ejpmr, 2017,4(10), 312-319

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY, ANTICANCER ACTIVITY, LC/MS ANALYSIS, AND SEQUENCE OF COCONUT HUSK RETTING SOIL ASSOCIATED ACTINOMYCETES IN MANDAIKADU, KANYAKUMARI DISTRICT

Sheena A. and P. A. Mary Helen*

Malankara Catholic College, Mariagiri.

***Corresponding Author: P. A. Mary Helen**

Malankara Catholic College, Mariagiri.

Article Received on 10/08/2017 **Article Revised on 30/08/2017** **Article Accepted on 20/09/2017**

ABSTRACT

Actinomycetes are a source of abroad variety of secondary metabolites with diverse biological activities, such as antifungal, antibiotic. Many of which have been developed for clinical use. Actinomycetes were isolated from the coconut husk retting soil sample by serial dilution agar plating method. The antimicrobial activity of actinomycetes extract was tested against pathogenic bacteria and fungi. The extract was tested against seven bacteria and five fungi. Then bioactive compounds were purified by silica gel column chromatography and it was identified by liquid chromatography-Mass spectrometry. Cytotoxic activity of actinomycetes extract on A375 cell line were analysed by MTT method. Three compounds such as 1-3-Di undecylprodiginine, Anthracene-9,10-dione, Isorenieratine were identified. The DNA of actinomycetes was isolated and the 16S rRNA gene was amplified using universal eubacteria 16S rRNA gene primers and it was phenotypically identified as *Streptomyces albaduncus.*

KEYWORDS: LC.MS, 16S rRNA, coconut husk retting soil.

INTRODUTION

Soil is comprised of minerals, soil organic matter, water and air. The composition and proportion of these components greatly influence soil physical properties like structure and porosity.[13] Microorganism are frequently present in soil, manure and decaying plant tissues which are able to degrade wastes that are correlated with the substrate organic matter.[8] Microorganisms produce some useful compounds that are beneficial to soil health, plant growth and play an important role in nutritional chains that are important part of the biological balance in the life in our planet. The microorganisms that are capable of biodegrading these toxic compounds may be absent at the contaminated site. If the necessary microorganisms are present, some limiting factor, such as a nutrient shortage, may create unfavourable conditions for the biodegradation of the contaminant. The second possibility is that the compound could be recalcitrant or resistant to biodegradation.[11]

The actinomycetes produce an enormous variety of bioactive molecules, e.g., antimicrobial compounds.[9] Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil. Actinomycetes are diverse group of Gram positive bacteria that usually grow by filament formation. They are free living, saprophytic bacteria and a major source for the production of antibiotics, widely distributed in natural and manmade environments and play an important role in the degradation of organic matter. The most important genus in Actinobacteria is *Streptomyces*; five hundred and thirty three species of *Streptomyces species* were described in Bergy s Manual of Systematic bacteriology.[7] The important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes.[10] The natural products (NPs) are one of the most prolific sources of therapeutics and because of their enormous structural diversity, they can serve as leads in finding novel drugs to be introduced in cancer therapy.[2]

In this context, a study was conducted on the isolation, characterization, identification of actinomycetes isolated from the coconut husk retting soil sample collected from Mandaikadu. The present study examined the antimicrobial activity against bacteria and fungi. This work also involves the purification and identification of antibiotic compound by silica gel column chromatography and LCMS analysis. The eluted fractions were tested for cytotoxic activity using cancer

cell lines. DNA was isolated from actinomycetes and 16S rRNA gene sequencing was carried out.

MATERIALS AND METHODS

Sample collection: Actinomycetes was isolated from coconut husk retting soil sample collected from Mandaikadu, Kanyakumari district, Tamil Nadu, South India by serial dilution agar plating method. The coconut husk retting soil samples were serially diluted up to 10^{-9} dilutions, in distilled water and 1 ml sample from 10^{-3} to 10^{-5} were pour plated in Actinomycetes isolation agar plates. The plates were kept for incubation at 25 to 30^0C for 48 hours in an inverted position. The colonies formed were enumerated using Quebec colony counter. Then the actinomycetes isolates were purified by pure culture techniques and refrigerated in agar slants for further studies.[4]

Antibiotic sensitivity with pathogens

The actinomycetes isolates were tested against pathogens and their antibiotic sensitivity were determined by Disc Diffusion method. The bacteria lawn of each organism was prepared on the nutrient agar plates. One drop of actinomycetes culture was added to sterile filter paper disc (size:5mm) and allow to dry after each addition. The disc were then placed on air dried surface of the medium. The plates were incubated at 37^0C for 24hours. After incubation the diameter of inhibition zones around the discs was measured.

