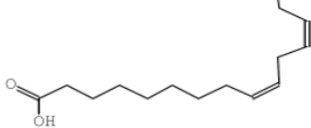
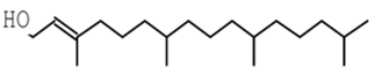
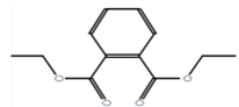
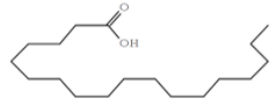
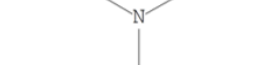
GC–MS Metabolite Profiling of *Nyctanthes arbor-tristis*

GC–MS analysis of *N. arbor-tristis* leaf extract showed a complex and varied phytochemical profile (Table 2). The predominant compound was methylamine, N,N-dimethyl- (30.77%), followed by 1-tetradecanamine, N,N-dimethyl- (23.40%) and tromethamine (18.04%). Additional compounds such as tetradecane derivatives, hexadecane, 1-chloro-, and n-hexadecanoic acid were present in moderate amounts, while phytol and octadecanoic acid were detected in lower concentrations (Fig. 3). The extract mainly consisted of amines, fatty acids, and hydrocarbon derivatives, indicating potential antimicrobial and bioactive properties. The study of compounds in *C. Gigantea* and *N. arbor-tristis* showed that they have some things in common and some things that are unique (Table 3). *C. Gigantea* has some compounds that *N. arbor-tristis* does not have, like α-tocopherol-β-D-mannoside and some other compounds that are good for fighting off things and are related to fats. On the hand *N. arbor-tristis* has a compound called trimethylamine that *C. Gigantea* does not have which means it has a different set of compounds that contain nitrogen. Some compounds are found in both *C. Gigantea* and *N. arbor-tristis* like phytol, N,N-dimethyltetradecan-1-amine and some related compounds, which shows that they both have some of the same good things.

Table 2
GC-MS analysis of *Nyctanthes arbor-tristis*

R.Time	Area	Area%	Name	Chemical Formula	Molecular Weight	PubChem CID
6.896	3021992	1.54	1-Pentanol, TBDMS derivative	C ₁₁ H ₂₆ OSi	202	529180
7.095	1515696	0.77	4-Hexen-1-ol, (E)-, TBDMS derivative	C ₁₂ H ₂₆ OSi	214	5364431
7.892	35439643	18.04	Tromethamine	C ₄ H ₁₁ NO ₃	121	6503
8.197	1299554	0.66	1-Tetradecanol	C ₁₄ H ₃₀ O	214	8209
8.774	1164789	0.59	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	C ₆ H ₁₄ O ₃	134	6510
8.952	1266793	0.65	1-Dodecanamine, N,N-dimethyl-	C ₁₄ H ₃₁ N	213	8168
9.112	616531	0.31	D-Allose	C ₆ H ₁₂ O ₆	180	439507
9.507	2117712	1.08	1-Hexadecanol	C ₁₆ H ₃₄ O	242	2682
10.030	6647290	3.38	Tetradecane, 1-chloro-	C ₁₄ H ₂₉ Cl	232	17043
10.178	45948367	23.40	1-Tetradecanamine, N,N-dimethyl-	C ₁₆ H ₃₅ N	241	8211
10.495	299627	0.15	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	11005
10.557	9150059	4.66	Tetradecane, 1-bromo-	C ₁₄ H ₂₉ Br	276	8208
10.720	556983	0.28	1-Pentadecanamine, N,N-dimethyl-	C ₁₇ H ₃₇ N	255	87232
11.150	8788978	4.48	Hexadecane, 1-chloro-	C ₁₆ H ₃₃ Cl	260	20993
11.261	60426874	30.77	Methylamine, N,N-dimethyl-	C ₃ H ₉ N	59	1146
11.340	26145	0.01	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	8181
11.515	1437818	0.73	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	985
11.638	12469505	6.35	Tetradecane, 1-bromo-	C ₁₄ H ₂₉ Br	276	8208
12.139	307277	0.16	n-Nonadecanol-1	C ₁₉ H ₄₀ O	284	80281
12.225	514001	0.26	1-Heptadecanamine, N,N-dimethyl-	C ₁₉ H ₄₁ N	283	16219
12.258	986733	0.50	Phytol	C ₂₀ H ₄₀ O	296	5280435
12.380	1673976	0.85	cis-9-Hexadecenal	C ₁₆ H ₃₀ O	238	5364643
12.474	722099	0.37	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	5281

Table 3: Comparative Table of Bioactive Compounds in *Calotropis gigantea* vs. *Nyctanthes arbor-tristis*

