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Molecular Docking, ADMET Property Analysis and Antibacterial Potency of Bioactive Compounds from Marine *Bacillus cereus* against ESPF *(E. Coli)*

Shalini T. S.*, Senthilraja P. and Manivel G.

Department of Bioinformatics, School of Life Science, Bharathidasan University, Tiruchirappalli-620024.

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***Corresponding Author**
Shalini T. S.
Department of Bioinformatics, School of Life Science, Bharathidasan University, Tiruchirappalli-620024.

ABSTRACT

Background: This research implies that the exploitation of *Bacillus cereus* could conceivably lay the groundwork for the formation of a pioneering antibacterial pharmaceutical agent. **Aim:** This study to indicate that *Bacillus cereus* could potentially explored for the development of antibacterial drug. **Methods:** The antibacterial activity of *Bacillus cereus* crude extract was demonstrated through molecular docking study and disc diffusion method. **Result:** *Bacillus cereus* crude extract, when compared to streptomycin, shows the most potent antibacterial activity against *E. coli* and *Salmonella typhi* at high concentrations. Compounds identified by pharmacokinetics properties their predicted biological targets are absorption, distribution, metabolism, excretion and toxicity (ADMET) parameters related to the screened compounds are included isobutyl acetate, methoxy acetic acid, 3-tetradecyl ester, and silicic acid diethyl bis (trimethylsilyl) ester and their interaction assessed by molecular docking of Pathogenic bacteria proteins like *E. coli* (PDB ID: 2YKT) and *Salmonella Typhi* (PDB ID: 5JW7) are used to evaluate their binding affinity.

KEYWORDS: Antibacterial activity, ADMET, *Bacillus cereus*, *E. coli*, GC-MS, Molecular docking.

INTRODUCTION

The marine environment possesses a rich source of biodiversity, approximately half of the total diversity (Costello, M. J., & Chaudhary, C. 2017). Marine microbes have recently received increased attention because of being a valuable source of biologically active compounds of pharmaceutical significance (Romano *et al.*, 2017). Products from marine organisms have shown anti-microbial, anticancer, anti-diabetic, and other pharmacological activities (Sharma *et al.*, 2019). A number of novel anti-microbial bioactive compounds have been isolated from marine bacteria, fungi, algae, sponges, etc (Wang, *et al.*, 2014). The marine bacteria supply novel leads against pathogenic microbes that are developing resistance to the existing pharmaceuticals (Awais *et al.*, 2007; Hassan *et al.*, 2014).

Microorganisms are more responsive for large-scale production of bioactive compounds in shorter period than plant or animal sources as because of their large-scale production (Bhumika, *et al.*, 2013). Until, marine microorganisms could be considered as a relatively unrecognized source for the discovery of bioactive compounds and could provide novel bioactive compounds in terms of structure and bioactivity (Ribeiro, *et al* 2014; Eagle, 1976). Among marine microbes, *Bacillus* species produce versatile secondary metabolites including lipopeptides, polypeptides, macro lactones, fatty acids, polyketides, and isocoumarins. (Mondol *et al.*, 2013) are the most important of natural product. It having structural variability of compounds has attracted the curiosity of biological activities (Estibaliz Sansinenea & Aurelio Ortiz 2011). It inspired the pharmaceutical industry to search for lead structures in microbial extracts. Screening of microbial extracts reveals the large structural diversity of bioactive compounds possessing varied biological activities including antibiotic, immunosuppressive, antioxidant, antiviral and enzyme inhibition properties (Abdelghani *et al.*, 2021). *Bacillus cereus*, a spore-forming Gram-positive bacterium frequently occurs in soil and marine sediments and its spores can survive thermal processing conditions (Bray *et al.*, 2018). Different traits among the species in the *Bacillus cereus* group could be interpreted as a sign of pathogenicity. Although *B. cereus* is regarded as a pathogen, isolates with antibiotic activity are said to be overrepresented in the species. (David *et al.*, 2015; Khalifa *et al.*, 2019). Reports have confirmed the flexibility of *Bacillus* species to supply antimicrobial compounds with potential biotechnological and pharmaceutical applications (Kumar *et al.*, 2014).

In recent years, the use of natural antibacterial agents has been proposed as another source for the deactivation of vegetative cells and bacterial spores. Due to the safety concerns of synthetic antimicrobials agents compared to natural source of the selection of generally recognized as safe (GRAS) (Cetin-Karaca & Newman 2018).

The exploration for novel antimicrobial compounds from natural sources is crucial to overcome drug resistance phenomena and harmful side effects associated with some verified artificial drugs (Jeong, 2008). Several natural products of marine microbial source possess antimicrobial activity in *in vitro* and *in silico* models against various mechanisms of action (Suzumura *et al.*, 2003).

In present study the antimicrobial activity of bioactive crude extracts from marine bacterium *Bacillus cereus* was assessed the *in vitro* antimicrobial evaluation and Minimal inhibitory concentration of resistant against four pathogenic bacterium such as *Escherichia coli, Salmonella typhi, Enterobacter cloacae* and *Enterococcus faecalis* and their *in silico* study are selected pathogenic bacterial growth proteins *E. coli* (PDB ID: 2YKT), *S. typhi* ((PDB ID: 5JW7), *E. cloacae* ((PDB ID: 4HFK) and *E. faecalis* ((PDB ID: 6ORI) are validated by molecular docking against the Isobutyl Acetate, Methoxy acetic acid, 3-tetradecyl ester and Silicic acid diethyl bis(trimethylsilyl) ester along with the prediction of activity are investigated in this study. Moreover, to confirm the stability and oral bioavailability of the drug, was prediction by ADMET pharmacokinetic and drug-likeness properties is described.