Pathogenic bacteria (Gram – positive and Gram-negative) and fungus were obtained from Microbial Type Culture Collection, (MTCC) Institute of Microbial Technology, Chandigarh. The bacterial strains used Vibrio cholarae(3906), Entero bacteria aerogenes(10102), Escherichia coli (40), Klebsiella pneumonia (4030), Shigellaflexneri(1457), Streptococcus epidermic(6810), Bacillus megaterium (453). The fungal strains used were Aspergillus niger (545), Aspergillus fumigates(4333), Candida albicans(183), Candida fumigate (8332) and Pencillium (7584). The bacterial and fungal strain cultures were maintained in nutrient and fungal broth a turbid growth and kept in refrigerator at 4^0Cfor further analysis. The observed structure was compared with Bergey's Manual of Determinative Bacteriology[1] and the organism was identified.

Purification of Antibiotics

The purification of antimicrobial substance was carried out using silica gel (2.5×50) chromatography. Chloroform and Methanol 95:5 (v/v)mixtures were used as an eluent. The column was left overnight until the silica gel (pro labo) was completely settled. 1 ml crude extract to be fractionated was added on the silica gel column surface and the extract was absorbed on top of silica gel. Read the absorbance at 272 nm. 10 eluents were collected (each of 5 ml) and tested for their anti-microbial activity.[6]

Antiproliferative Assay by MTT Method in A375 Cell Lines

A375 cell line from Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilisation Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the Formosan crystals. The absorbance values were measured by using micro plate reader at a wavelength of 540 nm.

LC MS Analysis

Mass spectrometer system equipped with a capillary column HP5-MS. (Thermo Fisher Scientific, San Jose, California, USA). Ion source polarity: Positive ion mode sheath gas pressure (N2):40 units Ion transfer tube temperature: 350^0C collision gas pressure (Ar): 1.0 mTorrq1 resolution:0.2 FWHM Q3 resolution: 0.7 FWHM D well time: 0.2 sScan type: SRM solvent: Methanol (Column purified – sephadex G24.5) sample: Animal Ext-Microbial origin LC MS conditions for the analytes.

DNA Preparation

The genomic DNA isolation from actinomycetes was described by the method. CTAB method was the most commonly used protocol for the preparation of actinomycetes genomic DNA.[5]

Randomly amplified polymorphic DNA (RAPD)

The Polymerase chain reactions (PCR) were carried out in a 200 ml thin walled PCR tubes. The PCR for RAPD were carried out in a 25 ml of reaction mixture as described by Abbasi et al. 1993. RAPD primers and PCR related chemicals were obtained from Bangalore genei, India. Amplification reactions were carried out in a total volume of 25 ml containing, 10_ PCR buffer with 1.5 mM MgCl2 and 10 mMTris, 2.25 U Taq polymerase, 0.20 mM each dNTPs, 1 mM primer and 50 ng template DNA. Amplification was carried out on Bio-Rad Thermal-Mycycler with the following programme as described by Shalini et al.[17] The samples were initially heated to 94 _C for 5 min and then subjected to 45 cycles of denaturation at 94 _C for 1 min, annealing at 37 _C for 1 min and 30 s and extension at 72 _C for 2 min followed by a final extension for 10 min. After the process, samples of 15 ml of the amplification products were assayed by electrophoresis in 1.5% agarose gel containing ethidium bromide, running with TBE buffer. The electrophoresis was carried out at 80 V (constant) for about 100 min using Bio-Rad submerge gel electrophoresis system. The gel was viewed under UV trans-illuminator for visualizing separated bands and photographed. The low range ruler (100 bp to 3 kb) was

used as molecular weight size markers. Amplifications were repeated once more for each RAPD primer and only consistent bands were considered for scoring. The NTSYS. PC (Numerical Taxonomy System Applied Biostatistics, Setauket, New York) system version 2.2 by Exeter Software was used for data analysis.[18] The data (band presence or absence) were introduced in the form of a binary matrix and a pair wise similarity matrix was constructed using the Jaccard's coefficient.

