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

Ram Kumar A\textsuperscript{1}, Rajagopal. K\textsuperscript{1}, Meenambiga SS\textsuperscript{2} and Kumaresan S\textsuperscript{3}\textsuperscript{*}

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

Abstract

Objective: Breast cancer is the most common cancer faced by women around the worldwide. The Estrogen receptor alpha (ER\textalpha) 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 were identified by GC-MS and the identified compounds were subjected to in silico docking studies against the targeted breast cancer protein Estrogen receptor alpha (ER\textalpha).

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 IC\textsubscript{50} value of 100\mu g/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 (ER\textalpha), GC-MS, Western Ghats.
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° 08’ to 13° 37’ N, Long 77° 27’ to 80° 4’ E) Western Ghats, Tamil Nadu, India. Isolation and enumeration of bacteria were performed by serial dilution method [8]. Briefly, one gram of soil was suspended in 9 ml of sterile double distilled water. The dilution was carried out up to 10^3 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 2; NaCl 1; Beef extract 1; Yeast extract 1; Agar Agar 15.0 and pH 7. The plates were incubated at 37°C for 2 days and bacterial colonies were purified by repeated streaking. The purified colonies were stored at 4°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°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°C for 10 minutes. 210 μl of 100% ethanol was added and mixed thoroughly using vortex mixture. The mixture was pipetted into NucleoSpin® Tissue column placed in a 2 ml collection tube and centrifuged at 11,000 rpm for one minute. 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. Finally the DNA was eluted by centrifuging at 11,000 rpm for one minute.

**16S rRNA analysis**

The primers 16S-Forward-CAGCCCTAACCACATGGCAAGTC and 16S-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°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. The 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

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

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].
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 IC₅₀ value of 100μg/ml (Figure- 3 and Table- 1). The standard anticancer compound 5- Fluorouracil showed IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ of 5.5μg/ml by MTT assay.

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                 |
| **IC₅₀ 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)**

| S.No. | RT   | Compounds Name                          | Molecular Formula | Molecular Weight | Peak Area |
|-------|------|----------------------------------------|-------------------|------------------|-----------|
| 1     | 6.38 | Pyrazine, tetramethyl-                 | C₆H₁₂N₂           | 136.198          | 0.35      |
| 2     | 9.08 | Benzeneacetic acid                     | C₆H₈O₂            | 136.15           | 5.06      |
| 3     | 11.06| 5-Tetradecene, (z)-                    | C₁₄H₂₈            | 196.378          | 1.45      |
| 4     | 11.16| Tetradeane                             | C₁₆H₃₂            | 198.394          | 0.81      |
| 5     | 11.83| Phosphine, methyl (1-methylethyl) phenyl-| C₁₀H₁₅P           | 166.204          | 2.73      |
| 6     | 12.00| Cyclohexaneone                         | C₆H₁₂O            | 98.145           | 3.85      |
| 7     | 12.58| Phenol, 2,4-bis (1,1-dimethylethyl)    | C₁₄H₁₈O           | 206.329          | 4.28      |
| 8     | 13.34| Benzenemethanol, 2- (2-aminoproxy)-3-methyl-| C₁₂H₁₄NO₂         | 195.262          | 0.54      |
| 9     | 13.61| Cetene                                 | C₁₆H₃₂            | 224.432          | 6.84      |
|10     | 13.70| Hexadecane                             | C₁₆H₃₄            | 226.448          | 2.34      |
|11     | 14.22| o-Acetylphenetidene                    | C₁₀H₁₆NO₂         | 179.219          | 4.01      |
|12     | 14.37| Benzene, 1-ethoxy-4-isothiocyanato     | C₁₀H₁₆NO₂         | 179.237          | 3.33      |
|13     | 14.98| p-Hydroxystamperidine                  | C₁₀H₁₆NO₂         | 151.209          | 0.46      |
|14     | 15.10| 1-n- Hexyladamantan                    | C₁₀H₃₈            | 220.4            | 1.41      |
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: 3ERT). Among the twenty six compounds, the compound Metaminol 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 Bacillus subtilis (MH198042) by Lipinski’s rule of five (Table 3). 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-like 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    | Benzenecarboxylic acid, bis(2-methylpropyl) ester | 215.38      | 2.76       | 2         | 0        | 0                |
| 3    | 5-Tetradecene, (z)-                 | 196.37      | 5.79       | 0         | 0        | 1                |
| 4    | Tetradecane                          | 198.39      | 5.93       | 0         | 0        | 0                |
| 5    | Phosphine, methyl (1-methylphenyl) phenyl- | 166.2       | 3.56       | 0         | 0        | 0                |
| 6    | Cyclohexane                          | 98.14       | 0.84       | 0         | 0        | 0                |
| 7    | Phenol, 2,4-bis (1,1-dimethylethyl)  | 206.32      | 3.87       | 1         | 1        | 0                |
| 8    | Benzenemethanol, 2- (2-aminoproxy)-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        | 0                |
| 11   | a-Acetylphenethidine                 | 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-dimethyl)propyl)-    | 164.24      | 3.05       | 1         | 1        | 0                |
| 19   | E-15-Heptadecan                     | 252.44      | 4.44       | 1         | 0        | 1                |
| 20   | Metaminol                            | 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-((Nonyloxy carbonyl) benzoic acid) | 292.37      | 3.67       | 4         | 1        | 0                |
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-picylhydrazyl, quercetin, kaempferol, kaempferol 3-bita-D-glucopyranoside and isorocilagin against breast cancer protein estrogen receptor alpha (ER-α) (PDB id: 3ERT). Among the five compounds, the compound isorocilagin 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    | Tetracane | -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    | Cyclohexaneone | -4.79 | Pro324, Glu353m, His356, Met357, Ile386, Leu387, Lys449 | 0.01 |
| 7    | Phenol, 2,4-bis (1,1-dimethylphenyl) | -6.31 | Glu323, Pro324, Pro325, Ile326, Glu353, Ile386, Leu387, Gly390, Trp393, Arg394, Phe445, Lys449 | 0.06 |
| 8    | Benzenemelhanol, 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)] undec-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, Phe445, Lys449 | 1.97 |
| 26   | 2-(Nonyloxybenzoyl) 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, Met357, Ile386, Leu387, Gly390, Leu391, Trp393, Arg394, Phe445, Lys449 | 0.82 |
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 focus 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.