MATERIALS AND METHOD

Sample collection

Marine bacterium *Bacillus cereus* PSMS6 strain (Accession No. OQ946984) was obtained from the Department of Bioinformatics, Bharathidasan University, Trichy, Tamil Nadu (India). The bacterium was inoculated by quadrant streaking on nutrient agar plate and incubated at 37ºC for 24 h for growth and multiplication. The pure culture was maintained in nutrient agar slants in a refrigerator till use.

Mass cultivation of *B. cereus*

B. cereus was grown in 100 mL Erlenmeyer flask containing 50 mL Luria broth (LB) and incubated at 37˚C for 24 h. This seed culture was transferred to 1000 mL flask containing LB medium for mass culture and incubated at 37˚C for 5 days on a rotary shaker (120 rpm).

Crude extract preparation

The crude extract was prepared as per the method of (Vijayaram Seerangaraj *et al*., 2017). The bacterial culture after incubation was centrifuged at 8,000 rpm for 20 min so as to harvest cell pellet. The supernatant was then filtered through a 0.22 µm membrane filter to remove bacterial cells. The cell-free supernatant was then mixed with equal volume of ethyl acetate (1:1 v/v) and kept in a shaker at 120 rpm for 24 h. The ethyl acetate phase was separated by using solvent extraction funnel, concentrated under rotary vacuum evaporator and dried by using 77 ˚C for magnetic stirring. The crude extract was then stored in -20˚C.

In vitro antibacterial assay

The antimicrobial activity of *B. cereus* crude extract was evaluated by agar disk diffusion assay (Vijayaram Seerangaraj *et al.*, 2017). The inhibitory effect of crude extract was assessed against four pathogens *viz*., *Escherichia coli*, *Salmonella typhi*, *Enterobacter cloacae* and *Enterococcus faecalis* are obtained from rhizosphere biology laboratory, Department of microbiology, Bharathidasan University, Trichy, Tamil Nadu, India. The overnight cultures of test bacterial pathogens were prepared and aseptically spread over Muller Hinton Agar (MHA) plates using sterile cotton swabs. Thereafter 6 mm diameter sterile disk were made over MHA plates using sterile forceps. The disk was impregnated with various concentration like (25, 50, 75 and 100 µL) of bacterial crude extract and streptomycin was used as a positive control disk. The plates were incubated at 37˚C for 24 h. The zone of inhibition formed around each disk was measured in millimetre (mm). The assay was carried out in triplicate. Data represented as mean and Standard deviation.

Determination of mminimal inhibitory concentration (MIC)

The MIC was assessed in 96 well polystyrene plates. The 100 µL crude extract of *Bacillus cereus* of different concentrations was prepared for test bacterial concentration as 10mg/ml. The wells were loaded with different concentration (10, 25, 50, 75, 100 and 125 µL of *Bacillus cereus* crude extract). In the same way, different concentrations of streptomycin (10, 25, 50, 75, 100 and 125 µL) were prepared and used as positive controls. Thereafter 100 µL of Muller Hinton broth was added into each well, crude extract, positive control and blank samples were coated individually in 96 well plates. Then 10 µL each of test bacterial cultures were added to the respective wells and incubated at 37˚C. The bacterial growth was observed after 24 h and growth measured in a spectrophotometer (Bio rad, imart-IN505509) at 570 nm. The experiment was performed in CRD and each treatment replicated three times. The percentage of inhibition concentration was calculated as under

$$\text{Percentage of inhibition (\%)} = (\text{Control OD} - \text{Test OD}) / \text{Control OD} \times 100.$$

In silico studies

Target selection and preparation: The crystallography structure of the proteins of *E. coli* ESPF (PDB ID: 2YKT), *Salmonella typhi* protein SopA (PDB ID: 5JW7), *Enterobacter* *cloacae* protein (PDB ID: 4HFK) and *Enterococcus faecalis* (PDB ID: 6ORI) were retrieved from Protein data bank (PDB) https://www.rcsb.org/. All protein structures were generated in PDBQT formats and unnecessary chains removed.

Ligand selection and preparation: All the selected *Bacillus cereus* chemical compounds were quantitatively analyzed by previous literature. (Vijayaram Seerangaraj *et al*., 2017) The 2D structure of the selected compounds was downloaded from PubChem. https://pubchem.ncbi.nlm.nih.gov/.

ADMET and molecular docking analysis

An attempt to predict pharmacokinetic properties such as absorption, distribution, metabolism, excretion and toxicity (ADMET) of the compounds, predicted by BOILED EGG model was used to predict whether the molecules could be absorbed by the oral bioavailability. Drug absorption depends on various factors such as human intestinal absorption (HIA), solubility, Blood brain barrier (BBB), skin permeability, carcinogenicity oral toxicity and substrate or inhibitor of P-glycoprotein. (Daina, A., & Zoete, V. 2016). Pharmacokinetics properties of ADMET were analysed by SwissADME (http://www.swissadme.ch/) and the toxicity by AdmidSAR v.0.2 (http://lmmd.ecust.edu.cn/admetsar2). The performance of molecular docking was evaluated by using (Auto dock Vina 4.0) (Manivel *et al.*, 2016) with pre-identified compounds for *B. cereus* and bacterial proteins (Vijayaram Seerangaraj *et al.*, 2017; Manikandaprabhu *et al.*, 2015). The macromolecule protein grid box of each protein was prepared on the basis of grid maps with different energies. The default algorithm program in Auto dock Vina 4.0 was used to find out the best-docked conformations between the prepared compounds and targeted receptors. After completion of docking, the binding affinities were calculated. For visualization of the structures of docked complexes, BIOVIA Discovery Studio Visualizer 2021 Client was employed to analyse both the docked poses and the 2D structure of amino acids that interacted with their respective proteins.