RESULTS AND DISCUSSION

Isolation of Actinomycetes

Actinomycetes were isolated from coconut husk retting soil sample collected from Mandaikadu, Kanyakumari district, Tamil Nadu, South India by serial dilution agar plating method. Three actinomycetes were selected for further studies.

Antibiotic sensitivity with Bacterial pathogens

The bioactive compound producing actinomycetes A3 was selected and tested its antibiotic sensitivity with bacterial pathogens. The largest inhibition zone was produced by actinomycetes strain A3 against *streptoccouss epidermice*. The inhibition zone diameter was 3.4 cm. The smallest zone was also produced by *Vibrio cholerae.* The inhibition zone diameter was 0.8 cm. The values were listed in the table no-1.

Antibiotic sensitivity with Fungi pathogens

The bioactive compound producing actinomycetes A3 was selected and tested its antibiotic sentivity with fungal pathogen. The largest inhibition one was produced by actinomycetes strain A3 against *pencillium sps.* The inhibition zone diameter was 3cm. The smallest inhibition produced by *Asperigillus fumigates* zone diameter was 2 cm. The values were listed in the table no – 2.

Table -1: Antibiotic sensitivity with Bacterial pathogens.

S.No	Bacterial Pathogens	Zone of diameter (cm)
1	*Vibrio cholerae* (3906)	0.8
2	*Bacillus megaterium* (453)	2.5
3	*Klebsilla pneumonia* (4030)	3.0
4	*Shigella flexneri* (1457)	2.5
5	*Enterobacteria aerogenes* (10102)	2.0
6	*Eschericha coli* (40)	2.9
7	*streptoccouss epidermice* (6810)	3.4

Table -2: Antibiotic sensitivity with Fungi pathogens.

S.NO	Fungal Pathogens	Zone of diameter (cm)
1	*Asperigillus fumigates* (4333)	2.0
2	*Candida fumigates* (8332)	2.0
3	*pencillium sps* (7584)	3.0
4	*Candida albicans* (183)	2.5
5	*Asperigillus niger* (545)	0

Purification of bioactive compounds by silica gel column chromatography

The bioactive compound were purified by silica gel column chromatography. Chlorofrom and Methanol 95:5 (v/v) mixtures were used as an eluting solvent. Crude extract of A3 was used for the purification of bioactive compound. Five eluents were collected from A3. The collected fractions were kept at 4^0C. The optical Density by eluent eight showed that the activity was increased that is 0.430. Decreased enzyme activity represented in the activity was showed by eluent two (0.147).

Antibacterial activity of eluent fractions of Actinomycetes

Seven elutions of Actinomycetes was used for the antibacterial activity against bacterial pathogens. The zone of inhibition of each elution was tabulated in **Table-3.**

Antifungal activity of eluent fractions of Actinomycetes

The anti fungal activity of eluent fractions of Actinomycetes was carried out in five fungal pathogens. **Table-4.**

Table-3 Antibacterial activity of eluent fractions of Actinomycetes.

S.No	Bacterial Isolates	Eluents Of Selected Actinomycetes – Zone Of the Diameter (cm)				
		I	II	III	IV	V
1	B1	1.5	1	1.3	1	0
2	B2	1.1	0	0	2	0
3	B3	1.2	0	0	0.9	0
4	B4	0	0.9	1.0	1.2	0
5	B5	0.9	0	0.9	0.8	0
6	B6	1.4	0	1	0	0
7	B7	0	1.2	1.3	0.6	0.8

Table-4 Antifungal activity of eluent fractions of Actinomycetes.

