

micrometres to a few hundred micrometres, depending on species. Microalgae serve as a valuable source for many bioactive compounds responsible for various biological activities [4].

They must evolve tolerance/defenses mechanisms to live against other pathogenic microorganisms. These techniques have led to the synthesis of antibacterial compounds with a significant variety and diverse mechanisms of action. Since they have a very basic structure and a high surface/volume ratio, which results in a high nutrient assimilation rate, the growth of green algae is about one hundred times as quick as the growth of terrestrial plants. The rapid growth and simple nutrient requirements attracted researchers to explore the potential of microalgae species all over the world [5].

Therefore, the present study is a screening process of identification of microalgal metabolites having antibacterial properties through *in silico* analysis. The compounds from *Oscillatoria sp.* and *Chlorella sp.* were retrieved and docked against dual bacterial targets DNA Gyrase and DNA topoisomerase. Further the compounds were subjected to drug likeness, PASS predictions and ADMET analysis.

MATERIALS AND METHODS

Target proteins preparation

The tyrosyl-tRNA synthetase (PDB ID: 1JIJ) and DNA Gyrase subunit b (PDB ID: 1KZN) are utilized as target proteins in this study and their 3D structures were acquired from the Protein Data Bank (<http://www.rcsb.org/>). The target proteins are visualized using PyMol tool and the protein associated water molecules, ligands and co-crystal ligands were removed (Figure 1).

The proteins were prepared in Auto Dock Tools, open source free software by adding charges and energy minimization was carried out in Swiss PDB viewer and further converted to pdbqt format.

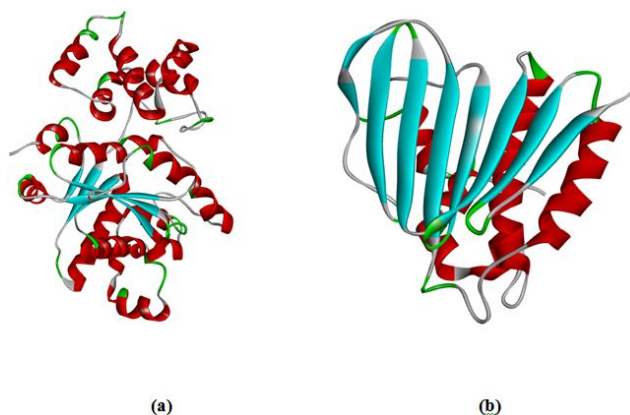


Figure 1: 3D structures of the Target proteins. (a) Tyrosyl RNA synthetase (b) DNA Gyrase.

Retrieval and preparation of ligands

The bioactive compounds present in *Oscillatoria sp.* and *Chlorella sp.* were identified and retrieved using KNApSACK database (<http://www.knapsackfamily.com/KNApSACK/>). Total of 39 bioactive compounds were used for the study (Table 1). The preparation of ligand is performed by identifying its torsion root, assigning charges, correcting torsion angle, optimising using UFF (universal force field) and lastly converting into pdbqt format to create 3D atomic coordinates of the molecules [6].

Table 1: Compounds used in the study.

S. No	Compound name	S. No	Compound name
1	beta-Echinone	21	Largamide B
2	Anatoxin alpha	22	Largamide C
3	Mutatochrome	23	Largamide D
4	Aphanizophyll	24	Largamide E
5	3'-Hydroxyechinenone	25	Largamide F
6	Aeruginosin 205A	26	Largamide G
7	Aeruginosin 205B	27	Largamide H
8	Anabaenopeptin F	28	Viridamide A
9	Anabaenopeptin G	29	Homoanatoxin-a
10	Anabaenopeptin H	30	12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid
11	Lipopurealin A	31	13-Hydroperoxyoctadecadienoic acid
12	Oscillamide H	32	13-Hydroxyoctadecadienoic acid
13	Oscillamide Y	33	9-Hydroperoxyoctadecadienoic acid
14	Prenylagaramide A	34	Ergosta-8,(9)14-dien-3beta-ol
15	Prenylagaramide B	35	Jasmonic acid

16	Raocyclamide A	36	Linolenic acid
17	Anabaenopeptin B	37	Loroxanthin
18	Microviridin I	38	Methyl jasmonate
19	Oscillacyclin	39	Poriferasterol
20	Largamide A		

Determination of functional sites of targets

Significant docking analysis requires precise evaluation of the active (Functional) site. The amino acid residues in the active pocket site formation of target proteins was identified using the CAST online server (Computed Atlas for Surface Topography) [7,8].

