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Original Research Article

In Vitro and *In Silico* Anti-Breast Cancer Analysis of Bioactive Metabolites of *Bacillus subtilis* Isolated From Soil

Ram Kumar A[1], Rajagopal. K[1], Meenambiga SS[2] and Kumaresan S[1*]

[1]PG and Research Department of Plant Biology and Plant Biotechnology, Ramakrishna Mission Vivekananda College (Affiliated to University of Madras), Mylapore, Chennai-600004, India
[2]Department of Bio-Engineering, School of Engineering, VISTAS, Pallavaram, Chennai-600117, India

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***Corresponding author:** S. Kumaresan

Abstract

Objective: Breast cancer is the most common cancer faced by women around the worldwide. The Estrogen receptor alpha (ERα) has been playing a major role in the stimulation of breast cancer. The present study aims to identify the anticancer activity of crude extract of Bacillus subtilis against breast cancer cell line by in vitro and in silico methods. ***Methods:*** The soil samples were collected from different regions in the reserve forests of Western Ghats of Nilgiris district in Tamil Nadu, India. Isolation of bacterial strain from the collected soil samples was performed by serial dilution method. Identification of bacterial strain was done by 16S rRNA sequencing analysis. The anti-cancer potential of crude extract of bacterial strain was tested against breast cancer cell line (MCF-7) by MTT assay. Further, the bioactive compounds found in the crude extract of bacterial strain was identified by GC-MS and the identified compounds was subjected to in silico docking studies against the targeted breast cancer protein Estrogen receptor alpha (ERα). ***Result:*** The bacterial strain isolated from the soil sample by serial dilution method was identified as *Bacillus subtilis* by 16S rRNA analysis. *In vitro* anti-breast cancer analysis of crude extract of *Bacillus subtilis* showed potential activity against MCF-7 breast cancer cell line with the IC50 value of 100µg/ml. The GC-MS analysis of the ethyl acetate extract of *Bacillus subtilis* showed twenty-six bioactive compounds. The compound Metaraminol showed maximum docking score -7.27 Kcal/mol against the target protein. ***Conclusion:*** The crude extract of soil bacterium showed potent anti-cancer activity against breast cancer cell lines. Further, the bioactive compounds showed good binding interactions with the virulence factor of breast cancer. Thus, the compounds of soil bacterium Bacillus subtilis could effectively used as leads for developing drugs against breast cancer.

Keywords: *Bacillus subtilis*, Breast cancer, Estrogen receptor alpha (ERα), GC-MS, Western Ghats.

INTRODUCTION

Cancer is the second leading cause of death globally with 8.8 million cancer-related deaths in 2015. Breast cancer is the most common cancer among women worldwide and the highest in women between 44 and 55 years of age. Although equally prevalent in developed and developing countries, breast cancer survival rates are higher (80%) in developed countries when compared to developing countries (less than 60%) and underdeveloped countries (less than 40%) [1]. The Estrogen receptors are the major prognostic markers used to identify tumors in breast tissue [2]. Estrogen receptor positive cancer is the most frequent subtype representing more than 70% of breast cancer. The Estrogen receptor consists of two subtypes namely Estrogen receptor alpha (ERα) and Estrogen receptor beta (ERβ) that have different affinities to estrogen. The Estrogen receptor alpha (ERα) is an activated ligand by a transcriptional regulator which is the main regulator of differentiation and proliferation of breast cells. Additionally, the Estrogen receptor α (ERα) plays an important role in the development and progression of dependent hormonal type breast cancer [3].

Soil is a highly complex environment possessing the highest microbial diversity on earth, which are important components of the biogeochemical cycles in terrestrial ecosystems [4-6]. In particular, soil bacteria is the key contributor to nutrient cycling and productivity in ecosystem processes, as it is the most abundant and diverse microbial community [7]. Besides, soil bacteria are not only the key factor to influence soil nutrients decomposition and mineralization [8, 9]. But it is also the main source of secondary metabolites, which have different

physiochemical biological properties and then in turn affect soil environment and plant growth [10, 4]. They produce unique biologically active metabolites, novel products like antibiotics, vaccines, steroids as well as other therapeutically useful compounds. A huge number of currently used antibiotics including erythromycin, streptomycin, rifamycin, and gentamycin, are all products isolated from soil bacteria [11, 12]. Members of the genus *Bacillus* are commonly found in soil and produce a variety of bioactive metabolites that are effective against many pathogenic microorganisms [13]. In particular, *B. amyloliquefaciens* and *B. subtilis* isolated from soil produce a variety of antibiotics with antimicrobial, anti-inflammatory, anti-viral, and anti-cancer properties [14, 15]. The primary aim of the present study is to identify the anticancer potential of crude extract of bacterial strain against breast cancer cell line (MCF-7) by in vitro analysis and the secondary aim is to identify the potent anti-breast cancer compound from the bacterial crude extract using in silico studies.

