Ocean is a promising source of novel bioactive compounds and the marine microorganisms produce bioactive substances. The microbes in the marine water and sediment show antimicrobial activities. The aim of the present study was to isolate microbes from Punnakayal mangrove ecosystem in Tuticorin coast of Gulf of Mannar, Tamil Nadu and to identify their antagonistic activity.

The Punnakayal estuary is the only perennial estuary in Tuticorin, located at 08º39’49”N, 78º07’23”E. The soil is marshy and canals are made to facilitate the colonization of mangroves. The estuarine area is having mangrove tress covering about 7 sq.km. area. The mangrove trees *Avicennia* sp. and *Rhizophora* sp. are available in this area and *Avicennia* sp. is dominant in total cover due to high saline condition and lack of adequate fresh water inflow except during the monsoon. Frequent inundation of seawater is seen, due to tidal fluctuation. Most of the mangrove areas are degraded due to both natural and anthropogenic stressors.

The rhizosphere and non rhizosphere soil of *Avicennia marina* were collected from Punnakayal mangrove ecosystem of Gulf of Mannar, Southeast coast of India during 2010-2011. The bacterial, fungal and actinomycetes population were 66.67 to 14.58x10^6, 33.92 to 11.26x10^3 and 52.81 to 16.61x10^4 cfu/g of sample. Among the microorganisms, actinomycetes are considered as potent antibiotic producers and a total of 31 actinomycete isolates showing different colony morphology were isolated, studied and were designated as SDMRI-1 to SDMRI-31. Among the 31 isolates, SDMRI-3, SDMRI-6, SDMRI-7, SDMRI-8, SDMRI-17, SDMRI-18, SDMRI-23, SDMRI-27 and SDMRI-31 isolates were selected for secondary screening and among
that SDMRI-3, SDMRI-6, SDMRI-17 and SDMRI-18 showed broad spectrum of activity against all the test standard strains.

Among the 9 isolates, SDMRI-3 actinomycetes isolate was found as superior antagoniser and was used for further studies. The most effective strain was identified as *Streptomyces parvulus* SDMRI-3 based on morphological, biochemical features and 16S rRNA sequencing.

In the biochemical test, SDMRI-3 isolate showed positive reaction for Grams staining, oxidase, methyl red, citrate utilization, urease production, carbohydrate fermentation, starch hydrolysis and negative reaction for catalase, indole production, voges proskauer test, casein hydrolysis, tween 20 and gelatin hydrolysis.

A BLAST search of the Gen Bank database results showed that the new isolate had the highest similarity (99%) with identity of *Streptomyces parvulus* (GenBank entry: AB18615.1), *Streptomyces* sp. SU238 (GenBank entry: AB246727), *Streptomyces* sp. HUBM 172305 (GenBank entry: EF608470) and *Streptomyces* sp. HUBM 71549 (GenBank entry: EU119180) thus, this innovative strain was designated as *Streptomyces parvulus* SDMRI-3.

For antibiotic extraction, a loopful of *Streptomyces parvulus* SDMRI-3 from the 5th day culture was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of ISP2. The flasks were incubated on a rotary shaker (200 rpm) at 30oC for 5 days. After fermentation, the broth and cells were separated using separating funnel with methanol, acetone, ethyl acetate, hexane, petroleum ether, chloroform, acetonitrile, and N-butanol.
solvent at the level of 1:0 - 5:1 (v/v). The maximum antibiotic yield (22.64 mg / 100 ml broth) was observed in residue extracted by using ethyl acetate at the level of 1:1 (v/v).

Effect of carbon sources on production of metabolite was detected by fermentation media with selected *Streptomyces parvulus* SDMRI-3 at different carbon sources such as, sucrose, D-glucose, D-fructose, mannitol, maltose, soluble starch and cellulose. In the present study, the results obtained demonstrated that the optimal carbon source for antibiotic production was D-glucose with 0.843±0.0207 g of biomass / 100 ml of broth and 21.253±0.322 mg of extraction yield /100 ml of broth.

