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ANTIMICROBIAL PROPERTY AND INSILICO MOLECULAR DOCKING STUDIES OF BIOACTIVE COMPOUNDS FROM *ALCANIVORAX XENOMUTANT* MN103744

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Abstract :The isolation, molecular identification and antimicrobial potential of marine bacteria *Alcanivorax xenomutant* was performed. The 16SrDNA sequences were deposited in GenBank with accession number MN103744. The bioactive profiling of the strain was done for the first time. The extraction of bioactive compounds was performed using ethyl acetate. The antimicrobial properties were investigated against *Klebsiella pneumonia* (MTCC 432), *Salmonella typhi* (MTCC 733), *Bacillus subtilis* (MTCC 441), *E.coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 1430). The results ***showed*** that the ethyl acetate extract showed higher and broad spectrum efficacy against all the tested organisms in comparison with the erythromycin standard. The purification of the bioactive compounds was done by thin layer chromatography using Chloroform:Methanol as a solvent system. The characterisation of compounds was done by GC- MS analysis and the identified compounds are 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl, 2,4-Di-tert-butylphenol, Ethyl homovanillate and Quinoline, 1,2-dihydro-2,2,4-trimethyl. These bioactive compounds were docked against the enzymes such as tyrosyl-tRNA syntehtase and DNA gyrase with the help of Autodock 4. Docking studies were carried out for all the ligands; the compounds binding to the active site of the enzymes and the binding energy was compared with that of the Erythromycin standard. The molecular docking study confirms the antimicrobial activity of ethyl acetate extract of marine bacteria *Alcanivorax xenomutant*.

Index Terms : Marine bacteria; Bioactive compound; Antimicrobial activity; Antioxidant activity; TLC; GCMS; Molecular docking.

I. INTRODUCTION

The emergence of drug resistance bacteria alone wiped off about 25% of the world population and it has become a serious threat to the biomedical field [1]. One of the main approaches of modern medicine to combat infections is by antibiotic treatment. The advent of multi-drug resistance among pathogenic bacteria has put at risk the worth of antibiotics, which have formerly transformed medical sciences [2]. Marine microorganisms are increasingly becoming an important source for the production of therapeutically important compounds. So many unique and biologically active metabolites have been reported from marine microorganisms [3]. They are considered as highly valuable, because they produce various antibiotics and therapeutic compounds with diverse biological activities [4]. The marine environment contains a wide range of distinct microorganisms which are not present in the terrestrial environment [5].

The world ocean is the interconnected system of earth's oceanic waters, and the bulk of the hydrosphere, which is about 71 % of earth's surface, is covered by the global ocean. From the beginning of science, it has been contributed novel compounds which are used widely for the curing of diseases [6]. The marine realm comprises various life forms including bacteria, fungi, actinomycetes, algae, sponges, and many aquatic flora and fauna [7].

As a result of the adaptation to extreme conditions and to prevent competition among themselves, marine organisms have to produce compounds which are proved to be bioactive. The unique biological properties of marine microorganisms made them to become a prominent target for the isolation and extraction of novel bioactive compounds [8].

For this study, marine sediment samples were collected from coastal areas of Mararikkulam and the strain *Alcanivorax xenomutants* was isolated. It was from the family Alcanivoracaceae, with order oceanospirillales, class Gammaproteobacteria, and phylum proteobacteria [9]. *Alcanivorax* are Gram negative, aerobic, motile rods that can tolerate high salt concentrations and many of the *Alcanivorax* species are shown to have bioremediation properties such as oil degradation [10].

In this study, the novel marine bacteria identified by molecular level as *Alcanivorax xenomutant* (MN103744) was selected for the determination of antibacterial activities. For the first time, the present study describes the production, purification, molecular characterization, antimicrobial potential and molecular docking of the bioactive compounds. The metabolites were identified and structural characterization was also done.

II. RESEARCH METHODOLOGY

2.1 Sample source and collection

Marine sediment samples were collected from the coastal areas of Mararikkulam (9° 36'0"N 76° 18'0"E) in Kerala. Collections were made from 0.4 km from coastal area and at 4 metre depth. The surface layers of sediments were removed and central portions of sediments were aseptically transferred into sterilized bottles.

2.2 Isolation of marine bacteria

0.1g of sediment was weighed and transferred into test tubes containing 10 ml of distilled water and it was then serially diluted to 10^{-8}. Then the samples were spread plated on Zobell's marine agar plates and incubated for 7 days at 37°C. The isolated colonies were purified by the streak plate method and control plates were kept together for overnight incubation at 37°C. The isolated colonies were sub-cultured and kept as stock culture at 4°C and subsequent sub culture was performed every two weeks [11]. Based on the antibacterial activity by cross streak method, the isolate MBTU_MBS2 was found to be more potent against human pathogens and selected for further studies.

2.3 Molecular characterization

The isolate was inoculated in zobell's marine broth and incubated overnight at 37°C in a shaking incubator at 120 rpm. The cell pellet was separated from the culture broth by centrifuged at 8000 rpm for 10 minutes. The genomic DNA of the isolate was extracted by the gram negative DNA isolation method [12]. The isolated DNA was dissolved in a 50µl TE buffer. Then it was loaded in respective wells and the agarose gel electrophoresis was carried out at 80V to visualize the isolated DNA. The amplification of 16s r DNA was carried out using primers, 27F (5'AGAGTTTGATCMTGGCTCAG3') and 1492R (5' AAGGAGGTGWTCCARCC 3'). A 50 µl reaction was carried out and the reaction mixture consists of 25 µl master mix (10 x assay buffer, dNTP's, Taq polymerase and $MgCl_2$) ,2µl forward primer, 2 µl reverse primer , 4µl of template DNA and 17 µl of milli-Q water. The reaction mixture was placed in a thermal cycler under the following conditions: an initial denaturation at 95°C for 4 minutes followed by 30 cycles of 95°C for 1 minute , 55°C for 1 minute, 72°C for 2 minutes followed by final extension at 72°C for 1minute and holding temperature at 4°C.