RESULTS AND DISCUSSION

Millions of people worldwide lose their lives each year due to microbial infections. Presently, the greatest obstacle to public health is the increasing resistance development of pathogens to antibiotic medications.

Antibacterial activity

The primary objective of this study was to discover substances that have antibacterial properties against harmful bacteria. These substances are derived from marine bacteria found in the sediment soil of the ocean. They have the potential to produce secondary metabolites in their natural environment. The raw extract obtained from *Bacillus cereus* exhibited antibacterial activity against various pathogenic bacteria, including *E. coli, salmonella typhi, Enterobacter cloacae*, and *Enterococcus faecalis* strains. The antibacterial activity was revealed that the effect was noted at concentration depended. such as 1 mL sample containing 10 mg crude extract which was dissolved by using dimethyl sulfoxide (DMSO) solution. The *B. cereus* crude extract strongly inhibited pathogenic resistance of 16 mm diameter in *Escherichia coli* and 14 mm diameter in *Salmonella typhi.* The moderate activity was recorded in 13 mm diameter in *Enterobacter cloacae and 12 mm diameter in Enterococcus faecalis*. Respectively compared to streptomycin antibiotic disc. *Salmonella typhi* did not showed resistance of streptomycin. (Table.1 & Fig.1 and 2). Notably, it showed maximum resistance with a diameter of 16mm against *E. coli* when compared to streptomycin antibiotic discs. This difference in antibacterial effectiveness can be attributed to other strains described by strain (S. N. Kumar, *et al*., 2014) which mentioned that cell-free culture filtrate from *Bacillus cereus* associated with an entomopathogenic nematode displayed strong antimicrobial activity according to strain (Farr, *et al*., 2014). Furthermore, *B. cereus* and *B. pumilis* were identified as having potent antibacterial properties. It has been reported that *B. cereus* is capable of producing enterotoxins along with compounds that possess both anti-fungal and antibacterial effects according to sources like source respectively (Nishanth, *et al*., 2022; Sharma, *et al*., 2012).

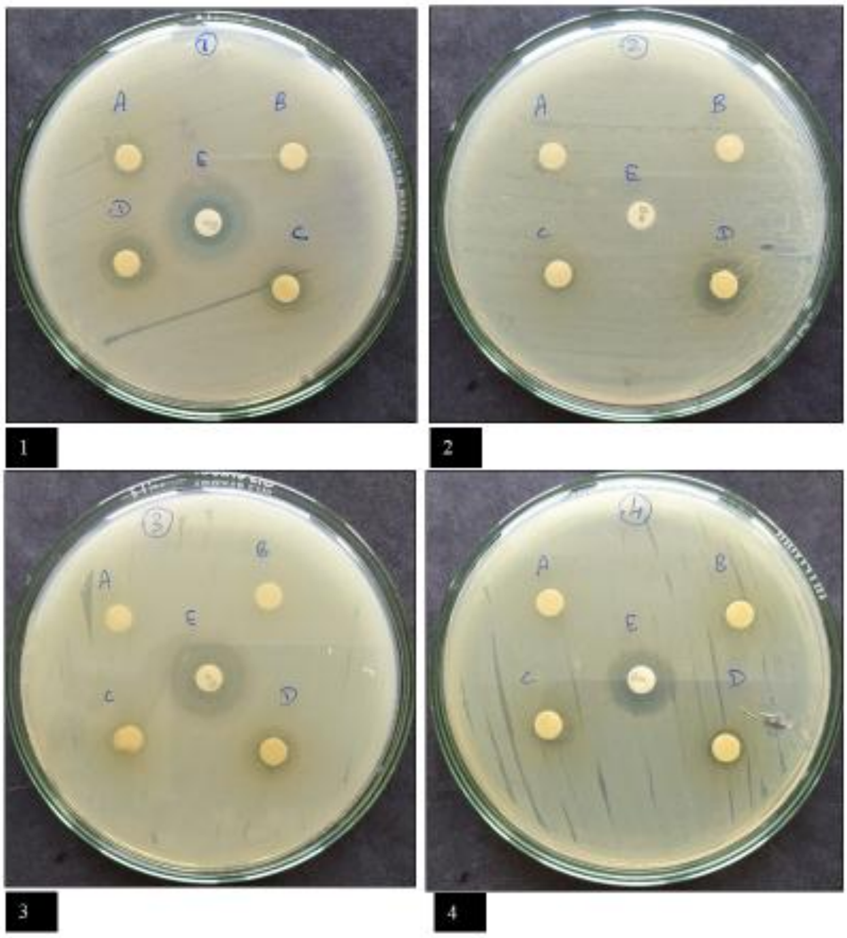

Fig. 1: Antibacterial activities of *B. cereus* crude extract 10 mg / ml concentration (A. 25 µL, B. 50 µL, C. 75 µL, D. 100 µL and *E. streptomycin* disk), 1. *Escherichia coli, 2. Salmonella typhi, 3. Enterobacter cloacae and 4. Enterococcus faecalis.*

Table 1: Antibacterial activity of *B. cereus* crude extract (10 mg / ml concentration) against various pathogenic bacteria and standard streptomycin.

S. No.	**Bacterial strains**	**Streptomycin (control)**	***B. cereus* crude extract (10 mg mL^{-1} concentration)**			
		10mcg	**25 µL**	**50 µL**	**75 µL**	**100 µL**
		Zone of inhibition in millimetre (mm)				
1.	*Escherichia coli*	18.6 ± 0.5	9.2 ± 0.2	11.2 ± 0.2	13.3 ± 0.2	16.2 ± 0.2
2.	*Salmonella typhi*	0	0	7.2 ± 0.2	10.2 ± 0.2	14.2 ± 0.2
3.	*Enterobacter cloacae*	16.6 ± 0.5	0	7.2 ± 0.2	10.2 ± 0.2	13.2 ± 0.2
4.	*Enterococcus faecalis*	14.3 ± 0.5	0	8.2 ± 0.2	10.1 ± 0.1	12.3 ± 0.2

Note. Mean of triplicate value [mean and SD. (n = 3)] 0 indicates no zone of inhibition.