PubChem CID	Compound Name	Present in Calotropis gigantea	Present in Nyctanthes arbor-tristis	Structure
597057	α -Tocopherol- β -D-mannoside or α -tocopheryl β -D-mannoside	Yes	No	
860	9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	Yes	No	
5280435	(2E,7R,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	Yes	Yes	
6781	Diethyl benzene-1,2-dicarboxylate	Yes	No	
8211	N,N-dimethyltetradecan-1-amine	Yes	Yes	
16217539	1- ¹³ C)octadecanoic acid	Yes	Yes	
1146	trimethylamine	No	Yes	

Physicochemical Properties and ADMET Analysis

The assessed phytochemicals possessed diverse physicochemical and pharmacokinetic characteristics (Supplementary Table 1). The maximum molecular weight (592 g/mol) belonged to α -tocopherol- β -D-mannoside, alongside an abundance of hydrogen bond acceptors (7) and donors (3); it was the only molecule violating two conditions of Lipinski's rule due to high LogP (~ 8.5), suggesting low absorbance but high lipid bioavailability and toxicity. The moderate molecular weight (278 g/mol) and one violation of Lipinski's rule pertained to linolenic acid, whereas phytol had a slightly higher molecular weight (296 g/mol) and a similar profile. Diethyl phthalate (222 g/mol) and trimethylamine (59 g/mol) did not have Lipinski's violations, signifying good drug-like behavior; yet, trimethylamine posed toxicity issues for larger doses. Similarly, N,N-dimethyltetradecan-1-amine and octadecanoic acid had only one Lipinski violation each, but both compounds were highly permeable while potentially toxic and poorly soluble in water.

In Silico Binding Affinity Analysis of Phytochemicals

From the docking results obtained for these ligands, it was evident that there existed a great difference in the binding affinity to different protein targets in bacteria by the selected phytochemicals (Table 4) thus highlighting that each target and ligand have unique binding capacity. In this study, the ligand with the greatest binding capacity towards bacterial proteins was α -tocopherol- β -D-mannoside, which had significant interactions with the *V.vulnificus* protein (7DHD - -9.900 kcal/mol), as well as moderate interactions with 8KA0 (-7.875 kcal/mol) and *C.acnes* protein 6A9N (-7.775 kcal/mol). In addition, linolenic acid showed a relatively high binding affinity towards 7DHD (-7.781 kcal/mol) and 8KA0 (-5.635 kcal/mol), highlighting moderate antibacterial properties. Phytol had a moderate binding capacity with 7DHD (-7.889 kcal/mol) and 6A9N (-6.550 kcal/mol), while diethyl phthalate also showed relatively high binding capacity with 7DHD (-6.615 kcal/mol) and 8KA0 (-6.262 kcal/mol). The bacterial protein that showed significant interactions with all the ligands was *Vibrio* protein (7DHD), making it the preferred protein target for docking studies, while there were no interactions with the protein of *E.coli* (1F47).

Table 4

In Silico Binding Affinity (kcal/mol) of Phytochemicals from *Calotropis gigantea* and *Nyctanthes arbor-tristis* Against Selected Bacterial Proteins

Bacteria	Protein (PDB ID)	α -Tocopherol- β -D-mannoside (C35H60O7)	Linolenic acid (C18H30O2)	Phytol (C20H40O)	Diethyl phthalate (C12H14O4)	N,N-dimethyltetradecan-1-amine (C16H35N)	Octadecanoic acid (C18H36O2)	Trimethylamine (C3H9N)
Aeromonas	6GRJ	-5.694	-5.113	-4.894	-4.762	-4.552	-3.592	-1.906
E. coli	1F47	-0.016	0	-0.006	-0.008	-0.000	0	0
	6G9P	-6.435	-5.967	-5.114	-5.061	-4.620	-4.260	-2.071
Vibrio	6A7H	-5.330	-4.333	-3.950	-4.065	-3.929	-3.217	-1.729
	7DHD	-9.900	-7.781	-7.889	-6.615	-6.283	-4.884	-2.212
	8KA0	-7.875	-5.635	-5.248	-6.262	-4.540	-4.457	-2.513
C. acnes	6A9N	-7.775	0	-6.550	0	0	-6.250	0

Protein–Ligand Interaction Analysis

The interaction study between the protein and the ligands also provided additional validation to the docking study results in terms of types of binding and types of interactions (Supplementary Table 2). It was observed that the compound α -tocopherol- β -D-mannoside had the most interactions, mainly with the protein 7DHD with 4 hydrogen bonds and 7 hydrophobic interactions with key amino acids such as Asp350, Tyr280, and Ser145, implying strong stability of the complex. The other compounds having strong interactions include proteins 8KA0 (H:3, Hy:6) and 6A9N (H:3, Hy:6). It was also found that linoleic acid and phytol possessed good interactions, specifically with protein 7DHD (H:2, Hy:6 for linoleic acid and H:1, Hy:6 for phytol) with amino acid residues like Leu300, Val275, and Ile290. The compound diethyl phthalate also possessed good interactions with different proteins (H:2, Hy:5 with 7DHD), while the compound N,N-dimethyltetradecan-1-amine possessed moderate interactions, mainly hydrophobic in nature. Octadecanoic acid possessed fewer interactions with 6A9N, with 2 hydrogen bonds and 4 hydrophobic interactions. Trimethylamine had few interactions (H:1, Hy:1–2), similar to the docking study results.