ACKNOWLEDGMENTS

Authors thank the Management of RKM Vivekananda College (Autonomous) for providing all the necessary facilities and encouragement.

REFERENCES

1. Coleman, M. P., Quaresma, M., Berrino, F., Lutz, J. M., De Angelis, R., Capocaccia, R., Baili, P., Rachet, B., Gatta, G., & Hakulinen, T.(2008). Cancer survival in five continents: A worldwide population-based study (concord). *Lancet Oncology*, 9, 730-736.
2. Mijatovic, T., Van Quaquebeke, E., Delest, B., Debeir, O., Darro, F., & Kiss, R., Cardiotonic. (2007). Steroids on the road to anti-cancer therapy. *Biochimica et Biophysica Acta*. 1776, 32-57.
3. Hafiz, H. (2017). Epigenetic mechanisms of tamoxifen resistance in luminal breast cancer. *Diseases*, 5(3), 1-11.
4. Tycz, O., Song, C., Dickschat, J. S., Vos, M., & Garbeva, P.(2017). The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. *Trends in Microbiology*, 25, 280-292.
5. Lin, Y. T., Whitman, W. B., Coleman, D. C., Jien, S. H., & Chiu, Y. (2017). Cedar and bamboo plantations alter structure and diversity of the soil bacterial community from a hardwood forest in subtropical mountain. *Applied Soil Ecology*, 112, 28-33.
6. Liu, X., Zhang, B., Zhao, W., Wang, L., Xie, D., Huo, W., Wu, Y., & Zhan, J.(2017). Comparative
effects of sulfuric and nitric acid rain on litter decomposition and soil microbial community in subtropical plantation of Yangtze River Delta region. *Science of the Total Environment*, 601-602, 669.

7. Delgadobaquerizo, M., Reich, P. B., Khachane, A. N., Campbell, C. D., Thomas, N., Freitag, T. E., Al-Soud, W. A., Sorensen, S., Bardgett, R. D., & Singh, B. K. (2016). It is elemental: soil nutrient stoichiometry drives bacterial diversity. *Environmental Microbiology*, 19, 1176.

8. Wardle, D. A. (2006). The influence of biotic interactions on soil biodiversity. *Ecology Letters*, 9, 870.

9. Zechmeister Boltenstern, S., Keiblunger, K. M., Mooshammer, M., Penuelas, J., Richter, A., Sardans, J., & Wanek, W. (2016). The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. *Ecological Monographs*, 85, 133-155.

10. Rocheli, D. S., Adriana, A., & Passaglia, L. M. P. (2015). Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology*, 38, 401-419.

11. Alexander, M. (1977). Introduction to soil microbiology. (2nd edition). John Wiley and Sons Inc, New York, USA.

12. Jeffrey, L. S. H. (2008). Isolation, characterization and identification of actinomycetes from agriculture soils at Semongok, Sarawak. *African Journal of Biotechnology*, 7(20), 59415.

13. Kuta, F. A. (2008). Antifungal effects of *Calotropis Procera* stem bark extract against *Trichophyton gypseum* and *Epidermophyton Floccosum*. *African Journal of Biotechnology*, 7(13), 2116-2118.

14. Kim, Y. S., Balaraju, K., & Jeon, Y. (2017). Biological characteristics of *Bacillus amyloliquefaciens* AK-0 and suppression of ginseng root-rot caused by *Cylindrocarpon destructans*. *Journal of Applied Microbiology*, 122, 166-179.