Effect of nitrogen sources on production of metabolite was detected by fermentation media with selected *Streptomyces parvulus* SDMRI-3 at different nitrogen sources (yeast extract, beef extract, peptone, casein, tryptone, sodium nitrate, potassium nitrate, ammonium sulphate, ammonium chloride and urea). The results obtained demonstrated that the optimal nitrogen source for antibiotic production was yeast extract with 0.819±0.006 g of biomass /100 ml and 21.440±0.278 mg of antibiotic / 100 ml.

The effect of pH (5-10) on antimicrobial activity was observed that the optimal pH range for the production of antibiotic was 8 with 0.812±0.010 g of biomass /100 ml and 22.217±0.227 mg of antibiotic / 100 ml.

The effect of temperature on metabolite was detected by fermentation media with selected *Sterptomyces parvulus* SDMRI-3 at different temperature (20, 25, 30, 35, 40 and 45°C). The results obtained demonstrated that the optimal temperature source for
antibiotic production was 30°C with 0.826±0.008 g of biomass / 100 ml and 20.830±0.719 mg of extraction yield / 100 ml of broth.

The effect of inoculums on metabolite was detected by fermentation media with selected *Sterptomyces parvulus* SDMRI-3 at different inoculums (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%). The results obtained demonstrated that the optimal inoculums source for antibiotic production was 2.5% with 0.795±0.008 g of biomass / 100 ml and 19.983±0.525 mg of extraction yield / 100 ml of broth.

The effect of NaCl on metabolite was detected by fermentation media with selected *Sterptomyces parvulus* SDMRI-3 at 10 different NaCl concentrations from 0.5 to 5%. The results revealed that the optimal NaCl concentration for antibiotic production was 2.5% with 0.871±0.016 g of biomass / 100 ml and 23.933±0.358 mg of extraction yield / 100 ml of broth.

The effect of incubation period on metabolite was detected by fermentation media with selected *Sterptomyces parvulus* SDMRI-3 at 10 different incubation periods from 24 to 240 hours. The results showed that the optimal incubation period for antibiotic production was 240 hours with 0.815±0.004 g of biomass in 100 ml and 216 hours with 20.543±0.415 mg of extraction yield / 100 ml of broth.

The effect of phosphate on metabolite was detected by fermentation media with selected *Sterptomyces parvulus* SDMRI-3 at 10 different phosphate concentrations from 0 to 1.0%. The results showed that the optimal phosphate concentration for antibiotic
production was 0.2% with 0.738±0.023 g of biomass in 100 ml and 0.3% with 20.397±0.320 mg of extraction yield / 100 ml of broth.

Plackett-Burman design was subjected to multiple linear regression analysis to estimate t-value, p-value and confidence level of each component by screening through relevant media for significant for antimicrobial compounds production. The results indicated that there was a variation in antimicrobial compound production in the 19 trials in the range from 133 to 220 µg/ml. These variations reflected the importance of medium optimization to obtain higher yield.

Among the 15 factors, 11 (glucose, NaNO₃, CaCO₃, K₂HPO₄.3H₂O, MnCl₂.4H₂O, inoculum level, incubation periods, sea water and pH) showed a positive sign (high level of coded variables), the other 4 factors like yeast extract, KCL, MgSO₄.7H₂O, ZnSO₄.7H₂O and FeSO₄.7H₂O, showed a negative sign (low level of coded variables) on antimicrobial production. Incubation periods, yeast extract, pH, K₂HPO₄.3H₂O and sea water were found to be highly significant for antimicrobial production. Determination coefficient ($R^2=0.9583$) is close to 1 for a good statistical simulation, which implies that the model can explain 95.83% variation in the experiment. This was explained by the model and revealed good agreement between the experimental results and the predicted values. The optimum level of these variables was further determined by following by response surface methodology.