Discussion

MIC/MBC data offer a functional perspective on correlating antibacterial action with the detected chemical constituents using the GC-MS method. In this research work, the correlation between GC-MS-assisted metabolite profiling and structure-based molecular docking is used to discover potential antibacterial candidates from *C. gigantea* and *N. arbor-tristis*. The GC-MS test results show a variety of phytochemicals including fatty acids, terpenoids, amines, and phenolic compounds that have been widely studied and found to exhibit considerable biological activities. This kind of diversity in terms of phytochemistry has also been observed in earlier studies, whereby phyto-metabolites like phytol, fatty acids, and tocopherols have been found to exert antimicrobial and antioxidant activities (Madhavan et al. 2020; Mishra et al. 2022; Gahtori et al. 2024). It is noteworthy that diethyl phthalate is abundant in *C. gigantea* while amines are abundant in *N. arbor-tristis*. Based on the bioactive compounds detected in both plants, it is clear that there is some similarity in how they exert their biological activity. One of the active compounds that have been identified and widely researched for its antimicrobial, anti-inflammatory, and antioxidant activities includes phytol (Sharma and Shourie 2025; Ahmad et al. 2023). Other fatty acids (linolenic acid and hexadecanoic acid) are also effective antibacterial agents due to their capability to damage microbial membranes (Nigussie et al. 2026; Rodríguez-Macías et al. 2025). The current study confirms that there is medicinal potential associated with both plants since these bioactive compounds have already been reported to exert some biological activity (Aggarwal et al. 2025; Govindasamy et al. 2025). Molecular docking analysis provided further support for the bioactivity of the selected phytochemicals as a result of their ability to interact with the protein targets of interest. Out of all analyzed compounds, α -tocopherol- β -D-mannoside showed the best binding to *Vibrio* bacteria, suggesting their potential as strong antibacterial agents. The stabilizing effect of tocopherol derivatives to biological membranes can be one of the reasons why these compounds can effectively interact with protein targets (Al-Rowaily et al. 2020). In the same vein, phytol and linoleic acid exhibited very potent and uniform binding affinities for all tested proteins, thus suggesting their broad-spectrum antibacterial activity. This is in line with recent docking studies on binding affinities between phytochemicals and microbial proteins (Sibuh et al. 2021; Akhtar et al. 2026; Ahmad et al. 2023). Differences in binding affinities for various proteins emphasize the need for target specificity in drug development. Interestingly, the *Vibrio* protein (7DHD) was found to interact very strongly with the majority of phytochemicals, thus making it an ideal target for new drugs. Alternatively, the lack of binding by some proteins may indicate problems in ligand accommodation due to limitations in structural/functional aspects. This has been noted in docking experiments, where the interaction between a ligand and a protein is one of the key factors that determines the potential for inhibition (Beg et al. 2020; Karthick et al. 2019). In combination with GC–MS profiling, molecular docking constitutes an effective strategy for the identification of active compounds. Combining both methods makes it possible to select bioactive molecules based on their abundance and biological significance, which contributes to a more effective process of drug lead discovery. Such strategies have been used in a number of recent studies of medicinal plants (Rodríguez-Macías et al. 2025; Mandal and Mandal 2024; Abhinand et al. 2023). What makes this research novel is its innovative strategy of comparing GC–MS profiling and multi-target molecular docking of two medicinal plants. Although various researchers conducted such independent studies on phytochemical composition or molecular docking on single plants (Sharma and Shourie 2025; Nigussie et al. 2026), the current study differentiates itself by the comparative analysis of *N.*

arbor-tristis and *C. gigantea*, which will help to recognize common as well as unique antibacterial phytochemicals in the studied species. In addition, the focus of the current research on the analysis of multi-target interactions is crucial for the development of antibacterial drugs due to the ability of the molecules under investigation to target several bacterial proteins. The results obtained through this study demonstrate the promise that plant-based phytochemicals hold as alternatives to traditional antibacterial medications. The high binding affinity and favorable interaction of certain compounds indicate that these compounds could be used in designing new medications. Moreover, the current study also lays down the groundwork for future experimental studies on these compounds.

Declarations

Acknowledgment

The authors express their sincere gratitude to the Govt. Institute of Science, Dr. Babasaheb Ambedkar Marathwada University, for their support and invaluable guidance throughout the study.

Conflict of Interest

Nil

Author's contributions

SC Conceptualization, methodology, experimental work, data analysis, and original draft preparation. RK: Supervision, molecular docking and bioinformatics analysis, manuscript review, editing, and final approval. Both authors read and approved the final manuscript

Ethical approval

Not applicable.