2.4 Sequencing and phylogenetic analysis

The sequence of the sample was determined using a DNA sequencing service provided at Kerala Agricultural University, Mannuthi. The obtained sequences were analysed using BLAST tool (www.nbi.nlm.nih.gov/blast) to get the relative identification of each bacterial species [13]. The pair wise evolutionary distances were computed with the help of the Kimura 2 parameter model as developed by [14]. To obtain the confidence values, the original data set was resampled 1000 times using the bootstrap analysis method. The bootstrapped data set was used directly for constructing the phylogenetic tree using the MEGA program The multiple distance matrix obtained was then used to construct phylogenetic trees using the neighbour joining (NJ) method of [15]. The 16s r DNA sequences of the selected isolates were deposited in Genbank (NCBI) to get the accession number.

2.5 Extraction of bioactive compounds

A single colony of the isolates growing on Zobell's Marine Agar plate were inoculated into 5 ml of Zobell's marine broth 2216 (HiMedia) and kept in on a rotary shaker at 120 rpm for overnight incubation at 37°C. After incubation it was then inoculated in to 95 ml Zobell's marine broth. After 48hr incubation, the culture was centrifuged at 8000 rpm for 15 minutes at 4°C, and the pellet was collected, washed and resuspended in phosphate buffer saline (PBS pH7). 1 % of one OD cells were transferred into 1000 ml of the same broth and the culture was kept in rotary shaker at 120rpm for 5 days at 37°C for the production of secondary metabolites. After fermentation, the culture broth was centrifuged at 10000 rpm for 10 minutes at 4°C. The extracellular compounds from the culture supernatant were extracted by adding equal amounts of ethyl acetate by a liquid-liquid extraction method. Both filtrate and organic solvent were mixed thoroughly by shaking in a separating funnel and allowed to stand for 30 minutes. The aqueous layer and the organic layer were separated, which contained the solvent and bioactive compounds. The process of extraction was repeated three times and the ethyl acetate extract was subjected to rotary evaporation (Cyberscan) [16].

2.6 Antibacterial activity by Disc diffusion method

Antibacterial activity was determined against *Klebsiella pneumonia* (MTCC 432), *Salmonella typhi* (MTCC 733), *Bacillus subtilis* (MTCC 441), *E.coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 1430) using disc diffusion method. Petri plates were surface inoculated with the pathogens. The sterile disc (6 mm) were impregnated with 20µl (1mg/ml) of ethyl acetate extract. Erythromycin was used as the positive control and 0.1% DMSO was used as the negative control. The discs were placed on the plates with proper spacing and incubated at 37°C for 24 hours. The diameter of the zone of inhibition was measured and recorded [17].

2.7 Purification of the ethyl acetate extract by thin layer chromatography (TLC)

Thin layer chromatographic technique was used for the purification of ethyl acetate extract. According to the solvent polarity chart, the solvent that gives maximum separation of the bioactive compounds on purification was standardized. The chloroform: methanol v/v (9:1) solvent system was selected which gives better separation of bioactive compounds. Briefly, the chromatographic chamber was filled with chloroform: methanol v/v (9:1) was kept for about 20 minutes to attain a state of equilibrium. On the silica gel plate, the extract was spotted at the bottom using a capillary tube. After air drying, it was then dipped into the chromatographic chamber. After running the solvent, the TLC sheet was carefully removed from the chamber, air dried and viewed under UV illuminator at 254 and 366 nm. The retention factor was calculated based on the formula.

Retention factor= Distance travelled by the compound / Distance travelled by the solvent front

2.8 Structure elucidation by GCMS

The Gas Chromatography/Mass Spectrometry (GC/MS) technique separates chemical mixtures and identifies the components at a molecular level. It is one of the most accurate tools for analyzing biological samples. The analysis was done by Shimadzu GC-MS –QP2020 on variant 1200 single quadrupole at a mass range 10- 800u. The carrier gas was helium and scanned at a resolution unit mass over entire range. WCOT fused silica stationary phase column was used as the database for detection.

2.9 Molecular docking

Molecular docking is a key tool in computer-assisted drug design that can predict the appropriate binding site of target proteins with small ligand molecules. Molecular docking mechanism uses computational techniques that can strongly support and help the design of potent inhibitors by revealing the mechanism of drug receptor interaction. Hence, the study was planned to evaluate the interaction of the selected ligand with a target protein expressed in pathogenic bacteria.

The structure of the antimicrobial compounds obtained after GCMS/MS based identification was downloaded from the chem-spider database. The identified ligands were transformed into pdb format using Open Babel software. The structure of Erythromycin as positive control is also downloaded.

The target enzyme structure required for docking was retrieved from the protein data bank repository (http://www.rcsb.org). The 3D structure coordinates tyrosyl-tRNAsynthetase and DNA Gyrase were obtained with PDB id's 1JIJ and 4CKK respectively. Prior to docking, water and ligand coordinates were deleted. The protein was optimized and its energy minimization was done using a program implemented in Swiss-PDBViewer (Johansson *et al.*, 2012). The energy minimized protein structure was included before docking to accommodate hydrogen atoms which were not included in the crystal structure. (http://swissmodel.expasy.org).