CASTp is a simple and handy tool to analysing protein topology and active site pockets. Determining the active site is a critical for setting the grid box prior to docking.

Molecular docking and protein-ligand interaction analysis

All the compounds were docked using the PyRx tool via auto dock wizard. Throughout the docking process, it was believed that the ligands were flexible and the protein was rigid.

The grid parameter configuration file is created in PyRx using the grid boxes for 6W41 (x=-12.59, y=-18.04, z=83.05) and 6LU7 (x=17.27, y=30.68, z=48.04) [9].

Followed by docking, the ligand with the greatest binding energy (most negative) was identified as having the highest binding affinity.

The ligands with a greater binding energy (-7 Kcal/mol) were identified, and the interaction between the ligand and the protein at the binding sites was analysed using Biovia Drug discovery studio 2019.

Screening of the of ligands for drug likeness

Swiss ADME (<http://swissadme.ch/index.php>) is used to evaluate the compound's drug likeness. The drug likeness of a molecule is a critical criterion for validating it as a possible agonist for therapeutic targets.

Compounds showing higher binding energy (<-7 Kcal/mol) were screened for Druglikeness property using the Lipinski's Rule of Five (RO5) [10]. Prediction of activity spectra for substances (PASS) for antibacterial activity

The antibacterial activity of the identified compounds was predicted using the Prediction of activity spectra for substances (PASS) programme [11]. PASS programme is used to predict a variety of physiological effects for a large number of substances.

The substance's activity is predicted and quantified as probable activity (Pa) and probable inactivity (Pi). The only components that have a Pa greater than Pi are those that are viable for the particular medical activity.

ADMET analysis

ADMET analysis utilises online-based algorithms to determine the Absorption, Distribution, Metabolism, Excretion, and Toxicity of particular compounds.

Numerous online databases and offline software programmes are available to aid in the prediction of drug candidate behaviour.

We used admit SAR to make ADMET predictions in this investigation [12]. The compounds with the highest binding energies were evaluated for their intestinal absorption, blood-brain barrier penetration, in vitro Caco-2 cell permeability, CYP450 2C9 substrate, and toxicity characteristics such as mutagenicity as assessed by the Ames test and carcinogenicity in rats.

Ciprofloxacin, a broad spectrum antibiotics used as a reference drug on which the compounds are compared.

RESULTS AND DISCUSSION

Binding site analysis and molecular docking

CASTp was used to determine the functional site pockets in tyrosyl-tRNA synthetase and DNA Gyrase. CASTp is a web-based tool for determining the amino acid residues in a protein's active pocket.

Figure 2 illustrates the CASTp results for tyrosyl-tRNA synthetase and Figure 3 illustrates the CASTp results for DNA Gyrase. From CASTp results, only the amino acids in the active site and their positions are listed as Table 2.

Grid box were generated covering the binding sites of the target protein.

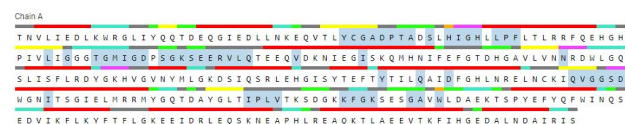


Figure 2: Binding sites of Tyrosylt RNA synthetase analysed using CastP.

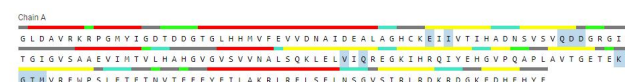


Figure 3: Binding sites of DNA Gyrase analysed using CastP.

Table 2: Amino acid residues in the active sites.

S. No	Target Protein	Amino acid residues in binding sites
1	Tyrosyl-tRNA synthetase (PDB ID: 1JJ)	TYR-36, CYS-37, GLY-38, ALA-39, ASP-40, PRO-41, THR-42, ALA-43, SER-45, HIS-47, ILE-48, GLY-49, HIS-50, LEU-52, PRO-53, PHE-54, LEU-70, GLY-72, THR-75, GLY-76, MET-77, ILE-78, GLY-79, ASP-80, SER-82, GLY-83, LYS-84, SER-85, GLU-86, GLU-87, ARG-88, VAL-89, LEU-90, GLN-91, VAL-96, ILE-103, ASN-124, TYR-170, GLN-174, ASP-177, GLN-190, 191-VAL, GLY-192, GLY-193, SER-194, ASP-195, GLN-196, ILE-200, ILE-221, PRO-222, LEU-223, VAL-224, 231-LYS, PHE-232, GLY-233, LYS-234, GLY-238, ALA-239, TRP-241
2	DNA gyrase (PDB ID: 1KZN)	GLU-58, ILE-60, GLN-72, ASP-73, ASP-74, VAL-133, GLN-135, LYS-162, THR-163, GLY-164, THR-165, MET-166