Funding

Not applicable

References

1. Abhinand CS, Ibrahim J, Keshava Prasad TS, Raju R, Oommen OV, Nair AS (2023) Molecular docking and dynamics studies for the identification of Nipah virus glycoprotein inhibitors from Indian medicinal plants. *J Biomol Struct Dyn* 41:9211–9218. 10.1080/07391102.2022.2153169
2. Aggarwal A, Mehta DK, Bhardwaj A, Das R (2025) *Nyctanthes arbor-tristis*: A multifaceted medicinal plant in traditional and contemporary medicine. *Planta Med.* 10.1055/a-2706-7358
3. Ahmad V, Khan MI, Jamal QMS, Alzahrani FA, Albiheyri R (2023) Computational molecular docking and simulation-based assessment of anti-inflammatory properties of *Nyctanthes arbor-tristis* Linn phytochemicals. *Pharmaceuticals* 17:18. 10.3390/ph17010018
4. Akhtar S, Ahmad V, Aatif M, Jamal QMS (2026) Computational molecular docking and simulation-based prediction of natural compounds from *Nyctanthes arbor-tristis* as potential antifungal agents. *J King Saud Univ Sci* 38. 10.25259/JKSUS_1667_2025
5. Albiheyri R, Ahmad V, Khan MI, Alzahrani FA, Jamal QMS (2024) Investigating the antiviral properties of *Nyctanthes arbor-tristis* Linn against the Ebola, SARS-CoV-2, Nipah, and Chikungunya viruses: A computational simulation study. *Pharmaceuticals* 17:581. 10.3390/ph17050581
6. Al-Rowaily SL, Abd-ElGawad AM, Assaeed AM, Elgamal AM, Gendy AENGE, Mohamed TA, Elshamy AI et al (2020) Essential oil of *Calotropis procera*: Comparative chemical profiles, antimicrobial activity, and allelopathic potential on weeds. *Molecules* 25:5203. 10.3390/molecules25215203
7. Beg MA, Ansari S, Athar F (2020) Molecular docking studies of *Calotropis gigantea* phytoconstituents against *Staphylococcus aureus* tyrosyl-tRNA synthetase protein. *J Bacteriol Mycol Open Access* 8:78–91
8. Bhandari D, Amatya M, Gautam S (2022) Phytochemical analysis and antimicrobial screening studies of *Calotropis gigantea* leaves. *J Pharmacogn Nat Prod* 8:9. 10.37421/2472-0992.2022.8.205
9. Clinical and Laboratory Standards Institute (2018) Performance standards for antimicrobial susceptibility testing, 28th edn. CLSI, Wayne
10. Dhivya S, Manimegalai K (2013) Preliminary phytochemical screening and GC-MS profiling of ethanolic extract of *Nyctanthes arbor-tristis* leaves. *Int J Pharma Bio Sci* 4:B1012–B1019
11. Fedai M, Kwansa AL, Yingling Y (2025) Cloud-based molecular dynamics simulations with AMBER: An interactive collabortutorial via Google Colab. *SSRN Electron J*
12. Gahtori R, Tripathi AH, Chand G, Pande A, Joshi P, Rai RC, Upadhyay SK (2024) Phytochemical screening of *Nyctanthes arbor-tristis* plant extracts and their antioxidant and antibacterial activity analysis. *Appl Biochem Biotechnol* 196:436–456. 10.1007/s12010-023-04552-4
13. Govindasamy C, Sikkandar S, Al-Numair KS, Alsaif MA, Loganathan C (2025) Identification of phytochemicals in *Nyctanthes arbor-tristis* leaf: Interpreting antidiabetic and antibacterial effect through in vitro and in silico analysis. *J Biomol Struct Dyn* 43:6435–6453.