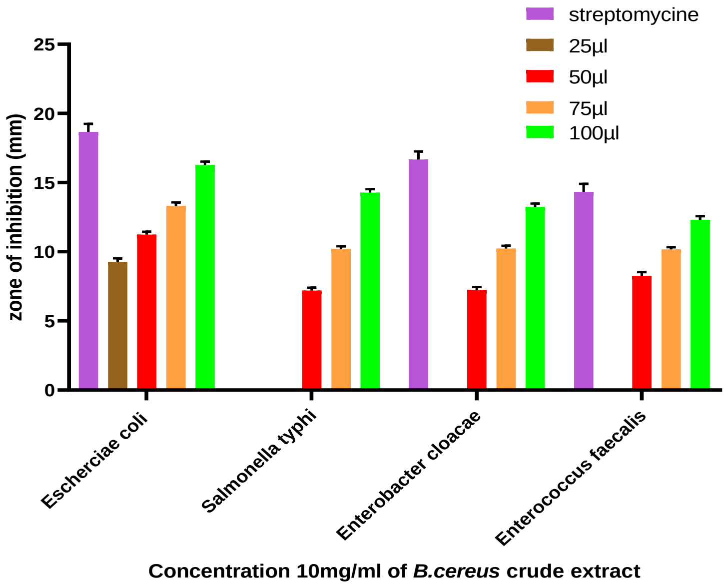

Fig. 2: Antibacterial activity of *B. cereus* crude extract against various pathogenic bacteria.

Minimal inhibitory concentration

MIC of *B. cereus* crude extract 10 mg mL^{-1} used against four pathogenic bacteria such as *Escherichia coli, Salmonella typhi, Enterobacter cloacae* and *Enterococcus faecalis.* In different concentration like (10, 25, 50, 75, 100 and 125 µL) revealed that in *E. coli* it minimum of 10µl of extract containing 49% inhibition effect and 100 µl of crude extract containing 91% of inhibition effect. Results are showed in (Table 2).

Table 2: MIC value of *B. Cereus* crude extract with various pathogenic bacteria.

S. No.	**Bacterial pathogen**	**Bacterial growth inhibition (%)**					
		Control	**10 µL**	**25 µL**	**50 µL**	**75 µL**	**100 µL**
1.	*E. coli*	1.1 ± 0	49 ± 0.5	85 ± 0.2	88 ± 0.2	89 ± 0.2	91 ± 0.5
2.	*Salmonella typhi*	0.8 ± 0	31 ± 0.6	76 ± 0.2	85 ± 0.5	87 ± 0.5	90 ± 0.4
3.	*Enterobacter cloacae*	0.9 ± 0	35 ± 0.5	74 ± 0.6	87 ± 0.7	88 ± 0.4	90 ± 0.5
4.	*Enterococcus faecalis*	1.1 0	55 ± 0.5	81 ± 0.5	88 ± 0.3	88 ± 0.5	90 ± 0.4

Note: Mean of triplicate value [mean and SD. (n = 3)] Control - Without crude extract.

ADMET pharmacokinetic analysis

A potential drug applicant, it must have some properties that are common for almost all of the approved drugs. Therefore, the drug-like properties are determined based on the ADMET parameters. It's a main checkpoint by the drug's outing from its point of entry to the site of action. Most importantly the compound should also have the capability of showing higher biological activity and lower toxicity too. So, it is important to predict the drug-like quality of compounds along to recognize its potentiality as a drug applicant. The marine bacteria *Bacillus cereus* identified compounds were selected based on totally 11 compounds were (Table 3) those compound structures are retrieved to PubChem. Then the selected compounds are physicochemical properties showed the better results as compared to the marketed drugs. The physicochemical and ADMET parameters of all the selected molecules (Figure 3 & 4) are depict in (Table 4). The drug molecules involve ensuring that they effectively bind to target receptors. However, it is important to note that oral administration does not guarantee immediate activity. Drug molecules must undergo various pharmacokinetic processes in order to reach their intended targets. These processes include absorption, distribution, metabolism, and excretion. It is worth considering that drug molecules with a molecular weight exceeding 1000 g/mol tend to have reduced membrane permeability, particularly when designing oral medications. The ADME (absorption, distribution, metabolism, excretion) process along with toxicity evaluation are critical factors in drug discovery and development. (Tibbitts, *et al.*, 2016; Matsson, *et al.*, 2017). They help provide assurance about the effectiveness and safety of evaluated compounds.

In silico molecular docking

Molecular docking plays a crucial role in the initial stages of drug development and formulation by predicting how ligands bind to target proteins. (Sureshkumar, *et al*., 2012) this enables researchers to determine the affinity of ligands for receptor complexes. The targeted pathogenic bacterial proteins 3D structures of are *Escherichia coli* protein ESPF (PDB ID: 2YKT), *Salmonella typhi* protein SopA (PDB ID: 5JW7), *Enterobacter cloacae* (PDB ID: 4HFK) and *Enterococcus faecalis* protein ESP (PDB ID: 6ORI) docking performance was done (Figure 5 to 10) (Table 5). The analysis of the docking results revealed that the ligand had a low binding free energy (BFE) value, indicating its ability to compete for binding with the target receptor and form a stable complex (Sahu, *et al.*, 2012). The affinity between a ligand and its target protein determines its effectiveness at the cellular level. Based on this study, Methoxy acetic acid, 3-tetradecyl ester demonstrated promising potential as an antibacterial agent against *E. coli* with a BFE value of -8.6 kcal/mol and interacted molecules are carbon hydrogen interaction of GLU189 (3.60A°) Alkyl interaction of LYS108 (4.80A°), PHE196 (4.77A°), ARG192 (4.08A°), ARG193 (3.60A°) and Pi-alkyl interaction of CYS195 (4.35A°) However, it is important to note that Methoxy acetic acid, 3-tetradecyl ester has not yet been explored for therapeutic applications in *B. cereus* until now.