2.9.1 Molecular Docking Simulation

Molecular docking of the target protein and the ligand was performed using AutoDock 4.0 software. The default parameters of the automatic settings were used to carry out the docking simulation. The ligand was manually docked into functional sites respective of the protein individually and the docking energy was monitored to achieve a minimum value. In the protein, polar hydrogen atoms are merged and total Kollman charge is added. The Lamarckian Genetic Algorithm (LGA) was employed for automated docking of proteins with ligands [20]. The torsion bonds and side chains of the ligands were allowed to rotate freely, while the target was kept rigid. Polar hydrogen atoms were added to targets, while Gasteiger charges were computed. LGA was implemented by creating an initial population of 150 individuals, applying random torsion to each of the 150 individuals, and performing a maximum of 500000 energy evaluations on each docking run. Further step is preparing PDBQT format files for both target and compound and creating Grid and Docking Parameter files (a.gpf and a.dpf) using AutoDock 4.2. During docking, a grid of 60x60x60 points in x, y, and z directions was built with a grid spacing of 0.375 Å using the Auto-grid component of the software. At least 10 such runs were performed for all ligands and the best conformation with the lowest docked energy was chosen. The final step is to perform molecular docking using Cygwin(http://www.cygwin.com/) and finally, the results are analyzed. The visualization of the best binding modes of the docked structure was performed using UCSF Chimera software.

2.9.2 Docking evaluation

The bioactive compounds showed the significant binding interaction with lowest binding energy against protein targets that were visualized by PyMOL software.

2.9.3 ADMET properties

The drug likeness and molecular property prediction of the compounds were analyzed using Molsoft (https://molsoft.com/mprop/).

III. RESULTS AND DISCUSSION

3.1 Source of marine bacteria

Marine sediment samples were collected from the coastal areas of Mararikkulam, Kerala. A total of 8 marine bacteria were isolated from sediments and screened for antimicrobial activity by streak plate method. Among these, the isolate MBTU_MBS2 which showed maximum antibacterial activity was selected for further study.

3.2 Isolation and identification of marine bacteria

The isolate MBTU_MBS2 was selected for molecular identification by 16SrDNA analysis. Phylogenetic analysis based on 16S rDNA gene sequences showed that the strain formed a 100% similarity to *Alcanivorax xenomutant* which was a novel strain isolated for the first time from the coastal area of Mararikkulam, Kerala, and the sequence was deposited in Gen Bank with accession number MN103744. The Phylogenetic tree is shown in fig1.

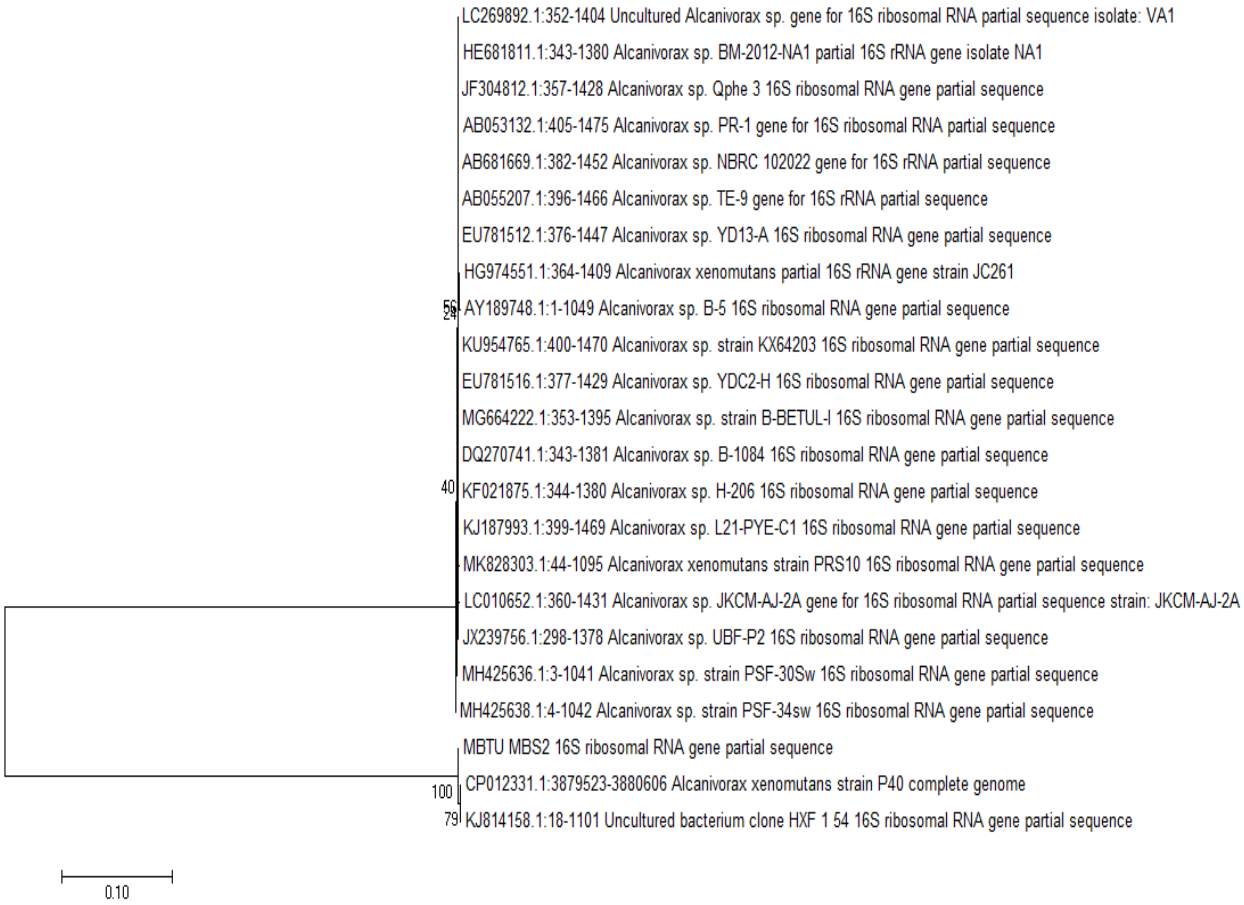


Fig1: Phylogenetic analysis of MBTU_MBS2

3.3 Extraction of bioactive compounds from *Alcanivorax xenomutant*

Large scale cultivation of *Alcanivorax xenomutant* was carried out and after centrifugation, the cell free supernatant was used for the extraction of bioactive compounds using ethyl acetate as the solvent system.