PyRx was utilised to dock all 39 compounds to their target proteins, tyrosyl-tRNA synthetase and DNA Gyrase. The binding energies of the compounds were determined, and those with a lower binding energy (-7.0

Kcal/mol) against dual targets were identified. Around 19 compounds demonstrated a substantial binding energy (-7.0 Kcal/mol) for both targets and compounds, which are listed in Table 3.

Table 3: Binding energies of the compounds against the target proteins.

S. No	Microalgae compounds	Binding energy (kcal/mol)	
		1JJ	1KZN
1	beta-Echinone	-6.4	-7.1
2	Anatoxin alpha	-5.8	-5.5
3	Mutatochrome	-6.9	-7.5
4	Aphanizophyll	21.1	-6.5
5	3'-Hydroxyechinenone	-6.2	-7.3
6	Aeruginosin 205A	-8.6	-6.4
7	Aeruginosin 205B*	-8.7	-7.6
8	Anabaenopeptin F*	-8.6	-8
9	Anabaenopeptin G*	-9.3	-7.3
10	Anabaenopeptin H*	-9.5	-7.4
11	Lipopurealin A	-7.6	-6.2
12	Oscillamide H*	-8.5	-8.3
13	Oscillamide Y*	-8.6	-9.6
14	Prenylagaramide A	-6.3	-8.1
15	Prenylagaramide B*	-9	-8.2
16	Raocyclamide A*	-9.1	-7.6
17	Anabaenopeptin B*	-8.1	-9
18	Microviridin I	9	-7.5
19	Oscillacyclin	-5.7	-7.6
20	Largamide A*	-9.8	-8.6
21	Largamide B*	-9.3	-7.8
22	Largamide C*	-9.4	-8.3
23	Largamide D*	-6.2	-6.8
24	Largamide E*	-7.2	-6.4
25	Largamide F*	-6.2	-7.7
26	Largamide G*	-9.6	-8.4

27	Largamide H	-1.4	-6.5
28	Viridamide A	-7.2	-5.4
29	Homoanatoxin-a	-6	-5.9
30	12, 13-trans-Epoxy-9-oxo-10E, 15Z-octadecadienoic acid*	-7	-7.3
31	13-Hydroperoxyoctadecadienoic acid*	-8.3	-7.7
32	13-Hydroxyoctadecadienoic acid	-6.3	-5.4
33	9-Hydroperoxyoctadecadienoic acid	-6.5	-5.7
34	Ergosta-8,(9)14-dien-3beta-ol*	-7.8	-8.3
35	Jasmonic acid*	-7.7	-7
36	Linolenic acid	-5.8	-5.1
37	Loroxanthin*	-6.4	-7.5
38	Methyl jasmonate*	-7.5	-7.8
39	Poriferasterol*	-8.5	-7.8

*Compounds showing binding energies higher than -7 Kcal/mol

Analysis of protein-ligand interaction

The best-docked compounds were further examined for binding interactions on active sites with amino acid residues using Biovia Accelrys Discovery Studio Visualizer software. Bonding type, number of hydrogen bonds and hydrophobic interactions are a very important determinant of protein-ligand interactions as equal as binding affinity. The number of hydrogen bonds formed

and amino acids involved in the interactions are tabulated in Table 4. The hydrogen bonds and other hydrophobic interactions of the ligands on the binding sites of the target proteins were shown in the Figure 4. All the 19 compounds showed H-bond formation on binding sites of the target proteins except Oscillamide Y, Prenylagaramide B, Largamide B, Largamide C and Largamide G. These compounds failed to show H-bond on binding sites of DNA Gyrase.

Table 4: Protein-ligand interactions.