MATERIALS AND METHODS

Isolation of bacteria from soil sample

The soil samples were collected from Nilgiri district (Lat 11^0 08' to 13^0 37' N, Long 77^0 27' to 80^0 4' E) Western Ghats, Tamil Nadu, India. Isolation and enumeration of bacteria were performed by serial dilution method[18]. Briefly, one gram of soil was suspended in 9 mL of sterile double distilled water. The dilution was carried out up to 10^{-5} dilutions. Aliquots (0.1 mL) of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were spread on the Nutrient Agar (NA) medium containing (g/L) Peptone 5.0; NaCl 5.0; Beef extract 1.5; Yeast extract 1.5, Agar Agar 15.0 and pH 7. The plates were incubated at 37^{o}C for 2 days and bacterial colonies were purified by repeated streaking. The purified colonies were stored at 4^{o}C for further analysis.

Molecular identification of bacterial isolate

Isolation of genomic DNA

The genomic DNA of active strain was isolated using NucleoSpin® Tissue Kit (Macherey-Nagel). Briefly, the pure bacterial culture was taken in a microcentrifuge tube. 180 µl of T1 buffer and 25 µl of proteinase K was added and incubated at 56 ^{o}C in a water bath until it were completely lysed. After lysis, 5 µl of RNase A (100 mg/ml) solution was added and incubated at room temperature for 5 minutes. Then 200 µl of B3 buffer was added and incubated at 70^{o}C for 10 minutes. 210 µl of 100% ethanol was added and mixed thoroughly using vortex mixturte. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11,000 rpm for one minute at room temperature. The NucleoSpin® Tissue column was transferred to a new 2 ml tube and washed with 500 µl of BW buffer. Wash step was repeated using 600 µl of B5 buffer. After washing the NucleoSpin® Tissue column was placed in a clean 1.5 ml tube and DNA was eluted out using 50 µl of BE buffer. Finaly the DNA was eluted by centrifuging at 11,000 rpm for one minute.

16S rRNA analysis

The primers 16S-RS-Forward-CAGGCCTAACACATGCAAGTC and 16S-RS-Reverse- GGGCGGWGTGTACAAGGC (5' → 3') were used to amplify 16S ribosomal sequences from genomic DNA were carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.6 [16].

Database searching and phylogenetic analysis

The obtained nucleotide sequence was compared with sequence details of other organisms in NCBI using BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree was constructed based on the Neighbor-joining method using MEGA (version 4.0) software [17].

Cultivation and extraction of bacterial metabolites

The isolated bacterial cultures inoculated in the 250ml of conical flask containing 100ml of Nutrient Broth medium (NB). After the, 72 hrs of incubation at 130rpm the fermented culture extracted with an equal volume of ethyl acetate (1:1). The crude extract concentrated and stored at 4^o C for further studies.

Cell line

Human breast cancer cell line (MCF-7) was obtained from National centre for cell sciences, Pune, India. Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS) and incubated at 37°C of 5 % CO2. Streptomycin and penicillin (100µg/ml) was used to avoid contamination.

Anticancer analysis by MTT assay

The anticancer property of crude bacterial extract was done by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) assay [18]. The cells were seeded in 96 well plates at a concentration of 1×10^5 cells/well. After 24 h, cells were washed twice with 100µl of serum-free medium and starved for 60m at 37°C. After starvation, cells were treated with different concentrations of crude extract (10-100µg/ml) and incubated for 24 h. After the incubation period, the medium was aspirated and serum free medium containing MTT (0.5mg/ml) was added and incubated for 4 h at 37°C in a CO2 incubator. After incubation MTT containing medium was discarded and the cells were washed with PBS (200µl). The insoluble formazan crystals were dissolved by adding 100µl of DMSO.

Spectrophotometrical absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). The 50% inhibitory concentration value (IC50) of the test compound was identified for treated cell line. The percentage of cell viability calculated using following formula

$$\text{Percentage}(\%)\text{of cell viability} = \frac{\text{Test OD}}{\text{Control OD}} \times 100$$

Gas Chromatography Mass Spectrometry (GC-MS) analysis

The ethyl acetate extract of the *Bacillus subtilis* metabolite was analyzed by Gas Chromatography Mass Spectrometry (GC-MS) to identify the compounds present. GC-MS analysis was performed in Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused capillary column (30 × 0.25 µm ID × 0.25 µm df). After analysis, the compounds were identified by matching with the known compound library.