3.4 Antibacterial activities of ethyl acetate extract *Alcanivorax xenomutant*

The antibacterial activity of ethyl acetate extract was determined using the Disc diffusion method. Erythromycin is used as the positive control and 0.1% DMSO as the negative control. The zone of inhibition was shown in fig 2: and table 1.

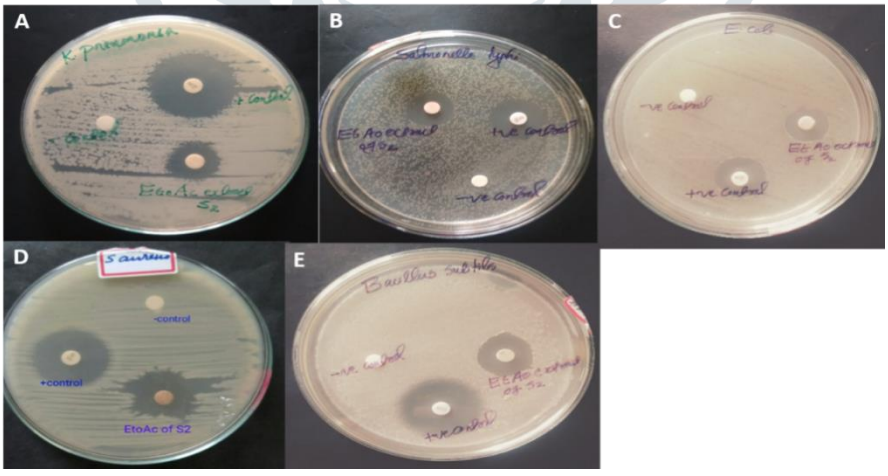


Fig 2: Antibacterial activities of ethyl acetate extract MBTU-MB S2 against pathogens: A- *Klebsiellapneumoniae*, B- *Salmonella typhi*, C- *Eschericia coli*, D- *Staphylococcus aureus*, E- *Bacillus subtilis*

Table 1: The zone of inhibition of ethyl acetate extract and positive control

Pathogens	Zone of inhibition of ethyl acetate extract 20µl (1mg/ml) in mm	Zone of inhibition of positive control (erythromycin 15mcg) in mm
Bacillus subtilis	20mm	30mm
Escherichia coli	16mm	18mm
Salmonella typhi	20mm	24mm
Staphylococcus aureus	22mm	26mm
Kebsiellapneumoniae	20mm	31mm

3.5 Thin layer chromatography

Bioactive compounds are separated by thin layer chromatography. According to the solvent separation system, maximum separation was observed in chloroform and methanol. Different ratios of chloroform and methanol were taken and chloroform: methanol (9:1) showed maximum separation. The TLC chromatogram was shown in fig: 7.

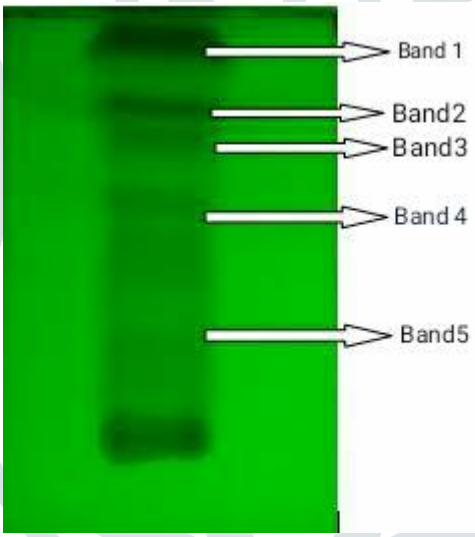


Fig 7: TLC chromatogram of marine bacteria MBTU_MBS2

3.6 GC-MS analysis

The isolated bands obtained from the TLC plate were scraped and subjected to GCMS/MS analysis. In this study, different compounds were identified from the TLC plate. They were Ethyl homovanillate ($C_{11}H_{14}O_4$), 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl ($C_{14}H_{18}N_2O_3$), 2,4-Di-tert-butylphenol ($C_{14}H_{22}O$) and Quinoline, 1,2-dihydro-2,2,4-trimethyl (C_9H_7N). The results are shown in the figures and tables below.