S. No	Microalgae compound	1JJ		1KZN	
		No of H-bond	Binding amino acid residue	No of H-bond	Binding amino acid residue
1	Aeruginosin 205B	8	A: 47-HIS; 50-HIS; 84-LYS; 193-GLY; 195-ASP; 233-GLY	1	A: 165-THR
2	Anabaenopectin F	5	A: 47-HIS; 50-HIS; 89-VAL; 91-GLN	5	A: 72-GLN; 73-ASP; 135-GLN; 165-THR
3	Anabaenopectin G	4	A: 47-HIS; 50-HIS; 88-ARG; 174-GLN	2	A: 165-THR; 73-ASP
4	Anabaenopectin H	7	A: 47-HIS; 49-GLY; 50-HIS; 75-THR; 80-ASP; 174-GLN; 196-GLN	1	A: 165-THR
5	Oscillamide H	5	A: 47-HIS; 50-HIS; 195-ASP; 232-PHE	2	A: 74-ASP; 135-GLN
6	Oscillamide Y	4	A: 195-ASP; 196-GLN; 232-PHE	-	-
7	Prenylagaramide B	4	A: 47-HIS; 50-HIS; 232-PHE	-	-
8	Raocyclamide A	3	A: 50-HIS; 84-LYS; 195-ASP	2	A: 74-ASP; 135-GLN
9	Anabaenopectin B	4	A: 50-HIS; 195-ASP; 233-GLY	1	A: 73-ASP
10	Largamide A	10	A: 38-GLY; 40-ASP; 47-HIS; 50-HIS; 193-GLY; 195-ASP; 232-PHE	1	A: 73-ASP

11	Largamide B	2	A: 47-HIS; 196-GLN	0	-
12	Largamide C	6	A: 47-HIS; 50-HIS; 84-LYS; 195-ASP; 232-PHE	0	-
13	Largamide G	6	A: 50-HIS; 75-THR; 84-LYS; ASP-177; ASP-195; PHE-232	0	-
14	12, 13-trans-Epoxy-9-oxo-10E, 15Z-octadecadienoic acid	2	A: 84-LYS; 190-GLN	1	A: 165-THR
15	13-Hydroperoxyoctadecadienoic acid	1	A: 174-GLN	1	A: 165-THR
16	Ergosta-8,(9)14-dien-3beta-ol	1	A: 224-VAL	1	A: 58-GLU
17	Jasmonic acid	3	A: 37-CYS; 38-GLY; 190-GLN	1	A: 165-THR
18	Methyl jasmonate	3	A: 37-CYS; 38-GLY; 174-GLN	1	A: 165-THR
19	Poriferasterol	1	A: 174-GLN	1	A: 58-GLU

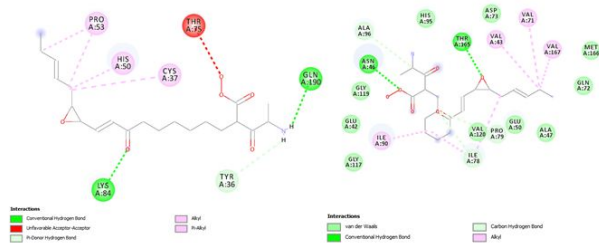


Figure 4: Interaction of 12, 13-Trans-Epoxy-9-oxo-10E, 15Z-octadecadienoic acid on target proteins.

Druglikeness analysis of the selected compounds

The antagonistic interaction of inhibitors with a receptor protein or enzyme cannot guarantee the suitability of an inhibitor as a drug; therefore, drug likeness of inhibitors is more important in the drug development. Therefore all the selected compounds are screened for its drug likeness property. From analysis of 19 compounds showing higher binding energies, 6 compounds showed drug likeness. The compounds satisfying RO5 are 12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid (MW: 308 g/mol; H-bond acceptors: 4; H-bond donors: 1; MLogP: 2.26), 13-Hydroperoxyoctadecadienoic acid (MW: 312 g/mol; H-bond acceptors: 4; H-bond donors: 2; MLogP: 3.55), Ergosta-8,(9)14-dien-3beta-ol (MW: 398

g/mol; H-bond acceptors: 1; H-bond donors: 1; MLogP: 6.43), Jasmonic acid (MW: 210 g/mol; H-bond acceptors: 5; H-bond donors: 3; MLogP: 1.68), Poriferasterol(MW: 412 g/mol; H-bond acceptors: 1; H-bond donors: 1; MLogP: 6.62), Methyl jasmonate(MW: 224 g/mol; H-bond acceptors: 6; H-bond donors: 3; MLogP: 1.95).12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid, 13-Hydroperoxyoctadecadienoic acid, Jasmonic acid and Methyl jasmonate satisfied Ro5 without any violations whereas Ergosta-8,(9)14-dien-3beta-ol and Poriferasterol satisfied with 1 violation. Other compounds failed to possess drug likeness. Thus the identified 6 compounds were subjected to PASS prediction and ADMET analysis.