Molecular Docking Analysis

Preparation of protein

The three-dimensional crystal structure of Human estrogen receptor alpha (PDB: 3ERT) was downloaded in PDB format from the protein data bank (http://www.rcsb.org/pdb).

Retrieval of ligands

The ligands (bacterial compounds) were retrieved from the PubChem database in SDF (structure data format) (http://www.pubchem.ncbi.nlm.nih.gov) and then converted into the PDB format using online SMILE translator.

Drug-likeness analysis

The drug-likeness analysis of the compounds was done by based on the Lipinski‟s rule of five. As per “Rule of 5”, the drug-like molecules have the number of hydrogen bond acceptors is not more than 10, number of hydrogen bond donors is not more than 5, Partition coefficient log P less than 5, molecular mass less than 500 daltons and number of violations not more than 2 [19].

Docking analysis

Molecular docking was carried out using AutoDock 4.2.6 software with their standard protocols and the visualization was done by Discovery studio 4.0.

Statistical Analysis

The results were expressed as mean±SD obtained from triplicates. Values were statistically significant (P≤0.05). The statistical analysis was done using Graph pad prism7 software.

RESULTS AND DISCUSSION

Isolation of soil bacteria

In the current investigation the bacterial strain was isolated from the soil samples collected from Nilgiri district, Western Ghats, Tamilnadu, India. Many scientists have chosen soil is a primary site of the isolation of novel antibiotics producing bacteria [20, 21]. Similary, Islam et al. [22] reported several bacterial communities isolated from the soil samples of Western Ghats, which produce efficient bioactive compounds.

Molecular identification of the bacterial isolate

The molecular identification method could be used to identify the organism at the species level. The isolated bacterial strain was identified as *Bacillus subtilis* based on the 16S rRNA analysis and the strain showed 99% similarity with *Bacillus subtilis* (NR102783) of the NCBI Gene bank data. The quality of the DNA isolated was checked using agarose gel electrophoresis. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad). The molecular standard used was a 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Figure - 1). The obtained gene sequence was analyzed using BLAST software in GenBank website and the phylogenetic tree were constructed using Mega blast software (Figure- 2). The nucleotide sequence of *Bacillus subtilis* has been deposited in the NCBI GenBank database with the accession number MH198042. This result provided strong support to earlier studies which have already proved *Bacillus* species as the most predominant bacteria found in soil [23, 24].