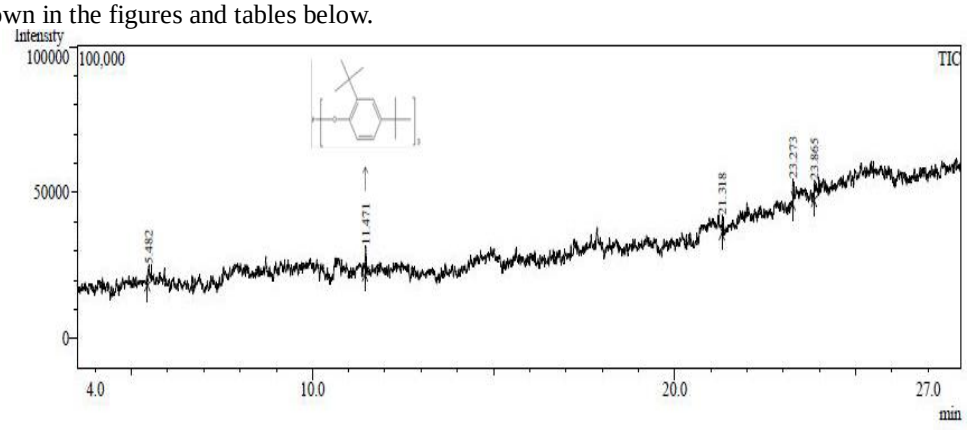
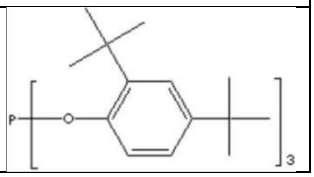


Fig: 8 GCMS chromatogram of band1

Table 4: Bioactive Compounds from band 1

Sl.No.	Compound	Molecular formula	Retention time	Peak area %	Structure
1	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	11.479	4.11	

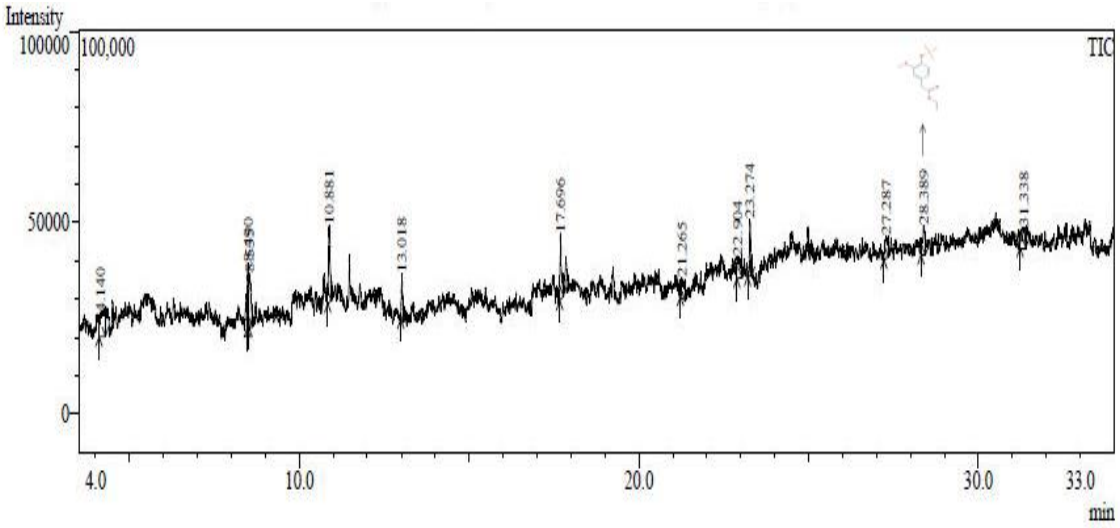
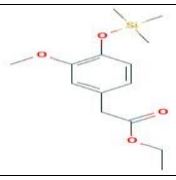


Fig 9: GCMS chromatogram of band 2

Table 5: Bioactive compounds from band 2

Sl.No.	Compound	Molecular formula	Retention time	Peak area %	Structure
1	Ethyl homovanillate	$C_{11}H_{14}O_4$	28.389	6.98	

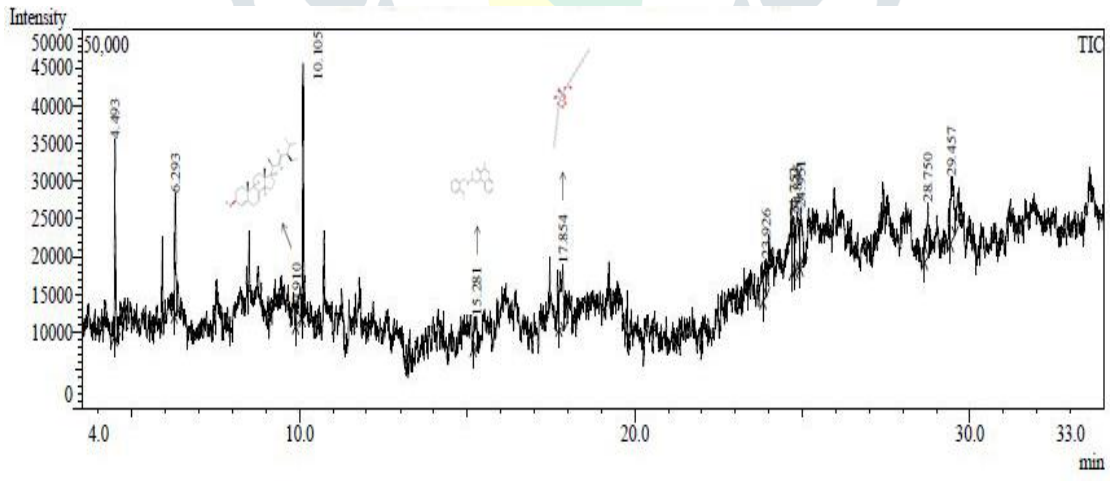
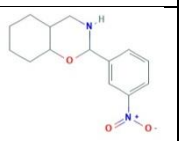


Fig 10: GCMS chromatogram of band 3

Table 6: Bioactive compounds from band 3

Sl. No.	Compound	Molecular formula	Retention time	Peak area %	Structure
1	2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl	$C_{14}H_{18}N_2O_3$	15.281	5.38	