This analysis aimed at determining whether *Bacillus cereus* are capable of producing secondary metabolites that possess significant antibacterial effects.

Table 3: GC-MS based identified compounds analysis.

S. No	Compound Name	PubChem ID	Chemical formula	Molecular weight	H. Donor	H. acceptor	XLog P value
1.	2-Butanone, 4-hydroxy-3-methyl-	18829	$C_5H_{10}O_2$	102.13	1	2	-0.2
2.	Isobutyl Acetate	8038	$C_6H_{12}O_2$	116.16	0	2	1.8
3.	Methoxy acetic acid, 3-tetradecyl ester	545726	$C_{17}H_{34}O_3$	286.4	0	3	6.5
4.	Phenol, 2,4-Bis(1,1-Dimethylethyl)	93344	$C_{22}H_{30}O$	310.5	1	1	7.2
5.	Hentriacontane	12410	$C_{31}H_{64}$	436.8	0	0	16.4
6.	Nonadecane, 9-Methyl	86566	$C_{20}H_{42}$	282.5	0	0	10.8
7.	Nonacosane	12409	$C_{29}H_{60}$	408.8	0	0	15.3
8.	Sulfurous Acid,Butyl Dodecyl Ester	6420769	$C_{16}H_{34}O_3S$	306.5	0	4	7
9.	Fumaric Acid, 3-Hexyl Tridecyl	444972	$C_4H_4O_4$	116.07	2	4	-0.3
10.	Phthalic Acid, Isobutyl Octadecyl	6423451	$C_{30}H_{50}O_4$	474.7	0	4	11.6
11.	Silicic acid diethyl bis(trimethylsilyl)ester	77092	$C_{10}H_{28}O_4Si_3$	296.58	0	4	0

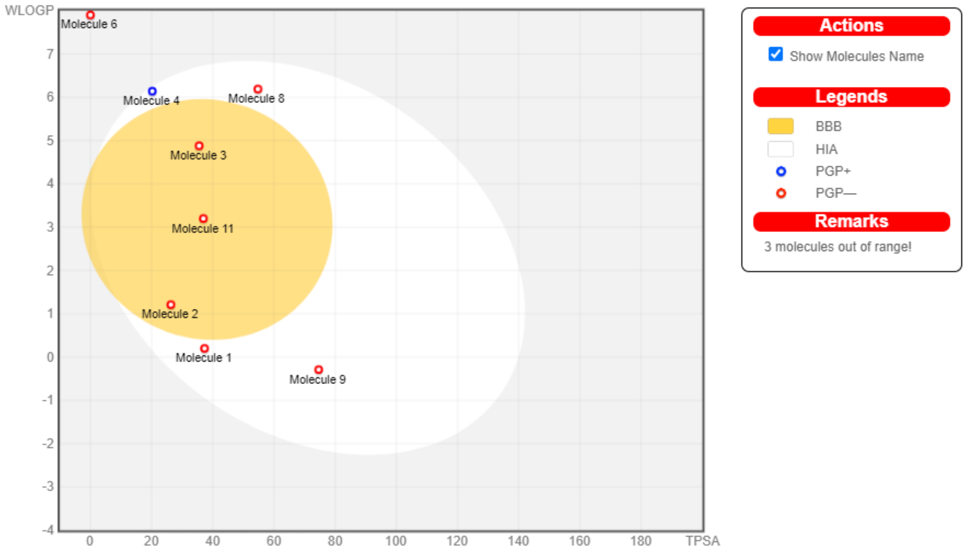

Fig. 3: The boiled egg plot of the selected target molecules.

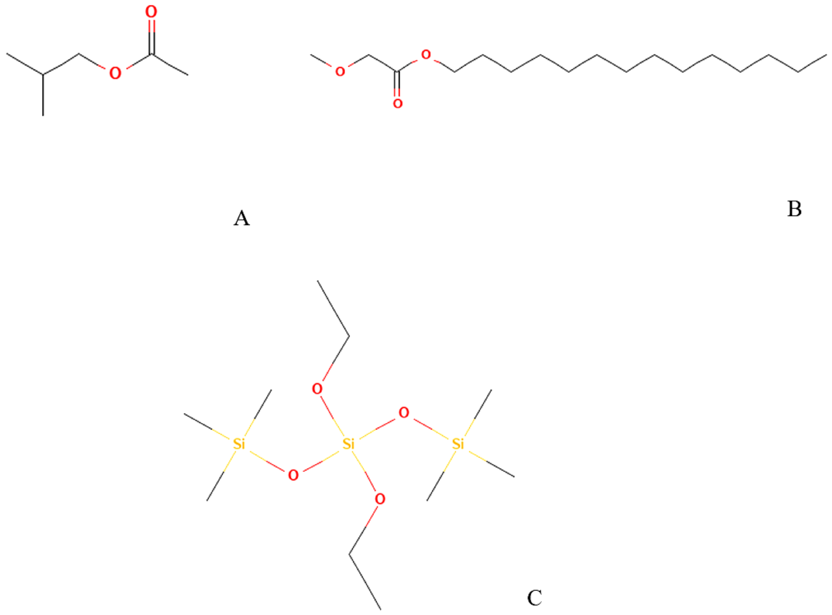

Fig. 4: The 2D structures of Egg blot based selected target *B.Cereus* compounds. A. Isobutyl Acetate, B. Methoxy acetic acid, 3-tetradecyl ester and C. Silicic acid diethyl bis(trimethylsilyl) ester.