S.No	Fungal Isolates	Eluents Of Selected Actinomycetes – Zone Of the Diameter (cm)				
		I	II	III	IV	V
1	F1	0.9	0.7	1.2	0.8	0
2	F2	0.8	1.1	1.2	1.2	0.8
3	F3	0	0	0	0	0
4	F4	0	0	0	0	0
5	F5	0	0	0	0	0

Antiproliferative Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 3 0µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico *et al.*, 2004). The values were listed in the **Table no: 5** and the results were showed in the **plate no: 1.**

Table No: 5 Antiproliferative Assay by MTT Method.

Sample volume (µl)	Average OD at 540nm	Percentage Viability
Control	0.4016	-
6.25	0.22905	57.03436
12.5	0.194	48.30677
25	0.1824	45.41833
50	0.16685	41.54631
100	0.14955	37.23855

LD 50 value – 11.2874µl

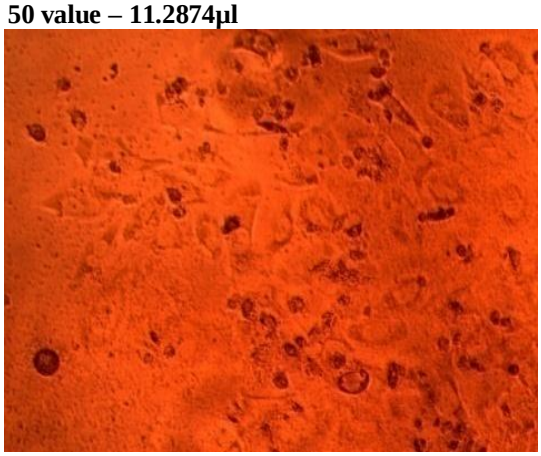


Plate No: 1 Antiproliferative Assay by MTT Method.

LC –MS Analysis

LC –MS Analysis indicated that the Actinomyceetes extract showed totally persnendage of peaks. Peaks were identified. They are 1-3 Di Undecylprodiginine, Anthracene-9, 10-dione, Isorenieratin. The values were listed in the table no: 6 and the results were showed in the plate no: 2.

Table No: 6 compounds present In the Peaks.

No.	Name of the Analyte(s)	% of Peak area	Mole. Formula	Mol. Wt	Peak Area	Retention Time
1.	1-3-Di undecylprodiginine	48.76	$C_{25}H_{35}N_3O$	393.575	2305671	20.14
2.	Anthracene-9, 10-dione	41.68	$C_{14}H_7O_2$	208.216	2257312	53.46
3.	Isorenieratine	48.37	$C_{40}H_{48}$	528.824	2536021	25.93