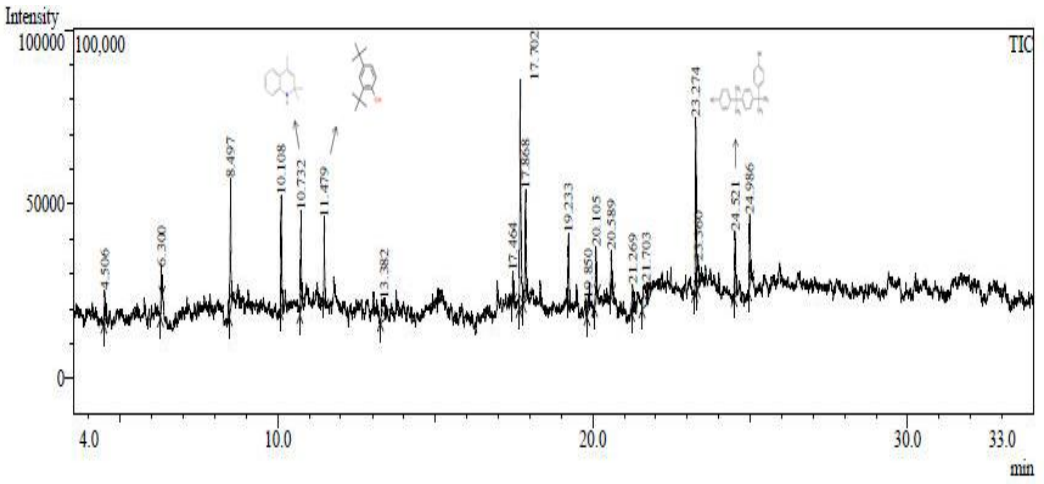
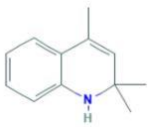


Fig 11: GCMS Chromatogram of band 4

Table 7: Bioactive compounds from band 4

Sl. No.	Compound	Molecular formula	Retention time	Peak area %	Structure
1	Quinoline, 1,2-dihydro-2,2,4-trimethyl	C_9H_7N	10.732	5.15	

3.7 Molecular docking

The identified bioactive compounds such as 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl, 2,4-Di-tert-butylphenol, Ethyl homovanillate and Quinoline, 1,2-dihydro-2,2,4-trimethyl were subjected to molecular docking with Tyrosyl-tRNAsynthetase and DNA gyrase respectively. Erythromycin was the positive control of the study.

3.7.1Docking with Tyrosyl -tRNAsynthetase

The compounds showed significant binding with Tyrosyl –tRNAsynthetase. The standard antibiotic Erythromycin is found to be bonded on the crucial binding pocket of amino acids such as His 50, Lys 84, Asp 195, Ser 194, Pro 222 and Phe 232. The identified compounds were also found to be bonded on the same region of the enzyme;the compound, 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl forms hydrogen bonds with Asp 40, Asp 80, Lys 84, Arg 88 and Tyr 170. The compound Ethyl homovanillate binds to the Asn 124 and Tyr 36 of the enzyme. Quinoline, 1,2-dihydro-2,2,4-trimethyl significantly binds to the Tyr 36, Gln 190 and Gly 193 of Tyrosyl –tRNAsynthetase. The results were shown in figures: 12 and table: 8.

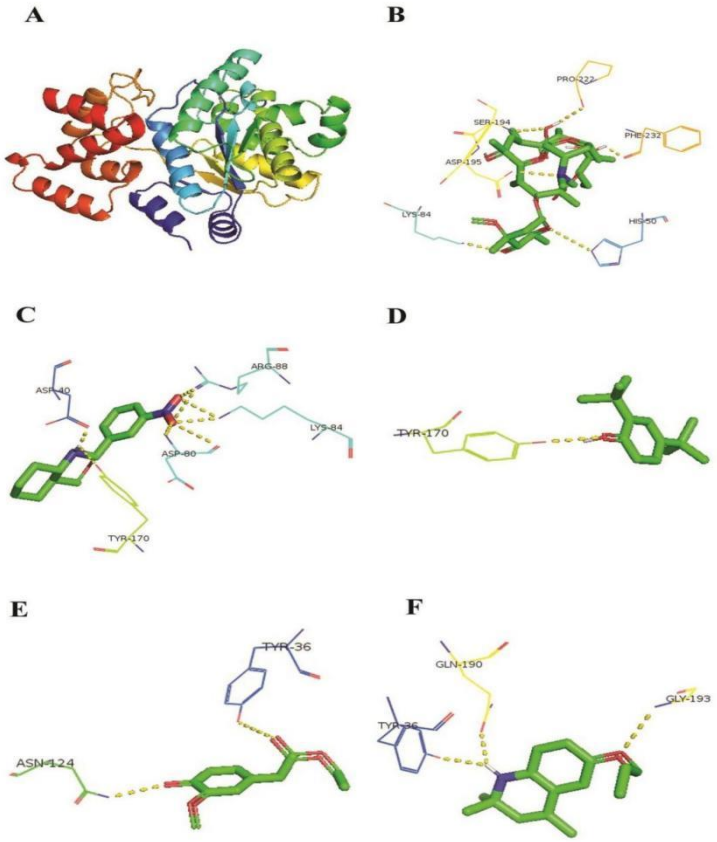


Fig 12: A- Structure of Tyrosyl- tRNAsynthetase, B) Docked pose with Erythromycin, C) Docked pose with 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl, D) Docked pose with Ethyl homovanillate and E) Docked pose with Quinoline, 1,2-dihydro-2,2,4-trimethyl.