14. Jamal A, Arif A, Kiran S, Shahid MN, Hossain MB (2025) Phytochemical investigations and pharmacological potential of organic extracts of *Calotropis gigantea* L. leaves. *Scientifica* 2025:1669969
15. Karthick V, Venkatareddy G, Dharani J, Ravi S (2019) Study on the chemical constituents of the essential oil from *Nyctanthes arbor-tristis* and its molecular docking studies. *Asian J Pharm Clin Res* 12:195–199
16. Kim G, Lee S, Levy Karin E, Kim H, Moriwaki Y, Ovchinnikov S, Mirdita M et al (2025) Easy and accurate protein structure prediction using ColabFold. *Nat Protoc* 20:620–642. 10.1038/s41596-024-01060-5
17. Madhavan SA, Vinotha P, Uma V (2020) Phytochemical screening and comparative GC-MS analysis of bioactive compounds present in methanolic leaf and latex extract *Calotropis gigantea* (L). *Asian J Adv Med Sci* 2:1–13
18. Mandal M, Mandal S (2024) Discovery of multitarget-directed small molecule inhibitors from *Andrographis paniculata* for Nipah virus disease therapy: Molecular docking, molecular dynamics simulation and ADME-Tox profiling. *Chem Phys Impact* 8:100493. 10.1016/j.chphi.2024.100493
19. Mishra AK, Tiwari KN, Saini R, Chaurasia JK, Mishra SK (2022) Assessment of antioxidant potential in seed extracts of *Nyctanthes arbor-tristis* L. and phytochemical profiling by gas chromatography-mass spectrometry system. *Braz J Pharm Sci* 58:e21180. 10.1590/s2175-97902022e21180
20. Naser EH, Kashmer AM, Abed SA (2019) Antibacterial activity and phytochemical investigation of leaves of *Calotropis procera* plant in Iraq by GC-MS. *Int J Pharm Sci Res* 10:1988–1994
21. Nigussie G, Zaib S, Chanyalew M, Dekebo A, Meressa A, Abebe M, Endale M et al (2026) GC-MS metabolite profiling and multi-target docking analysis of *Calotropis procera* and *Euphorbia tirucalli* stem extracts for cytotoxicity and antioxidant activity. *Sci Rep*
22. Ohue M (2023) MEGADOCK-on-Colab: An easy-to-use protein–protein docking tool on Google Colaboratory. *BMC Res Notes* 16:229. 10.1186/s13104-023-06505-w
23. Rajamohan S, Kalaivanan P, Sivangnanam H, Rajamanickam M (2014) Antioxidant, antimicrobial activities and GC-MS analysis of *Calotropis gigantea* white flowers. *J Phytopharmacol* 3:405–409
24. Rani R, Sharma D, Chaturvedi M, Yadav JP (2019) Phytochemical analysis, antibacterial and antioxidant activity of *Calotropis procera* and *Calotropis gigantea*. *Nat Prod J* 9:47–60
25. Rodríguez-Macías JD, Saurith-Coronell O, Martínez Parra L, Carrascal-Hernández DC, Fuentes-Gandara F, Insuasty D, Márquez-Brazón EA (2025) Integrated in vitro and in silico evaluation of the antimicrobial and cytotoxic potential of *Calotropis procera* leaf ethanolic extract: From GC-MS profiling to molecular docking and dynamics. *Int J Mol Sci* 26:10574. 10.3390/ijms262110574
26. Sah AK, Verma VK (2012) Phytochemicals and pharmacological potential of *Nyctanthes arbor-tristis*: A comprehensive review. *Int J Res Pharm Biomed Sci* 3:420–427
27. Sangeetha K, Steffi PF, Selvi BT, Priyadarshni S (2020) Phytochemical evaluation, GC-MS analysis of phytoactive compounds and antibacterial activity studies from *Calotropis gigantea*. *J Pharm Sci Res* 12:789–794
28. Sarkar S, Singh RP (2022) *Nyctanthes arbor-tristis* L.: Perspective of phytochemical-based inhibition of fatty acid biosynthesis in *Mycobacterium tuberculosis*. *Int J Plant Based Pharm* 2:166–175
29. Sattar T, Intisar A, Kousar R, Ramzan A, Niazi ARK, Qadir MA, Abid MA et al (2024) Constituents and antibacterial activity of essential oil of *Nyctanthes arbor-tristis* flower. *J Anal Chem* 79:1345–1351
30. Sharma P, Shourie A (2025) Antimicrobial activity, phytochemical characterization and molecular docking studies of *Nyctanthes arbor-tristis* L. extracts. *Int J Second Metab* 12:472–485
31. Sharma S, Kumari A, Sharma M (2016) Comparative GC-MS analysis of bioactive compounds in methanolic extract of *Calotropis gigantea* (L.) W.T. Aiton leaf and latex. *Int J Pharmacogn Phytochem Res* 8:1823–1827
32. Sibuh BZ, Khanna S, Taneja P, Sarkar P, Taneja NK (2021) Molecular docking, synthesis and anticancer activity of thiosemicarbazone derivatives against MCF-7 human breast cancer cell line. *Life Sci* 273:119305. 10.1016/j.lfs.2021.119305
33. Singh N, Gupta P, Patel AV, Pathak AK (2014) *Calotropis gigantea*: A review on its phytochemical and pharmacological profile. *Int J Pharmacogn* 1:1–8
34. Ravikumar MJ et al (2026) Genomic analysis of Classical Swine Fever Virus: Single nucleotide polymorphism profiling, phylogenetic insights and implications for disease control. *J Clin Immunol Microbiol* 7:1–9
35. Pires DEV, Blundell TL, Ascher DB (2015) pkCSM: Predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. *J Med Chem* 58:4066–4072. 10.1021/acs.jmedchem.5b00104