Table 4: The selected three compound pharmacokinetics properties.

S. No.	**Classifications**	**Probability**		
		Isobutyl Acetate	**Methoxy acetic acid, 3-tetradecyl ester**	**Silicic acid diethyl bis (trimethylsilyl) ester**
1.	Human Intestinal Absorption	0.9962	0.9852	0.7873
2.	Caco-2	0.5708	0.8361	0.7201
3.	Blood Brain Barrier	0.9750	0.8250	0.7500
4.	Human oral bioavailability	0.5143	0.6857	0.8714
5.	Subcellular localization	0.7279	0.6354	0.6300
6.	OATP2B1 inhibitors	0.8502	0.8494	0.8552
7.	OATP1B1 inhibitors	0.9608	0.9348	0.9692
8.	OATP1B3 inhibitors	0.9362	0.9347	0.9475
9.	MATE1 inhibitors	0.9400	0.9400	0.9800
10.	OCT2 inhibitors	0.9000	0.8500	0.8750
11.	BSEP inhibitors	0.8963	0.6103	0.9450
12.	P-glycoprotein inhibitors	0.9689	0.8028	0.9715
13.	P-glycoprotein substrate	0.9792	0.9218	0.9840
14.	CYP3A4 substrate	0.6995	0.5700	0.6825
15.	CYP2C9 substrate	0.6168	0.5862	0.8000
16.	CYP2D6 substrate	0.8859	0.8667	0.7844
17.	CYP3A4 inhibition	0.9857	0.9719	0.9856
18.	CYP2C9 inhibition	0.9431	0.9428	0.8576
19.	CYP2C19 inhibition	0.9508	0.9365	0.8261

20.	CYP2D6 inhibition	0.9547	0.9367	0.9341
21.	CYP1A2 inhibition	0.8137	0.8247	0.8922
22.	CYP inhibitory promiscuity	0.9360	0.9473	0.9311
23.	UGT catalysed	0.0000	0.0000	0.0000
24.	Carcinogenicity (binary)	0.5557	0.6823	0.5600
25.	Carcinogenicity (trinary)	0.5872	0.6496	0.5821
26.	Eye corrosion	0.9913	0.8775	0.8150
27.	Eye irritation	0.9901	0.9818	1.0000
28.	Ames mutagenesis	0.8300	1.0000	0.8300
29.	Human Gene inhibition	0.7815	0.4795	0.7559
30.	Micronuclear	1.0000	0.9800	0.6300
31.	Hepatotoxicity	0.5401	0.5250	0.5862
32.	skin sensitisation	0.6988	0.8181	0.8997
33.	Respiratory toxicity	0.9667	0.9889	0.7778
34.	Reproductive toxicity	0.8667	0.6111	0.7444
35.	Mitochondrial toxicity	0.9750	1.0000	0.9625
36.	Nephrotoxicity	0.6487	0.7918	0.7614
37.	Acute Oral Toxicity (c)	0.6227	0.5543	0.6474
38.	Estrogen receptor binding	0.9387	0.9356	0.8208
39.	Androgen receptor binding	0.8743	0.7724	0.8538
40.	Thyroid receptor binding	0.9393	0.6311	0.7443
41.	Glucocorticoid receptor binding	0.9633	0.8953	0.7461
42.	Aromatase binding	0.8575	0.8531	0.7761
43.	PPAR gamma	0.9475	0.7111	0.7631
44.	Water solubility (logS)	-1.272	-2.285	-1.077
45.	Plasma protein binding	0.537	0.607	0.319
46.	Acute Oral Toxicity log(1/(mol/kg))	0.923	1.532	2.459

Table 5: Molecular docking of selected compounds.

S. No	**Compound Name**	**(Kcal/mol)**			
		E. coli **(2YKT)**	***S. typhi*** **(5JW7)**	***E. cloacae*** **(4HFK)**	***E. faecalis*** **(6ORI)**
1.	Isobutyl Acetate	-8.2	-7.1	-4.1	-4.3
2.	Methoxy acetic acid, 3-tetradecyl ester	-8.6	-7.5	-3.8	-3.4
3.	Silicic acid diethyl bis(trimethylsilyl) ester	-6.2	-6.4	-6.3	-3.1

Note. *E. coli* protein 2YKT shown best binding value (-8.6 kcal/mol) of Methoxy acetic acid, 3-tetradecyl ester.

The *Escherichia coli* ESPF protein crystal structure of (PDB ID: 2YKT) with Isobutyl Acetate the binding affinity was -8.2 kcal/mol and interacted molecules are alkyl interaction of TYR15 (5.13A°), LYS108 (4.07A°) and pi-alky interactions of Pi-Alkyl, LEU111 (4.99A°). The 3D and 2D intractions are shown Fig. 5.