Table 8: Molecular docking parameters

Sl no:	Positive control and compounds	Binding energy (Kcal/mol)	Inhibition constant (Ki)	Hydrogen bond interaction with amino acids
1	Erythromycin	-11.79	2.29 nM	His 50, Lys 84, Asp 195, Ser 194, Pro 222 and Phe 232
2	2H-1,3-Benzoxazine, octahydro-2-(3-nitrophenyl	-9.39	131.50 nM	Asp 40, Asp 80, Lys 84, Arg 88 and Tyr 170
3	2,4-Di-tert-butylphenol	-6.64	9.67 µM	Tyr 170
4	Ethyl homovanillate	-6.42	19.82 µM	Asn 124 and Tyr 36
5	Quinoline, 1,2-dihydro-2,2,4-trimethyl	-7.19	5.36 µM	Tyr 36, Gln 190 and Gly 193

3.7.2 Docking with DNA gyrase

DNA gyrase was used as another target enzyme and the compounds showed significant binding interaction. Erythromycin binds to the Arg 497, Glu 504 and Gln 512 of the active site. 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl forms hydrogen bonds with Lys 372 and Glu 369. The compound, Quinoline, 1,2-dihydro-2,2,4-trimethyl binds to the Glu 133 of the active site of the target. Ethyl homovanillate binds to the active residue Lys 372 of the DNA gyrase. The results were shown in figure: 13 and table: 9.

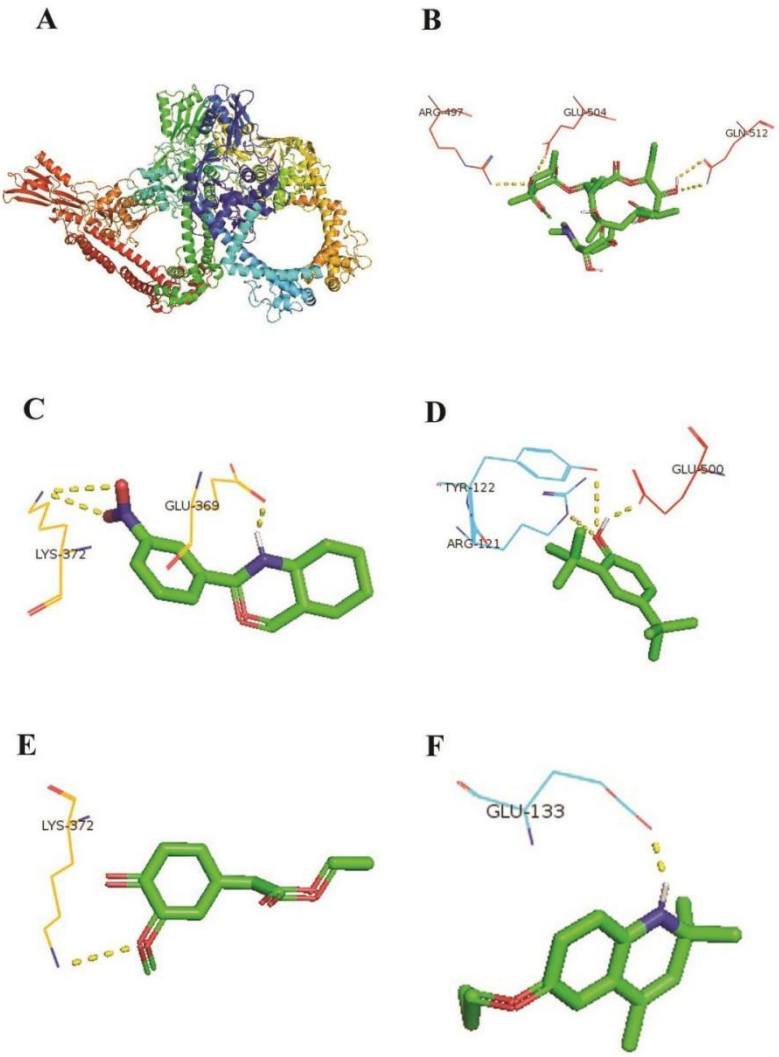


Fig 13: Fig 12: A- Structure of DNA Gyrase B) Docked pose with Erythromycin, C) Docked pose with 2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl, D) Docked pose with Ethyl homovanillate and E) Docked pose with Quinoline, 1,2-dihydro-2,2,4-trimethyl.

Table 9: Molecular docking parameters

Sl no:	Positive control and compounds	Binding energy (Kcal/mol)	Dissociation constant	Hydrogen bond with amino acids
1	Erythromycin	-8.22	950.07 nM	Arg 497, Glu 504 and Gln 512
2	2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl	-7.79	668.10 nM	Lys 372 and Glu 369
3	2,4-Di-tert-butylphenol	-4.80	300.69 µM	Arg 121, Tyr 122 and Glu 500
4	Ethyl homovanillate	-4.75	328.90 µM	Lys 372
5	Quinoline, 1,2-dihydro-2,2,4-trimethyl	-4.93	244.12 µM	Glu 133

3.8 ADMET properties

The all identified compounds follow the Lipinski's rule of five, hence the metabolites were considered to be orally active drug.