Figures

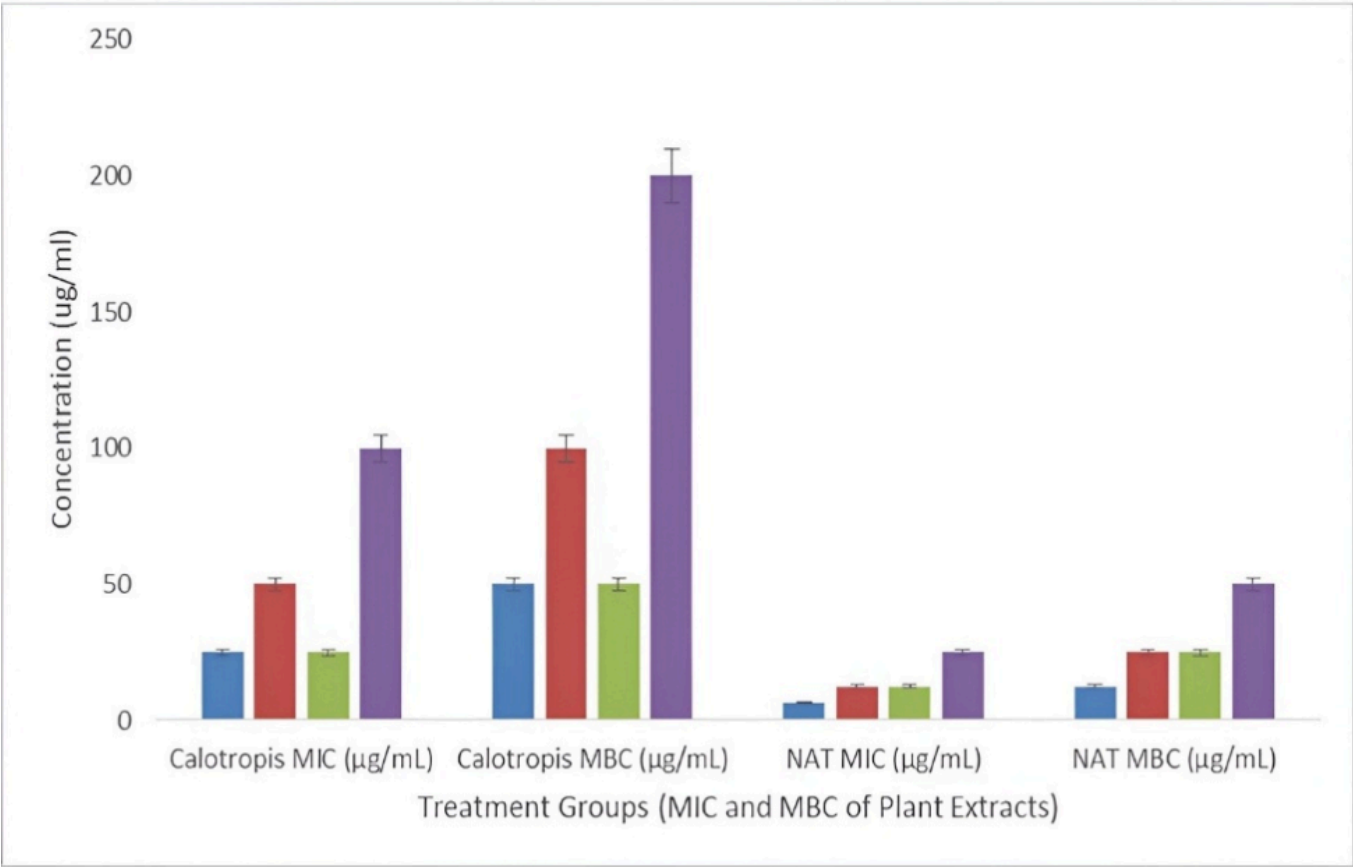


Figure 1

Comparative MIC and MBC Values of *Calotropis* and (NAT) *Nyctanthes arbor-tristis* Against Selected Bacterial Strains