In silico PASS prediction

In silico PASS prediction: All nine compounds were evaluated to determine their antibacterial activity and their probable activity (Pa) and probable inactivity (Pi) values are reported. The probability of particular pharmacological action is high if Pa values are greater than Pi (Pa>Pi). Antibacterial prediction was determined for 6 compounds and presented in the Table 5. Among them 12,13-trans-Epoxy-9-oxo-10E, 15Z-octadecadienoic acid and Ergosta-8,(9)14-dien-3beta-ol showed higher Pa:Pi ratio. Notably, all the compounds showed antibacterial action i.e. Pa>Pi values.

Table 5: PASS predictions.

S. No	Microalgae compound	Pa	Pi
1	12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid	0.452	0.022
2	13-Hydroperoxyoctadecadienoic acid	0.173	0.143
3	Ergosta-8,(9)14-dien-3beta-ol	0.315	0.055
4	Jasmonic acid	0.313	0.055

5	Methyl jasmonate	0.274	0.07
6	Poriferasterol	0.209	0.099

ADMET properties

The ADMET characteristics of the compounds have an influence on their absorption, distribution, metabolism, excretion, and toxicity inside and throughout the human body. ADMET is a term that refers to the pharmacokinetic characteristics of a pharmacological molecule and is critical for determining its pharmaco-dynamic action. Azithromycin, a broad spectrum antibiotic is used as control drug for comparison. Azithromycin had

probability values of 0.97, 0.55, 0.75, 0.67, and 0.83 for BBB, HIA, in vitro Caco-2 permeability, distribution, and non-substrate of CYP450 2C9 respectively. Other compounds showed substantial ADME values and presented in Table 6. From the toxicity analysis, AMES toxicity and mutagenicity of the compounds were evaluated. All the compounds were found to be non-mutagenic and non-carcinogenic (Table 7).

Table 6: ADME analysis.

S.No	Microalgae Compounds	In vivo blood-brain barrier penetration (C. brain/C. blood)	Human intestinal absorption (%)	In vitro Caco-2 cell permeability (nm/sec)	Distribution	CYP450 2C9
1	Azithromycin	0.97	0.55	0.75	0.67	NS (0.83)
2	12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid	0.88	0.92	0.5	0.79	NS (0.77)
3	13-Hydroperoxyoctadecadienoic acid	0.88	0.92	0.54	0.67	NS (0.82)
4	Ergosta-8,(9)14-dien-3beta-ol	0.98	1	0.83	0.49	NS (0.79)
5	Jasmonic acid	0.9	0.98	0.64	0.83	NS (0.77)
6	Methyl jasmonate	0.95	0.99	0.7	0.81	NS (0.85)
7	Poriferasterol	0.97	1	0.79	0.46	NS (0.84)

Table 7: Toxicity analysis.

S.No	Microalgae Compounds	AMES Non-toxic	Non-Carcinogen
1	Azithromycin	0.91	0.93
2	12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid	0.82	0.88
3	13-Hydroperoxyoctadecadienoic acid	0.79	0.67
4	Ergosta-8,(9)14-dien-3beta-ol	0.89	0.93
5	Jasmonic acid	0.9	0.88
6	Methyl jasmonate	0.87	0.84
7	Poriferasterol	0.91	0.91

DISCUSSION

Microalgae metabolites serve as the potential source with wide range of biological activities. In the search of new antimicrobials, microalgae metabolites can be the best solution in context of bioavailability and reactivity. Since there are thousands of metabolites identified in microalgae, *in silico* studies paves way for quick and effective screening of best compounds. An effective compound with target specificity and better ADMET properties can be identified. Thus, *in silico* screening is the best technique for identification of new drugs [13].

Today, several drug-resistant bacteria have developed with their own unique mechanisms of resistance,

resulting in antibiotics that are mostly useless and a looming global threat. Antibiotic resistance (AMR) was identified by the WHO as an increasing crisis in the world, and therefore, the need for novel antimicrobial treatments to fight AMR [14]. Therefore, Microalgae metabolites from *Oscillatoria sp.* and *Chlorella sp.* were screened for the antibacterial action against potential bacterial targets tyrosyl-tRNA synthetase and DNA gyrase in the present study.