Sl no	Properties	2H-1,3-Benzoxazine, octahydro-2-3-nitrophenyl	2,4-Di-tert-butylphenol	Ethyl homovanillate	Quinoline, 1,2-dihydro-2,2,4-trimethyl
1	Molecular formula	C15 H15 N O3	C14 H22 O	: C11 H14 O4	C15 H21 N O
2	Molecular weight	257.11	206.17	210.09	231.16
3	Number of Hydrogen bond	3	1	4	1

	acceptors				
4	Number of Hydrogen bond donors	1	1	1	1
6	MolLogP	1.93	4.71	1.94	3.42
7	MolLogS	-3.09 (in Log(moles/L)) 210.05 (in mg/L)	-5.04 (in Log(moles/L)) 1.89 (in mg/L)	-1.65 (in Log(moles/L)) 4738.53 (in mg/L)	4.13 (in Log(moles/L)) 17.22 (in mg/L)
8	MolPSA	43.86 A^2	16.79 A^2	44.58 A^2	18.85 A^2
9	MolVol	262.08 A^3	257.37 A^3	210.61 A^3	285.26 A^3
10	Number of stereo centers	0	0	0	0
11	Rule of 5 violations	0	1	0	0

IV. DISCUSSION

Marine microorganisms are good sources for the production of bioactive compounds. They live in the extreme conditions of temperature, salinity, pressure, pH, and light. These conditions made them produce bioactive compounds as an adaptive measure.

A novel marine bacteria *Alcanivorax xenomutant* was isolated from the marine sediments of Mararikkulam coastal area and it was named as MBTU_MBS2. The bacteria was already known for the degradation of oil [10]. The molecular identification reveals that the strain was 100% similar to *Alcanivorax xenomutant* and the sequence was deposited in Gen Bank with accession number MN103744.

In the current study we used this strain for the production of bioactive secondary metabolites. Large scale cultivation of *Alcanivorax xenomutant* was carried out and extraction of bioactive compounds was done by using the organic solvent ethyl acetate by Liquid- liquid separation method. It was a common method used for the extraction of bioactive compounds.

We studied the antibacterial activity of ethyl acetate extract of MBTU_MBS2 against *Klebsiella pnuemoniae, Salmonella typhi, Streptococcus aureus, Bacillus subtilis, and Escherichia coli*. Significant antibacterial activity was obtained from the ethyl acetate extract against these pathogens as compared to the erythromycin standard.

The antibacterial properties of bioactive compounds have been proved by many researchers. Compounds isolated from *Pseudomonas* have showed potent antimicrobial activities [21]. The novel lipo-peptides such as tauramamide and ethyl esters were isolated from the marine *Brevibacillus laterosporus*, which showed potent inhibitory activity against the human pathogen *Enterococcus sp*[22].The isolated compounds from the new bacterial genus *Marinispora* have shown a broad spectrum of antimicrobial activities, similar to those of the commercial antibiotic linezolid [23].

The purification of bioactive compounds present in the ethyl acetate extract was done using the TLC chromatographic technique. TLC is a relatively simple and inexpensive method used for the identification of components present in a mixture. The extract was subjected to thin layer chromatography, using chloroform and methanol (9:1) as the solvent system. The presence of separate bands was detected and visualized under UV light at 254 and 366 nm.

The Gas Chromatography/Mass Spectrometry (GC/MS) is one of the most accurate tools for analyzing bioactive compounds. After GC-MS, three compounds were obtained which showed bio activity such as Ethyl homovanillate (28.389), Stigmasta-4,7,22-trien-3.beta.-ol (9.910) and 2H-1,3-Benzoxazine, octahydro-2-(3-nitrophenyl (15.281), In our study, the identified compounds as per the report have antimicrobial, anti-inflammatory, antioxidant, anticoagulant, properties.

The identified compounds were subjected to molecular docking. The goal of ligand-protein docking is to predict the predominant binding models of a ligand with a protein of known three-dimensional structure. Hence, a study was planned to evaluate the interaction of the selected ligand with a target protein expressed in pathogenic bacteria. Autodock4 software was used for the study. The enzymes used for protein targeting were DNA gyrase and TyrosyltRNA synthetase. Using these target proteins, molecular docking studies were performed.

DNA gyrase is considered as an important target for the development of antimicrobial drugs. Fluoroquinolins are broad spectrum antibacterial antibiotics and they are highly effective against various antibacterial infections. It forms complexes with DNA gyrase and is responsible for stoppage of DNA replication. Molecular docking confirmed the formation of hydrogen bonds and good binding affinity with DNA gyrase [26]. So, in the present study we selected DNA gyrase as one of the target proteins. The identified compounds significantly bind with the active site of DNA gyrase, same as that of the antibiotic Erythromycin.

Tyrosyl tRNA synthetase was used as a target for this study because it was a prominent target for many antimicrobial agents. Molecular docking of an aminofuran derivative significantly binds into tyrosyl-tRNA synthetase active site and may well explain its excellent inhibitory activity [27]. Erythromycin showed excellent binding affinity for the enzyme and the identified compounds also showed promising hydrogen bonds against the target with the lowest free energy of binding. Drug likeness of the compound was studied and found to follow Lipinski's rule of 5 and hence the compounds were corresponding to orally active drugs.

Our study discussed that all the identified compounds showed significant binding interaction with DNA gyrase and tyrosyl- tRNA synthetase. The docking study confirmed the antimicrobial activity of ethyl acetate extract of marine bacteria *Alcanivorax xenomutants*.

V. CONCLUSION

The main objective of the study was the isolation, characterization, and identification of marine bacteria having antibacterial properties. Based on antimicrobial activity, the MBTU_MBS2 strain was selected for further studies. Molecular identification confirmed that the strain was *Alcanivorax xenomutant* MN103744. The extraction of bioactive compounds was carried out using ethyl acetate as the solvent and evaluated its antimicrobial activity. The extract was further purified by TLC and characterized by GC-MS analysis. The GC-MS study reveals the structural characterization of the bioactive compounds. From the molecular docking study it was revealed that the compounds possess significant binding affinity with DNA gyrase and tyrosyl tRNA synthetase. The molecular docking study confirms the antimicrobial activity of ethyl acetate extract of marine bacteria. This is the first attempt and report of *Alcanivorax xenomutant* MN103744 which produces the antibacterial compounds.

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VII. REFERENCES

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