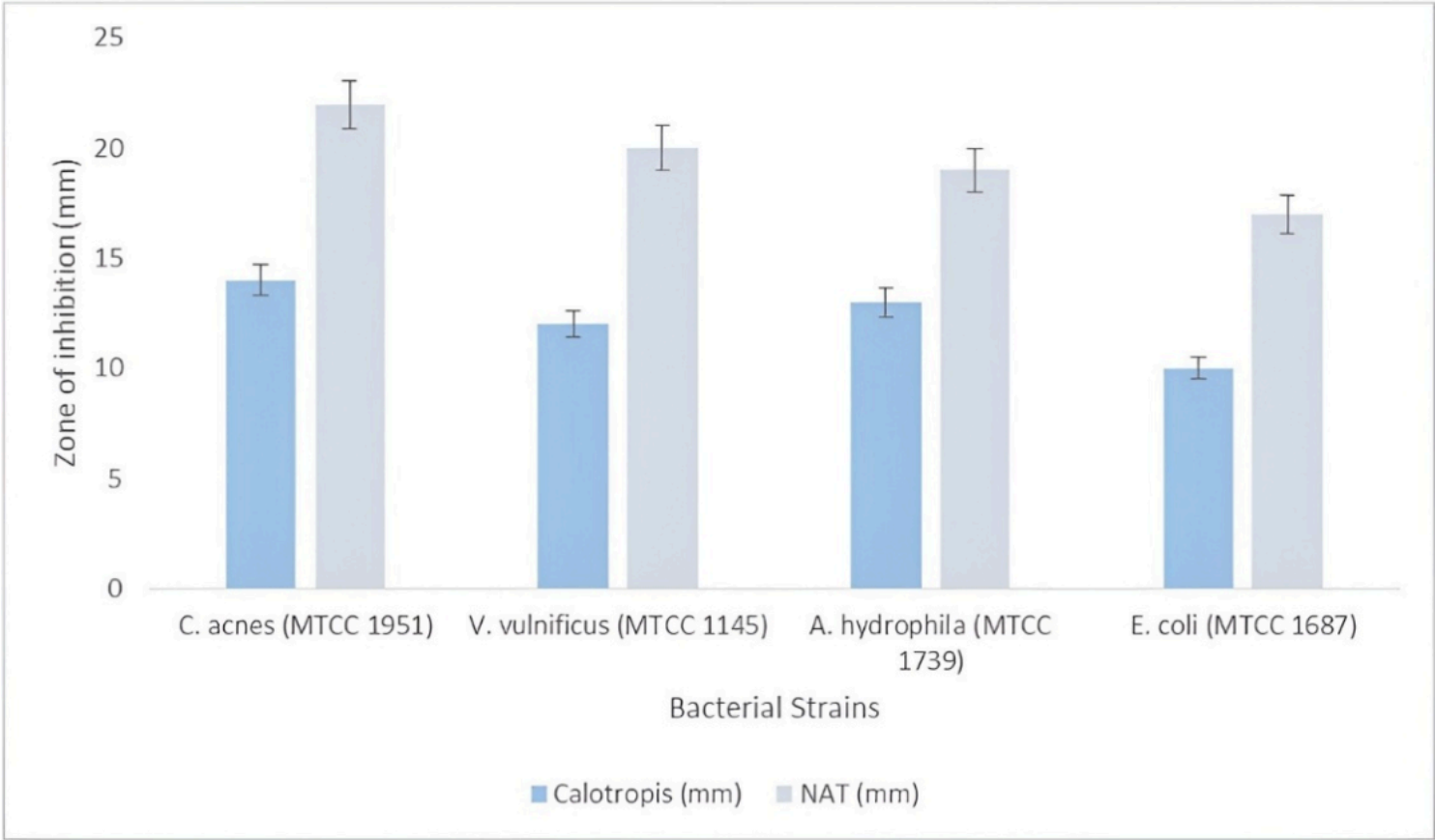


Figure 2

Antibacterial effects of plants extracts against the pathogenic bacteria

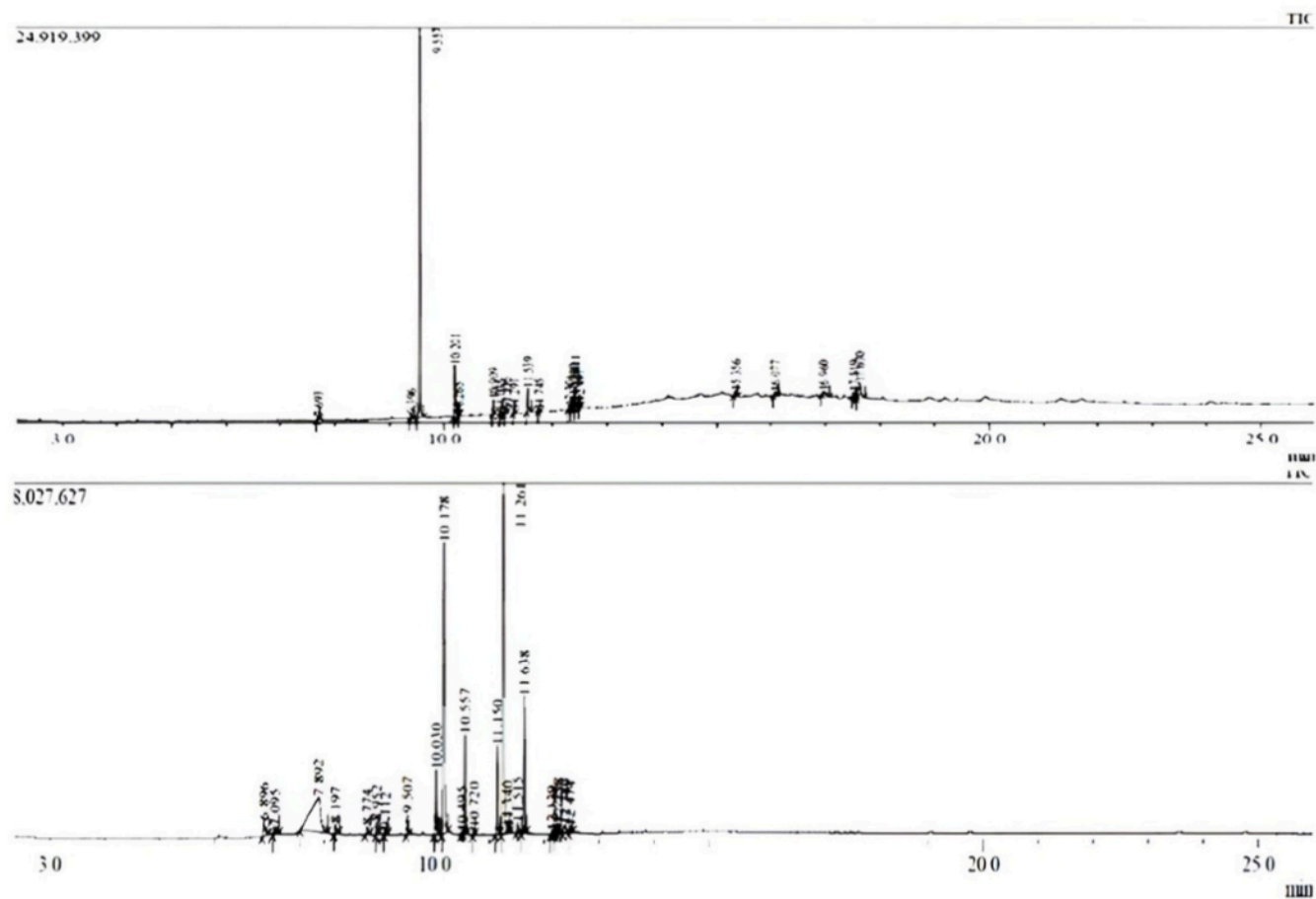


Figure 3