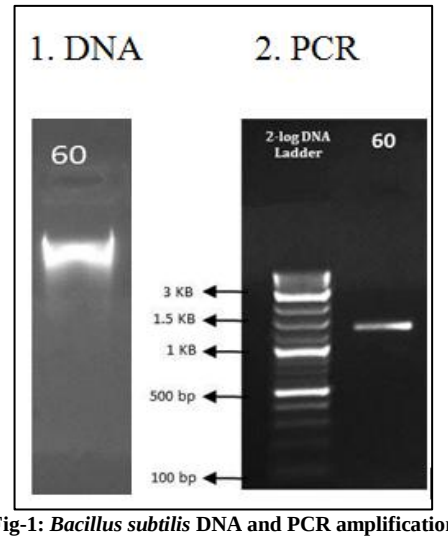


Fig-1: *Bacillus subtilis* DNA and PCR amplification

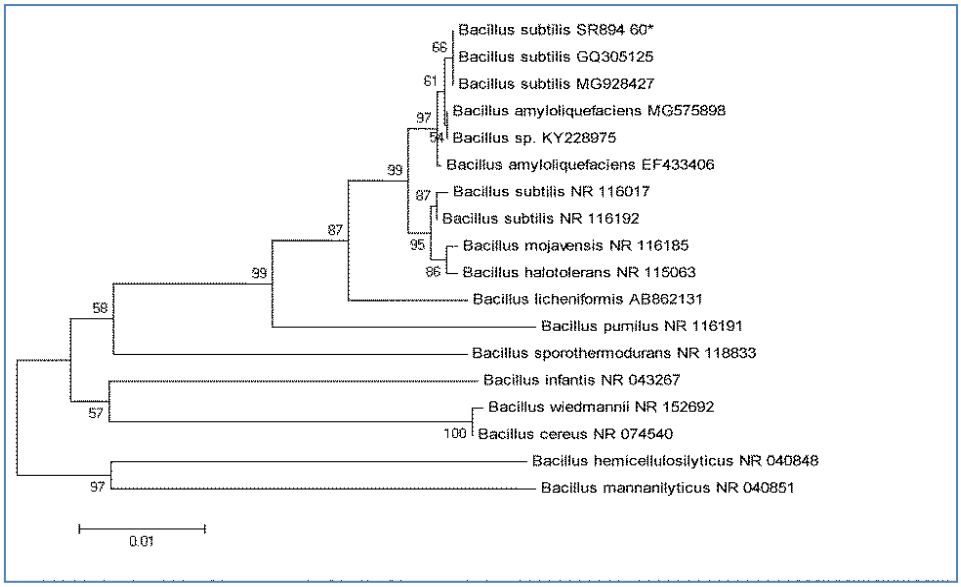


Fig-2: Phylogenetic tree of *Bacillus subtilis*

Anticancer analysis

The anticancer analysis of different concentrations (10 to 100µg/ml) of crude extract of *Bacillus subtilis* (MH198042) were tested against the breast cancer cell line (MCF-7) by MTT assay. The result showed 100µg/ml concentration of the crude extract showed maximum anticancer activity against breast cancer cell line with the IC_{50} value of 100µg/ml (Figure- 3 and Table- 1). The standard anticancer compound 5- Fluorouracil showed IC_{50} value of 25.63µg/ml against the breast cancer cell line. Seerangaraj *et al*. [25] reported, the crude extract of *Bacillus subtilis* SVSK5 showed strong anticancer activity against breast cancer cell line (MCF-7) with the IC_{50} value of 150µg/ml by MTT assay. In the present study, the crude extract of *Bacillus subtilis* (MH198042) showed strong activity against breast cancer cell line, when compared with the crude extract of *Bacillus subtilis* SVSK5 isolated from *Oreochromis mossambicus* and *Labeo rohita*. Similarly, Ramasubburayan *et al*. [26] reported crude extract of *Bacillus subtilis* subsp. *subtilis* RG, isolated from the rhizospheric soil of a mangrove plant species, *Excoecaria agallocha* at South east coast of India, showed significant anticancer activity against human breast cancer (MCF-7) cell line with the IC_{50} value was 46.64 ± 0.79µg/ml as determined through MTT assay. Further, Aboul-Ela *et al*., [27] reported crude extracts of *Bacillus subtilis* strain FS05, isolated from Red sea sponge *Amphimedon ochracea*, showed potential anticancer activity against MCF-7 (breast carcinoma) with an IC_{50} of 5.5µg/ ml by MTT assay.

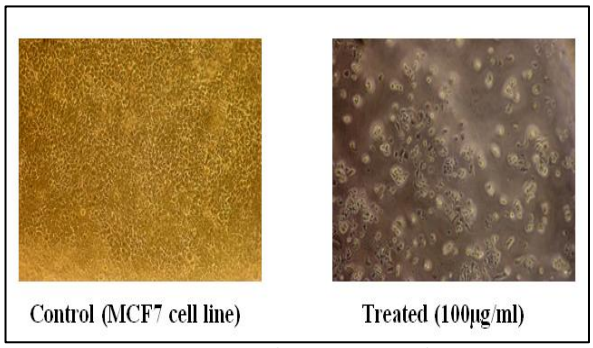


Fig-3: Anticancer analysis of crude extract of *Bacillus subtilis*

Table-1: Anticancer analysis of crude extract of *Bacillus subtilis* (MH198042) against breast cancer cell line (MCF-7)

S.No.	Concentrations of the crude extract (µg/ml)	Percentage (%) of viability
1	10	94.02±0.06
2	20	88.44±0.12
3	30	83.02±0.56
4	40	76.02±0.80
5	50	71.64±0.26
6	60	63.31±0.39
7	70	60.74±0.35
8	80	55.02±0.36
9	90	48.11±0.56
10	100	36.80±0.52
	IC_{50} Value	**100µg/ml**

GC-MS analysis of crude extract of Bacillus subtilis

GC-MS chromatogram of the ethyl acetate extract *Bacillus subtilis* (MH198042) showed 26 peaks indicating the presence of twenty six different compounds in the crude extract (Figure- 4). On comparison of the mass spectra of the constituents with the NIST library the twenty six bacterial compounds were characterized and identified (Table- 2). Among the twenty six compounds, the compound Cetene is the major peak compound found in the crude extract of *Bacillus subtilis* (MH198042) with the retention time of 13.61, followed by Phenol 2, 4-bis (1,1-dimethylethyl) with the retention time of 12.58. Recently, Phuong *et al*. [28] reported GC-MS analysis of ethyl acetate extract of *Bacillus subtilis* HD16b showed eight bioactive compounds. Additionally, Weiwei Liu *et al*. [29] reported the soil *Bacillus subtilis* G8 showed thirty bioactive compounds by GC-MS analysis.