By following the response surface methodology, Box-Behnken Design results indicated that there was a variation of lipase production in the thirty one trials in the range from 171.63 to 214.67 µg/ml. The smaller p-value indicates more significance in
the corresponding coefficient. It was observed that the coefficient for overall effect of the variables had high significance (p=0.000) on antimicrobial production. The individual and quadratic effect of incubation periods, yeast extract, pH, K$_2$HPO$_4$.3H$_2$O and sea water and interaction effect of yeast extract vs. pH and yeast extract vs. K$_2$HPO$_4$.3H$_2$O were found to be most significant factor on antimicrobial production. Determination coefficient ($R^2$=0.9713) is close to 1 for a good statistical simulation, which implies that the model can explain 97.13 % variation in the experiment. The response optimizer in MINITAB 15.0 software was used to optimum value of the variables for maximum antimicrobial production by marine isolate of *Streptomyces parvulus* SDMRI-3. The optimum value of the variables in actual unit was predicted as of incubation periods (8.38 days), yeast extract (1%), pH (8.15), K$_2$HPO$_4$.3H$_2$O (1.69%) and sea water (34.09%) with the predicted maximum antimicrobial production of 220.80 µg/ml of fermented media. A mean value of 221.52±0.2286 µg/ml of fermented media was acquired from real experiments.

Thin layer chromatography for evaluating the component composition of the antimicrobial substances produced by *Streptomyces parvulus* SDMRI-3 was executed. The plate was developed in Butanol: ethyl acetate (95:5). The spots on the plates were developed and observed under UV light at 254 nm and also observed by developing spot with iodine vapors and Rf values were 0.78. The chromatography technique was used for the isolation and purification of secondary metabolite. The purification process in the course of column chromatography packed with silica gel eluted with a mixture of butanol and ethyl acetate 95:5 (v/v). Thirty five fractions at 20 min interval collected by column chromatography technique were checked for their antimicrobial activity. The
purification of the compound was confirmed by TLC. The active fraction had an Rf value 0.78. The pure compound thus acquired was stored at 4°C. The pure compound was identified as actinomycin D using UV spectrum, FTIR, NMR and LCMS studies.

The minimum inhibitory concentration (MIC) of the purified compound was determined by micro-titre broth dilution method using two-fold dilution in nutrient broth at 28°C. *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most sensitive to antibiotic produced by SDMRI-3 with MIC value of 75 µg/ml of antibiotic.

The BLASTP analysis of target sequences of the DNA dependent RNA polymerase from *Bacillus cereus, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* against PDB database resulted the crystal structures as the most homologous sequences.

The docking interactions revealed that the actinomycin D is having strong interaction with rpoB of *Staphylococcus aureus*. The low interaction was observed in *Pseudomonas aeruginosa*. The docking score ranges from -7.1652 kJ/mol to -9.2351 kJ/mol. The docking interactions envisaged that the Actinomycin D is interacting with the means of two bonded interactions and four non-bonded interactions with rpoB of *Bacillus cereus, Escherichia coli, Enterobacter*, and *Klebsiella pneumoniae*, whereas, in the case of *Pseudomonas aeruginosa* it is observed that 5 amino acids were contributing the hydrophobic interactions and eight amino acids in the case of *Staphylococcus aureus*. In *Bacillus cereus*, it was observed that keto group of glutamine (gln1072) is favouring the H bond formation with the hydroxyl group and amino group of...
actinomycin D and the second H bond is favoured by means of keto group from Lysine (Lys 1098) with the amide group of actinomycin D. In *E. coli*, it is observed that keto group of Phenyl alanine (Phe224) is favouring the H bond formation with the hydroxyl group and amino group of actinomycin D and the second H bond is favoured by means of amino group from Glysine (Gly 344) with the keto group of actinomycin D, whereas in the case of *Enterobacter aerogenes* and *Klebsiella pneumoniae* the Glysine (Gly344 and Gly355) and Glutamic acid (Glu226 and Glu237) favours the H bonds respectively. In *Pseudomonas aeruginosa*, the keto and amino groups of Phenyl alanine (Phe228 and Phe351) favors the H bond formation with the hydroxyl and keto groups of Actinomycin D. The amino groups of Leucine (Leu887) and Histidine (His790) in *Staphylococcus aureus* favors the H bond formation with hydroxyl and keto groups of actinomycin D molecule.

The results of the present study showed that the sediments of Punnakayal mangrove ecosystem possess potential antibiotic producing bioactive *Actinomycetes*. A new strain of *Actinomycetes* was identified and named as *Streptomyces parvulus* SDMRI 3 and registered in gen bank. This isolate is a natural source of the therapeutic molecule, having highly active metabolite of actinomycin D and is beneficial in the production of novel pharmaceuticals for human beings.