GCMS- Spectral chromatogram of both plant ethanol extract

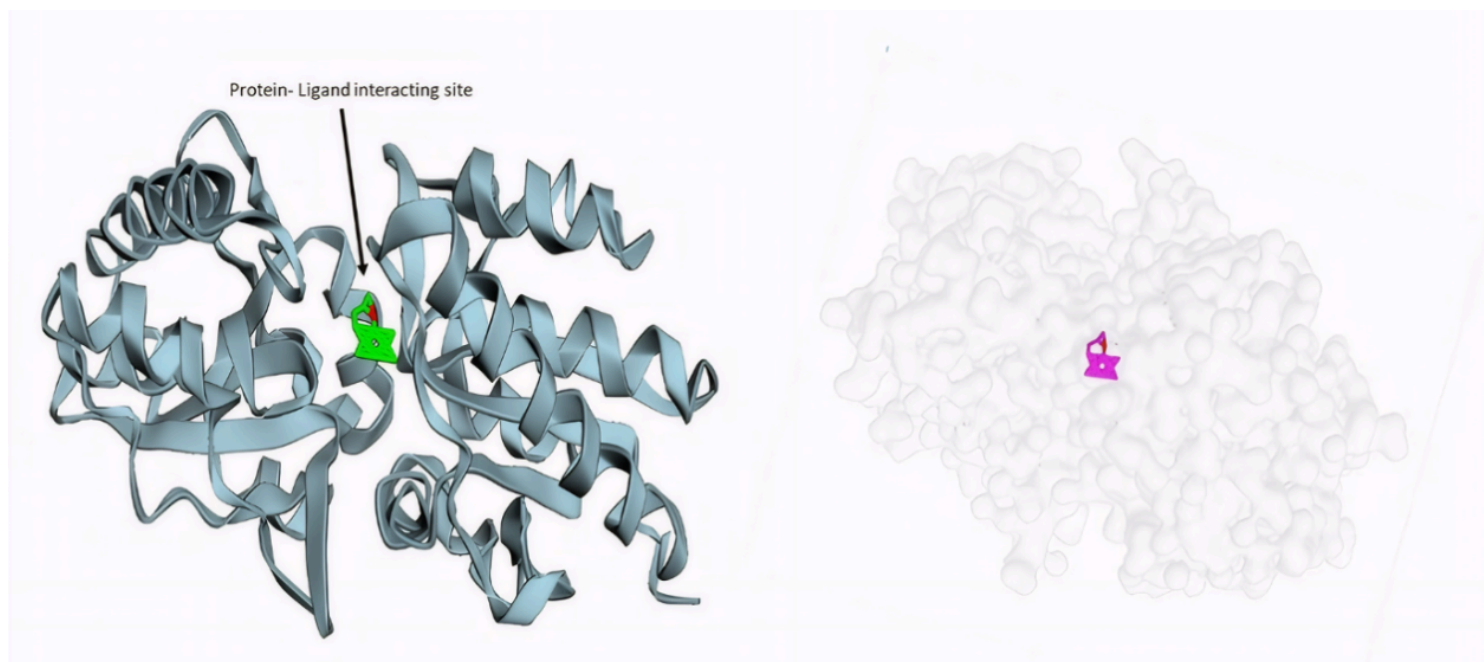


Figure 4

In-Silico Docking Results of Phytochemicals with *Vibrio*Target Proteins