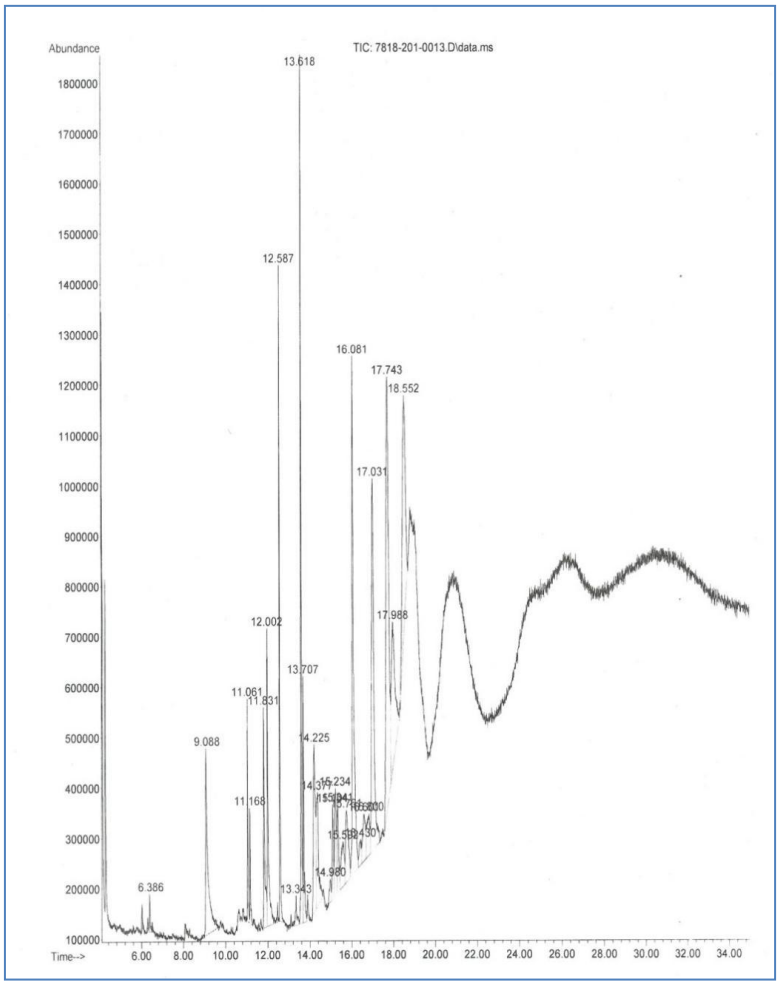


Fig-4: GC-MS chromatogram of crude extract of *Bacillus subtilis* (MH198042)

Table-2: Bioactive compounds identified from ethyl acetate extract of *Bacillus subtilis* by GC-MS analysis

S.No.	RT	Compounds Name	Molecular Formula	Molecular Weight	Peak Area
1	6.38	Pyrazine, tetramethyl-	$C_8H_{12}N_2$	136.198	0.35
2	9.08	Benzeneacetic acid	$C_8H_8O_2$	136.15	5.06
3	11.06	5-Tetradecene, (z)-	$C_{14}H_{28}$	196.378	1.45
4	11.16	Tetradecane	$C_{14}H_{30}$	198.394	0.81
5	11.83	Phosphine, methyl (1-methylethyl) phenyl-	$C_{10}H_{15}P$	166.204	2.73
6	12.00	Cyclohexanone	$C_6H_{10}O$	98.145	3.85
7	12.58	Phenol, 2,4-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	206.329	4.28
8	13.34	Benzenemethanol, 2- (2-aminopropoxy)-3-methyl-	$C_{11}H_{17}NO_2$	195.262	0.54
9	13.61	Cetene	$C_{16}H_{32}$	224.432	6.84
10	13.70	Hexadecane	$C_{16}H_{34}$	226.448	2.34
11	14.22	o-Acetylphenetidine	$C_{10}H_{13}NO_2$	179.219	4.01
12	14.37	Benzene, 1-ethoxy-4-isothiocyanato	C_9H_9NOS	179.237	3.33
13	14.98	p-Hydroxyamphetamine	$C_9H_{13}NO$	151.209	0.46
14	15.10	1-n- Hexyladamantane	C_6H_{28}	220.4	1.41