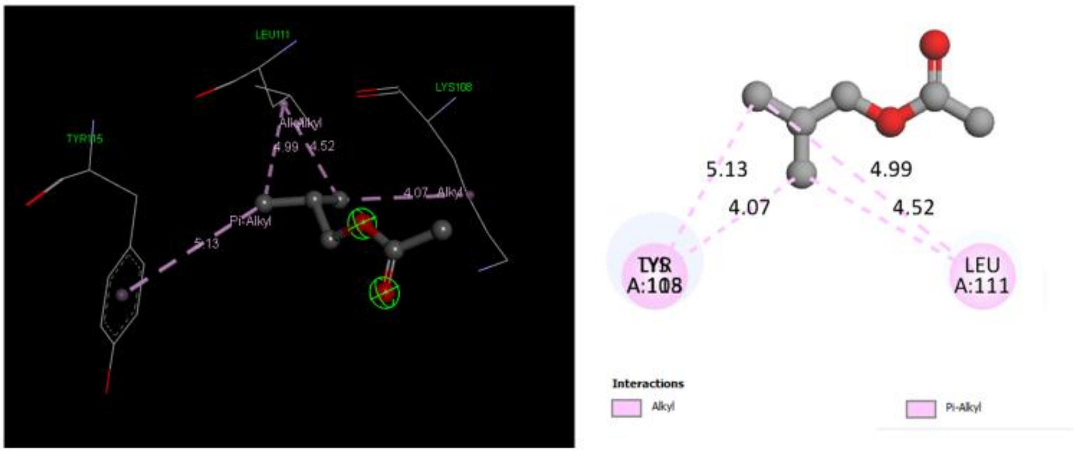

Fig. 5: The 3D & 2D intraction between the 2YKT amino acid with Isobutyl Acetate.

The *Salmonella typhi* protein SopA (PDB ID: 5JW7) interaction between Isobutyl Acetate the binding affinity was -7.1 kcal/mol and interacted molecules are Alkyl interaction of ALA186 (4.18A°), conventional interaction of LEU190 (2.30A°) and ARG194 (2.94A°). The 3D and 2D intractions are shown Fig. 6.

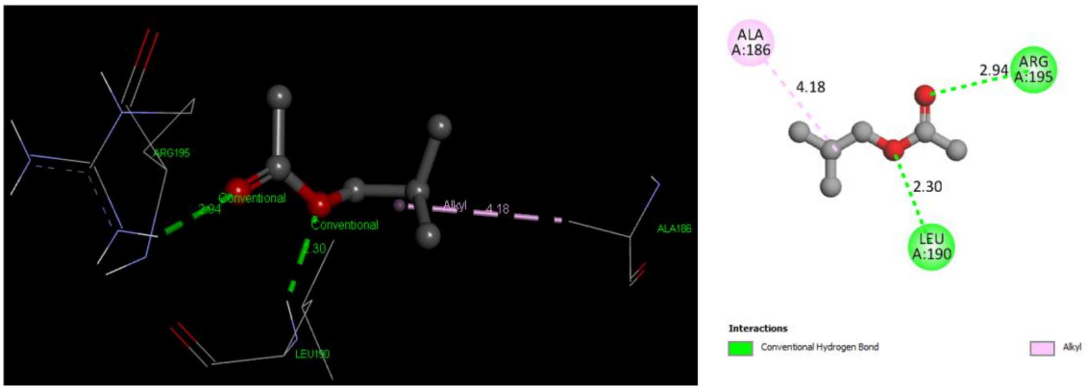

Fig. 6: Structures of 3D & 2D intraction between 5JW7 amino acid with Isobutyl Acetate.

The *Escherichia coli* protein ESPF crystal structure (PDB ID: 2YKT) interaction with Methoxy acetic acid, 3-tetradecyl ester the binding affinity was -8.6 kcal/mol and interacted molecules are carbon hydrogen interaction of GLU189(3.60A°) Alkyl interaction of LYS108(4.80A°), PHE196(4.77A°), ARG192(4.08A°), ARG193(3.60A°) and Pi-alkyl interaction of CYS195(4.35A°). The 3D and 2D interactions are shown Fig. 7.

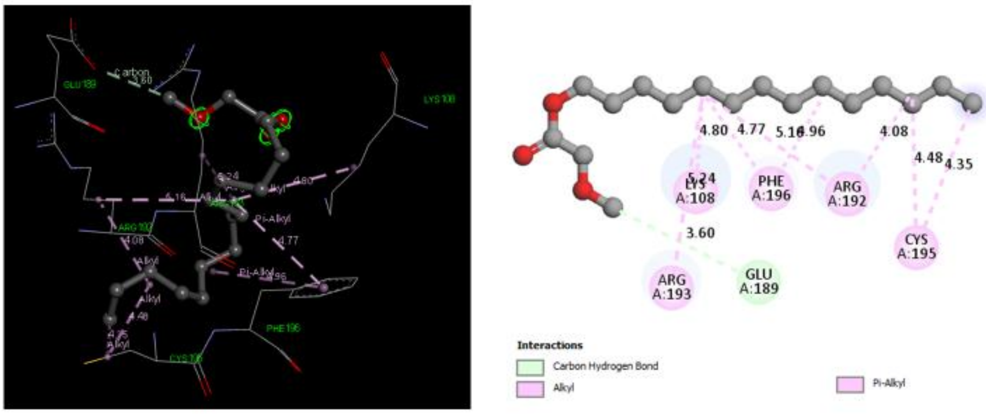

Fig. 7: The structure of 3D and 2D intraction between the 2YKT amino acid with Methoxy acetic acid, 3-tetradecyl ester.

The *Salmonella typhi* protein SopA (PDB ID: 5JW7) interaction with Methoxy acetic acid, 3-tetradecyl ester the binding affinity was -7.5 kcal/mol and interacted molecules are conventional hydrogen bond of SER198(2.75A°), ASP196(3.25A°) alkyl interaction of PRO167(4.60A°), TRP173(5.30A°) Pi-alkyl interaction of LYS176(5.26A°), PHE197 (5.01A°) and carbon hydrogen interaction of ALA193(3.48A °). The 3D and 2D interaction are shown Fig. 8.