15. Zhao, H. B., Shao, D. Y., Jiang, C. M., Shi, J. L., Li, Q., Huang, Q. S., Rajoka, M. S. R., Yang, H., & Jin, M. L. (2017). Biological activity of lipopeptides from *Bacillus*. *Applied Microbiology and Biotechnology*, 101(15), 5951-596.

16. Drummond, A. J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Duran, C., Heled, J., Kearse, M., Markowitz, S., Moir, R., Stones-Havas, S., Sturrock, S., Swidan, F., Thirier, T. and Wilson, A., Geneious v5.6, 2012, Available from http://www.geneious.com.

17. Tamura, K., Dudley, J., Nei, M., & Kumar, S., MEGA4.(2007). Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24, 15969.

18. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63.

19. Lipinski, C. A. (2004). Lead and drug-like compounds: the rule-of-five revolution. *Drug Discovery Today Technologies*, 1(4), 337-341.

20. Nike, A. R., Hassan, S. A., Ajjilakewu, & Bosede. A. F. (2013). Soil screening for antibiotic producing microorganisms. *Advances in Environmental Biology*, 7, 7-11.

21. Yunus, F. N., Khalid, Z. Z., Rashid, F., Ashraf, A., Iqbal, M. N., & Hussain, F. (2016). Isolation and screening of antibiotic producing bacteria from soil in Lahore city. *PSM Microbiology*, 1, 1-4.

22. Islam, M. R., Jeong, Y. T., Lee, Y. S., & Song, C. H. (2012). Isolation and identification of antifungal compounds from *Bacillus subtilis* C9 inhibiting the growth of plant pathogenic fungi. *Mycobiology*, 40, 59-66.

23. Cavalcanti, M. A. D. Q., Oliveira, L. G. D., Fernandes, M. J. and Lima, D. M. (2006). Filamentous fungi isolated from soil in districts of the Xingo region, Brazil. *Acta Botanica Brasiliaca*, 20, 831-837.

24. Singh, A. P., Singh, R. B., & Mishra, S. (2015). Studies on isolation and characterization of antibiotic producing microorganisms from industrial waste soil sample. *Open Nutraceuticals Journal*, 5, 169-173.

25. Seerangaraj, V., Kannan, S., Vijayakumar, U., Meganathan, B., Seerangaraj, V., Selvam, S. and Selvaraj, J. (2017). Isolation and characterization of bioactive compounds from *Bacillus cereus* and *Bacillus subtilis* from *Oreochromis mossambicus* and *Labeo rohita*. *International Journal of Pharmaceutical Sciences Review and Research*, 43(2), 71-77.

26. Ramosubburayen, R., Sumathi, S., Bercy, D. M., Immanuel, G., & Palavesam, A. (2015). Antimicrobial, antioxidant and anticancer activities of mangrove associated bacterium *Bacillus subtilis* subsp. *subtilis* RG. *Biocatalysis and Agricultural Biotechnology*, 4(2), 158-165.

27. Aboul-Ela, H. M., Shreadah, M. A., Abdel-Monem, N. M., Yakout, G. A., & Soest R. W. M. (2012). Isolation, cytotoxic activity and phylogenetic analysis of *Bacillus* sp. bacteria associated with the red sea sponge *Amphimedon ochracea*. *Advances in Bioscience and Biotechnology*, 3(7), 815-823.

28. Phuong, T. V., Han, P. N. and Diep, C. N.(2018). Bioactive compounds from marine bacterium *Bacillus subtilis* strain HD16b by Gas Chromatography-Mass Spectrometry. *The Pharmaceutical and Chemical Journal*, 5(2), 110-118.

29. Weiwei, Liu, Wei M.U., Bingyu, Z., & Feng, L. (2008). Antifungal activities and components of VOCs produced by *Bacillus subtilis* G8. *Current Research in Bacteriology*, 1(1), 28-34.

30. Naeem, M., Khan, N., Aman, Z., Nasir, A., Samad, A., & Khattak, A.(2008). Pattern of breast cancer: Experience at lady reading hospital, Peshawar.
Journal of Ayub Medical College Abbottabad, 20, 22-25.

31. Hayashi, S., Eguchi, H., Tanimoto, K., Yoshida, T., Omoto, Y., Inoue, A., Yoshida, N., & Yamaguchi, Y. (2003). The expression and function of estrogen receptor alpha and beta in human breast cancer and its clinical application. Endocrine-Related Cancer, 10(2), 193-202.

32. Ravi, L., Ragunathan, A., & Krishnan, K. (2017). Marine Streptomyces paradoxus VITALK03 derived gancidin W mediated cytotoxicity through Ras-Raf-MEK-ERK signaling pathway. Indian Journal of Biotechnology, 16, 164-175.

33. Shanta, A., Nazim, U., Kazi Zahra, M., Tarannur Kabir, N., Fayejun, N., Shermin, A., Sahida, A., Sajal, C., Dipannita, C., & Nasrin, A. (2018). In silico molecular docking approach of some selected isolated phytochemicals from Phyllanthus Emblic against breast cancer. Biomedical Journal of Scientific & Technical Research, 10(2), MS.ID.001917.