15	15.23	Acetamide, N-(3-methylphenyl)	$C_9H_{11}O$	149.193	2.38
16	15.34	Benzaldehyde diethyl acetal	$C_{11}H_{16}O_2$	180.247	1.56
17	15.59	Tricyclo[4.3.1.1(3,8)] undecan-1- amine	$C_{11}H_{19}N$	165.28	1.81
18	15.75	Phenol, 4-(1,1-dimethylpropyl)-	$C_{11}H_{16}O$	164.248	2.40
19	16.08	E-15-Heptadecenal	$C_{17}H_{32}O$	252.442	10.07
20	16.43	Metaraminol	$C_9H_{13}NO_2$	167.208	0.70
21	16.60	Methylpent-4-enylamine	$C_6H_{13}N$	99.177	1.56
22	16.79	Acetamide, N-(alpha-methylphenethyl)-	$C_{11}H_{15}NO$	177.247	1.62
23	17.03	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278.348	10.45
24	17.74	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	$C_{18}H_{24}O_4$	304.386	14.72
25	17.98	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334.456	6.37
26	18.55	2- (Nonyloxycarbonyl) benzoic acid	$C_{17}H_{24}O_4$	292.375	8.93

Molecular docking analysis

Drug-likeness analysis

Assessment of drug-likeness properties of identified compounds from *Bacillus subtilis* (MH198042) were tested for Lipinski‟s rule of five. The result showed all the twenty six compounds can satisfy Lipinski rule (Table -3). Hence, all the twenty six compounds were selected for molecular docking analysis against breast cancer protein Estrogen receptor alpha (ERα).

Table-3: Drug-likeness analysis of bioactive compounds of *Bacillus subtilis* (MH198042) by Lipinski's rule of five (RO5)

S.No.	Compounds Name	MW(<500) Da	Log P (<5)	HBA (<10)	HBD (<5)	No. of violations
1	Pyrazine, tetramethyl-	136.19	0.55	2	0	0
2	Benzeneacetic acid	136.15	1.66	2	1	0
3	5-Tetradecene, (z)-	196.37	5.79	0	0	1
4	Tetradecane	198.39	5.93	0	0	1
5	Phosphine, methyl (1-methylethyl) phenyl-	166.2	3.56	0	0	0
6	Cyclohexanone	98.14	1	1	0	0
7	Phenol, 2,4-bis (1,1-dimethylethyl)	206.32	3.87	1	1	0
8	Benzenemethanol, 2- (2-aminopropoxy)-3-methyl-	195.26	1.24	3	2	0
9	Cetene	224.43	6.29	0	0	1
10	Hexadecane	226.44	6.44	0	0	1
11	o-Acetylphenetidine	179.21	1.54	2	1	0
12	Benzene, 1-ethoxy-4-isothiocyanato	179.23	3.20	2	0	0
13	p-Hydroxyamphetamine	151.21	1.53	2	2	0
14	1-n- Hexyladamantane	220.39	6.04	0	0	1
15	Acetamide, N-(3-methylphenyl)	149.19	1.84	1	1	0
16	Benzaldehyde diethyl acetal	180.24	2.53	2	0	0
17	Tricyclo[4.3.1.1(3,8)] undecan-1- amine	165.28	2.74	1	1	0
18	Phenol, 4-(1,1-dimethylpropyl)-	164.24	3.05	1	1	0
19	E-15-Heptadecenal	252.44	4.44	1	0	1
20	Metaraminol	167.21	0.65	3	3	0
21	Methylpent-4-enylamine	99.17	1.39	1	1	0
22	Acetamide, N-(alpha-methylphenethyl)-	177.24	2.17	1	1	0
23	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278.34	3.43	4	0	0
24	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	304.38	3.52	4	0	0
25	1,2-Benzenedicarboxylic acid, butyl octyl ester	334.45	4.37	4	0	1
26	2- (Nonyloxycarbonyl) benzoic acid	292.37	3.67	4	1	0

Molecular docking analysis of bioactive compounds against targeted breast cancer protein Estrogen receptor alpha (PDB id: 3ERT)

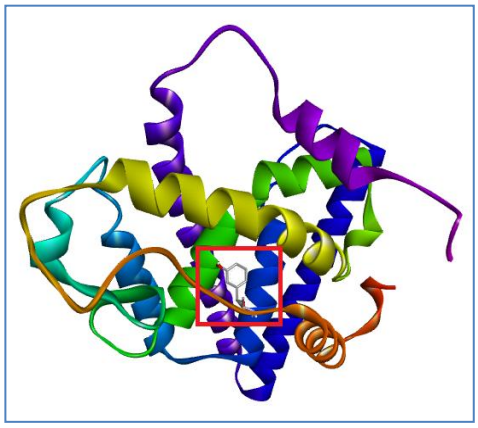
Breast cancer is known as a death sentence and second major cause of death in world. Ratio of breast cancer in is one in nine in case of women [30]. Main cause of breast cancer is overexpression of estrogen receptor alpha [31]. Therefore ER-α is used as a target for prevention of breast cancer. In the present investigation, all the twenty six compounds were screened against the breast cancer protein Estrogen receptor alpha (PDB id: 3ERT). Among the twenty six compounds, the compound Metaraminol showed maximum docking score -7.27 Kcal/mol (Figure-5) with the targeted protein followed by 1-n-Hexyladamantane and Tricyclo[4.3.1.1(3,8)] undecan-1- amine with the docking scores of -7.19 and -7.01 Kcal/mol respectively (Table- 4). So, these compounds may be reason for the *in vitro* anticancer analysis of crude extract of *Bacillus subtilis* (MH198042) against breast cancer (MCF-7) cell line. Similarly, Ravi *et al*. [32] reported the compound gancidin W isolated from the bacterium *Streptomyces paradoxus* VITALK03. The compound showed good docking score -7.55