Bacterial gyrase focused therapeutics is exclusive because it is the universal enzyme required for the survival of bacterial and is missing in higher eukaryotes. DNA gyrase (gyrase), a bacterial topoisomerase, is known

to regulate DNA-dependent processes by introducing transient breaks in both DNA strands and relieving torsional stress in the DNA molecule by introducing negative supercoils. DNA Gyrase is a heterotetrameric protein made up of two GyrA subunits that contain the DNA cleavage site and two GyrB subunits that provide the energy for the enzyme's catalytic function by hydrolyzing ATP. Thus, drugs that target bacterial topoisomerases function in one of two ways: either by stabilising the complex between the DNA molecule and the enzyme's GyrA active site (e.g. quinolones) or by inhibiting the GyrB subunit's ATPase activity (e.g. Aminocoumarin class of inhibitors) [15].

Aminoacyl-tRNA synthetase (aaRSs) is a type of significant enzymes that catalyse the transport of amino acids to their corresponding tRNAs during protein synthesis. They are necessary to translate coded information into protein structures in nucleic acids since they recognise this information, which includes coincident tRNA molecules and amino acid structures. TyrRS are present in all living species as members of the aaRS family. TyrRS is a member of the class I tRNA synthetase family, which has two strongly symmetric sequence motifs, HIGH and KMSKS, at the active site. TyrRS in bacteria and TyrRS in humans vary in a number of ways. Small-molecule TyrRS inhibitors with these properties may be promising drug candidates for high selectivity and broad-spectrum antibacterial agents. TyrRS plays an important role in protein biosynthesis, and inhibiting these enzymes is harmful to cells. TyrRS is also highly conserved among prokaryotes, making it a good target for the production of broad-spectrum antibiotics [16].

Total of 39 compounds were identified KNApSack database and these compounds were subjected to docking against the dual targets. Compounds showing higher binding energies (>-7 kcal/mol) were investigated for protein-ligand interaction. Interaction of ligands on binding sites of the targets proteins are very much essential on comparing with the higher binding energies. Formation of H-bonds proves the stability of the docked complex. Therefore the, H-bond formation on binding sites of the target proteins was evaluated. 14 compounds showed significant interactions with H-bonds on binding sites. Druglikeness analysis was carried out based on the Lipinski rule of five (RO5). RO5 is a rule of thumb to determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely active drug in humans. Only six compounds showed drug likeness property and further subjected to PASS predictions and ADMET analysis. All the compounds possess $Pa > Pi$ values for antibacterial action and had significant ADMET range on compared with Azithromycin.

Antibacterial activity of the *Chlorella vulgaris* and *Oscillatoria geminata* was investigated by Salem et al. [17]. Methanolic extracts of *Chlorella vulgaris* showed significant antibacterial activity against *B. subtilis*, *K. pneumoniae*, *S. aureus* and *S. lutea*; and *Oscillatoria*

geminata against *K. pneumoniae* and *S. aureus*. The MIC of the *Oscillatoria sp.* SSCM01 extracts was found to be 31.2 $\mu\text{g/mL}$ against *S. aureus* and 7.8 $\mu\text{g/mL}$ against *S. typhi* [1]. Similarly, *Chlorella vulgaris* showed MIC of 5 mg/ml against *S. mutans* [18]. Ethanol extracts of *C. vulgaris* had significant inhibition against *E. coli*, *Klebsiella sp.* and *Pseudomonas sp.* [19]. These results well correlates the present study.

CONCLUSION

Thirty-nine compounds corresponding to *Oscillatoria sp.* and *Chlorella sp.* were retrieved from KNApSack database and used as ligands in the study. The antibacterial bacterial targets tyrosyl-tRNA synthetase and DNA Gyrase subunit b were obtained from Protein data bank. Docking analysis was performed and compounds showing higher binding energies were subjected to further Drug likeness, PASS predictions and ADMET analysis. From the analysis, we identified six compounds (12, 13-Trans-Epoxy-9-oxo-10E, 15Z-octadecadienoic acid, 13-Hydroperoxyoctadecadienoic acid, Ergosta-8, (9)14-dien-3beta-ol, Jasmonic acid, Methyl jasmonate and Poriferasterol) with potential binding energy, interactions and drug likeness properties. All the selected compounds showed significant ADME properties on compared with standard drug Azithromycin and found to be non-toxic. Thus, these compounds can be used further for development of antibacterial drugs for treatment of multidrug resistant (MDR) infections.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest relevant to this article.

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