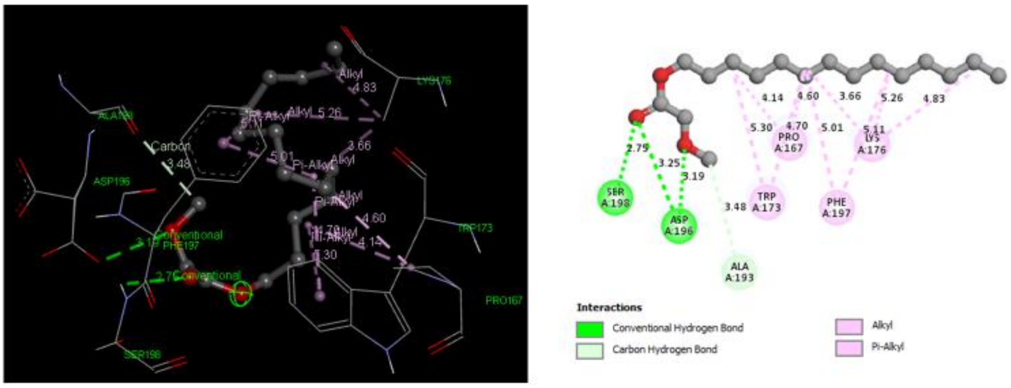

Fig. 8: The structure of 3D and 2D intraction between the 5JW7 amino acid with Methoxy acetic acid, 3-tetradecyl ester.

The *Escherichia coli* protein ESPF (PDB ID: 2YKT) interaction between Silicic acid diethyl bis(trimethylsilyl) ester the binding affinity was -6.2 kcal/mol and interacted molecules are pi-pi interactions of TYR115(4.30A°) alkyl interaction of ARG114(4.17A°), ARG192(4.40A°) and pi-alkyl interactions of LYS108(3.60A°), ARG193(4.29A°) and PHE196(4.79A°). The 3D and 2D interactions are shown Fig. 9.

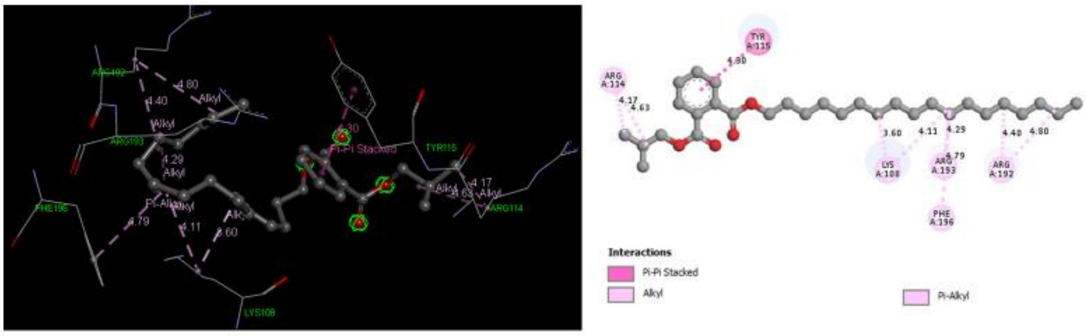

Fig. 9: The structure of 3D and 2D intraction between 2YKT amino acid with Silicic acid diethyl bis(trimethylsilyl) ester.

The *Salmonella typhi* protein SopA (PDB ID: 5JW7) interaction between Silicic acid diethyl bis(trimethylsilyl) ester the binding affinity was -6.4 kcal/mol and interacted molecules are Alkyl interactions of PRO167(5.05Aº), LYS 176(4.32Aº) and pi-alkyl interactions of PHE197(3.68Aº) and ALA180(4.46Aº). The 3D and 2D interactions are shown Fig. 10

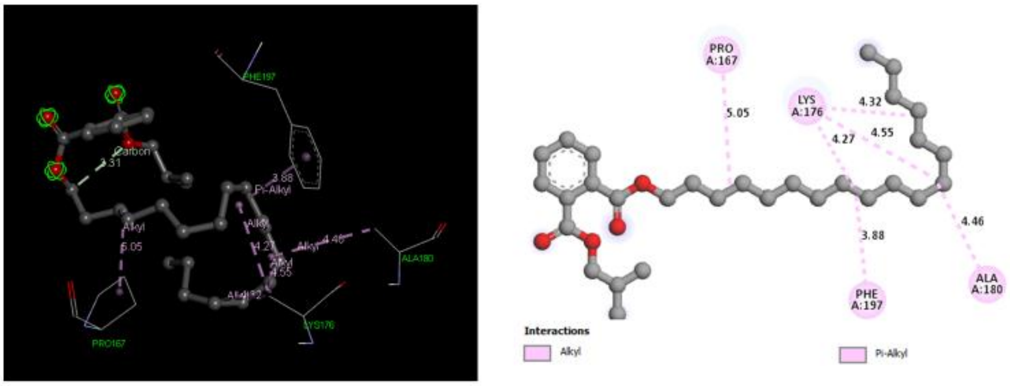

Fig. 10: The structure of 3D and 2D intraction between 5JW7 amino acid with Silicic acid diethyl bis(trimethylsilyl) ester.

CONCLUSION

The results of this study reveal that Marine bacterium *Bacillus cereus* has the ability to produce a bioactive compound known as Methoxy acetic acid 3-tetradecyl ester. This compound has shown promising potential as an antibacterial agent against *E. coli*, which is responsible for various extraintestinal illnesses such as urinary tract infections, traveller's diarrhoea, and abdominal infections. The efficacy of Methoxy acetic acid 3-tetradecyl ester was confirmed through molecular docking analysis, which demonstrated a strong binding affinity (-8.6 kcal/mol) with the *E. coli* protein (PDB ID: 2YKT). These findings suggest that further in vivo studies should be conducted to explore the potential benefits of this novel lead compound in treating bacterial infections caused by *E. coli.*

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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