Kcal/mol against the targeted breast cancer protein ERas of pERK pathway. Additionally, Shanta *et al*., [33] reported to screen the five bioactive compounds namely 1,1-diphenyl-2- picrylhydrazyl, quercetin, kaempferol, kaempferol 3-bita-D-glucopyranoside and isocorilagin against breast cancer protein estrogen receptor alpha (ER-α) (PDB id: 3ERT). Among the five compounds, the compound isocorilagin showed maximum docking score -7.90 Kcal/mol against the targeted protein.

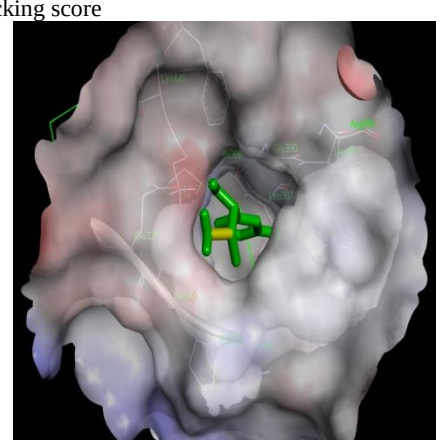
Table-4: *In silico* docking analysis of bioactive compounds of *bacillus subtilis* (MH198042) against breast cancer protein Estrogen receptor alpha (PDB id: 3ERT))

S.No	Compounds Name	Binding Energy (Kcal/mol)	Interacting Aminoacid residues	RMSD (Å) Value
1	Pyrazine, tetramethyl-	-4.99	Pro324, Glu353, His356, Met357, Trp360, Ile386, Leu387, Gly390, Arg394, Lys449	0.09
2	Benzeneacetic acid	-4.57	Pro324, Glu353, Met357, Ile386, Leu387, Ile389, Gly390, Arg394, Phe445, Lys449	1.99
3	5-Tetradecene, (z)-	-5.05	Glu323, Pro324, Ile326, Glu353, Met357, Trp360, Ile386, Leu387, Gly390, Leu391, Trp393, Arg394, Phe445, Lys449	0.99
4	Tetradecane	-4.78	Pro324, Pro325, Ile326, Glu353, Met357, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	0.98
5	Phosphine, methyl (1-methylethyl) phenyl-	-5.31	Pro324, Glu353, His356, Met357, Ile386, Leu387, Gly390, Leu391, Arg394, Phe445, Lys449	0.04
6	Cyclohexanone	-4.79	Pro324, Glu353m, His356, Met357, Ile386, Leu387, Lys449	0.01
7	Phenol, 2,4-bis (1,1-dimethylethyl)	-6.31	Glu323, Pro324, Pro325, Ile326, Glu353, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	0.06
8	Benzenemethanol, 2- (2-aminopropoxy)-3-methyl-	-5.56	Glu323, Pro324, Glu353, Met357, Trp360, Ile386, Leu387, Gly390, Leu391, Arg394, Phe445, Lys449	1.97
9	Cetene	-4.79	Glu323, Pro324, Pro325, Ile326, Glu353, Met357, Trp360, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	1.21
10	Hexadecane	-4.99	Glu323, Pro324, Pro325, Ile326, Glu353, Met357, Ile386, Leu387, Gly390, Arg394, Phe445, Lys449	1.13
11	o-Acetylphenetidine	-5.56	Glu323, Pro324, Pro325, Glu353, Met357, Trp360, Ile386, Leu387, Gly390, Arg394, Phe445, Lys449	0.08
12	Benzene, 1-ethoxy-4-isothiocyanato	-4.79	Pro324, Glu353, Met357, Trp360, Ile386, Leu387, Gly390, Leu391, Arg394, Lys449	0.34
13	p-Hydroxyamphetamine	-5.54	Glu323, Pro324, Glu353, Ile386, Leu387, Gly390, Leu391, Trp393, Arg394, Phe445, Lys449	1.83
14	1-n- Hexyladamantane	-7.19	Glu323, Pro324, Pro325, Ile326, Met357, Trp360, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	0.57
15	Acetamide, N-(3-methylphenyl)	-5.14	Glu323, Pro324, Glu353, Met357, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	0.08
16	Benzaldehyde diethyl acetal	-4.96	Glu323, Pro324, Glu353, Met357, Ile386, Leu387, Gly390, Leu391, Trp393, Arg394, Phe445, Lys449	1.39
17	Tricyclo[4.3.1.1(3,8)] undecan-1- amine	-7.01	Pro324, Pro325, Glu353, Ile386, Leu387, Gly390, Leu391,Arg394, Lys449	0.03
18	Phenol, 4-(1,1-dimethylpropyl)-	-5.31	Glu323, Pro324, Pro325, Ile326, Glu353, Met357, Ile386, Leu387, Gly390, Leu391, Arg394, Lys449	0.11
19	E-15-Heptadecenal	-4.97	Glu323, Pro324, Pro325, Ile326, Glu353, Met357,Trp360, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	1.81
20	**Metaraminol**	**-7.27**	**Glu323, Pro324, Pro325, Glu353, Ile386, Leu387, Gly390, Leu391, Arg394, Phe445, Lys449**	**0.10**
21	Methylpent-4-enylamine	-3.87	Pro324, Glu353, Met357, Trp360, Ile386, Leu387, Arg394, Lys449	0.47
22	Acetamide, N-(alpha-methylphenethyl)-	-5.81	Glu323, Pro324, Glu353, His356, Met357, Ile386, Leu387, Gly390, Leu391, Arg394, Phe445, Lys449	0.30
23	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	-5.47	Glu323, Pro324, Pro325, Ile326, Glu353, His356, Met357, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449	1.55
24	1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester	-6.61	Glu323, Pro324, Ile326, Glu353, Met357, Ile386, Leu387, Gly390, Leu391, Trp393, Arg394, Lys449	0.65
25	1,2-Benzenedicarboxylic acid, butyl octyl ester	-5.74	Pro324, Glu353, Met357, Leu384, Ile386, Leu387, Met388, Gly390, Leu391, Arg394, Leu428, Phe445, Lys449	1.97
26	2- (Nonyloxycarbonyl) benzoic acid	-4.50	Glu323, Pro324, Pro325, Ile326, Glu353, Met357, Ile386, Leu387, Met388, Ile389, Gly390, Leu391, Trp393, Arg394, Phe445, Lys449	1.34
27	5-Fluorouracil (Standard compound)	-4.19	Glu323,Pro324,Ile326,Glu353,Met357Ile386,Leu387,Gly390,Leu391, Trp393Arg394, Phe445, Lys449	0.82

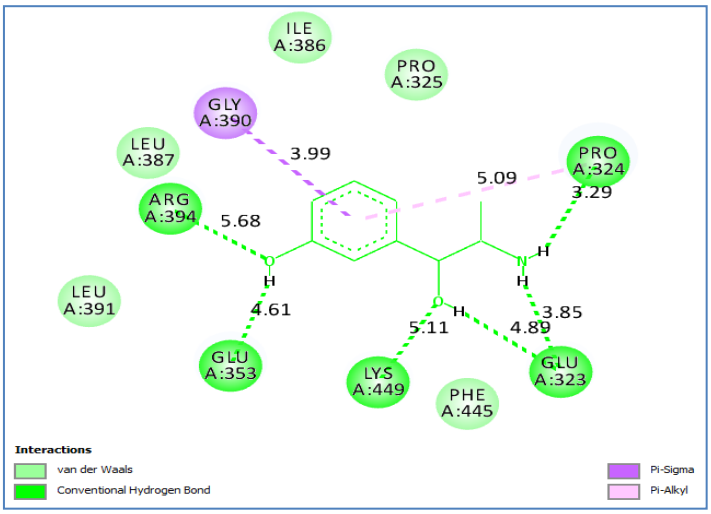
Bold value represents maximum docking score



Interaction of compound with protein



Compound docked with protein cavity



2D interaction of the of the compound with protein

Fig-5: Interaction of the compound Metaraminol with the targetd breast cancer protein estrogen receptor alpha (PDB id: 3ERT)

CONCLUSION

The current study showed that bioactive metabolites obtained from *Bacillus subtilis* (MH198042) showed *in vitro* anticancer activity against breast cancer (MCF-7). The *in silico* analysis of the bioactive compounds showed good docking scores against the targeted breast cancer protein Estrogen receptor alpha. Among all the compounds, Metaraminol showed best docking score towards estrogen receptor alpha. Hence, the further study should focuse on exploration of the functions and molecular mechanisms of the compound which will facilitate a better understanding for the control of breast cancer and in development of anticancer drugs.

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