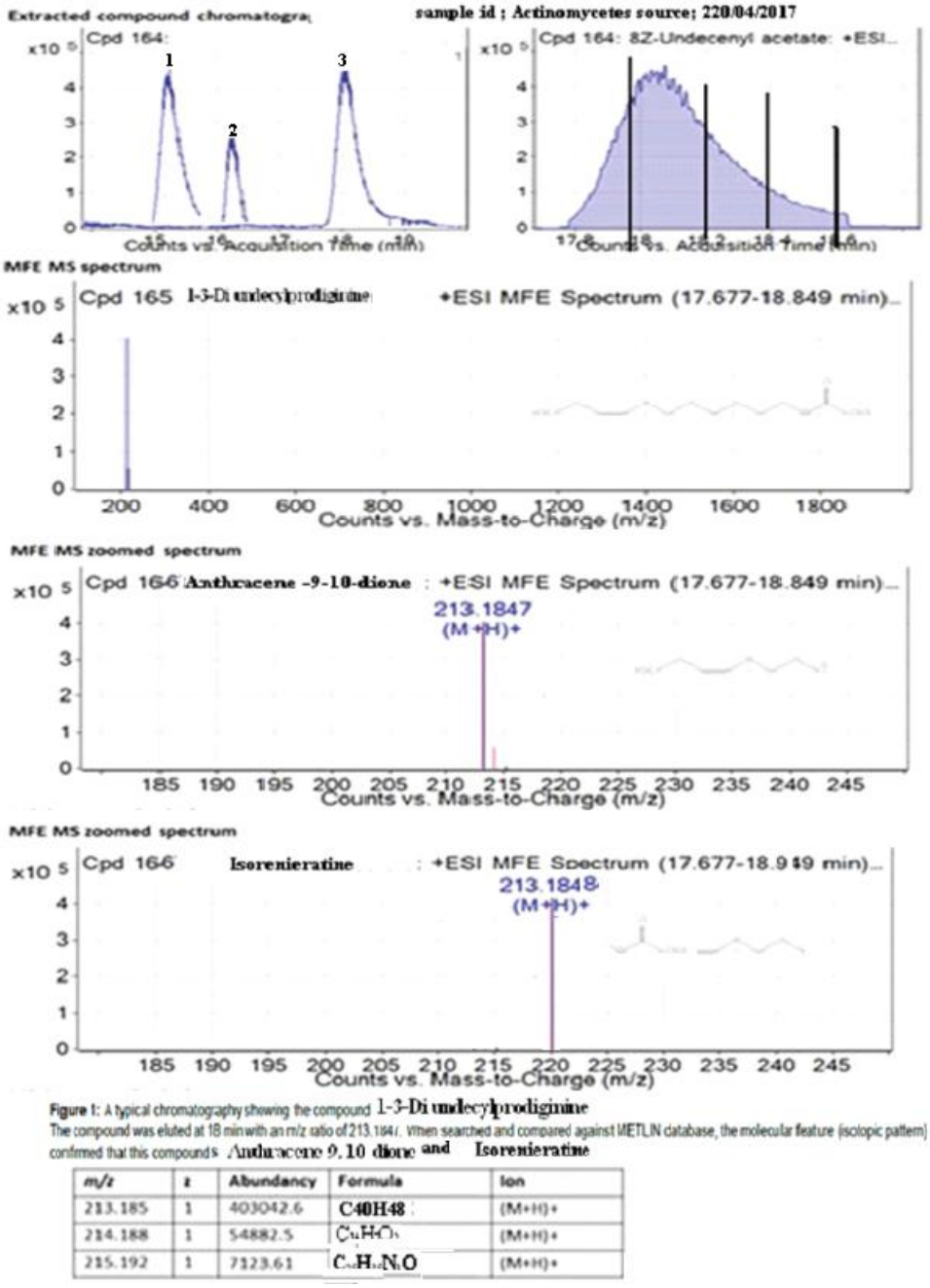


Figure 1: A typical chromatography showing the compound 1-3-Di undecylprodiginine
The compound was eluted at 18 min with an m/z ratio of 213.1847. When searched and compared against METLIN database, the molecular feature (isotopic pattern) confirmed that this compound is Anthracene-9, 10-dione and Isorenieratine

m/z	z	Abundancy	Formula	Ion
213.185	1	403042.6	C40H48	(M+H)+
214.188	1	54882.5	$C_{14}H_8O_2$	(M+H)+
215.192	1	7123.61	$C_{25}H_{34}N_3O$	(M+H)+

Plate No: 2 Chromatogram For Selected Actinomycetes.

Amplification of 16S rRNA Sequencing

The actinomycetes DNA was isolate and amplified used **16S rRNA**gene and it was phenotypically identified as *Streptomyces albaduncus* of the size 0.6kbp. The amplified product was visualized in ethidium bromide stained gel. The result was displayed in plate no-30 followed by the seguence of the identified actinomycetes.

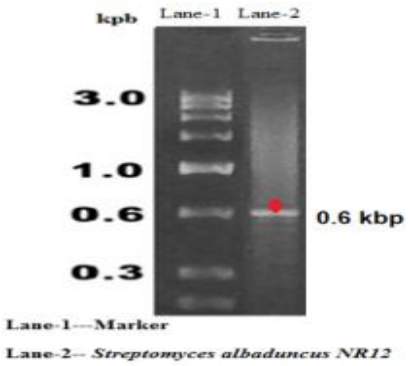


Plate No: 4 16SrRNA Gene Amplification.

Species name: *Streptomyces albaduncus*
*Streptomyces albaduncus*16SrRNA gene, partial sequence
Size: 0.6 kbp.

Forward Cycle

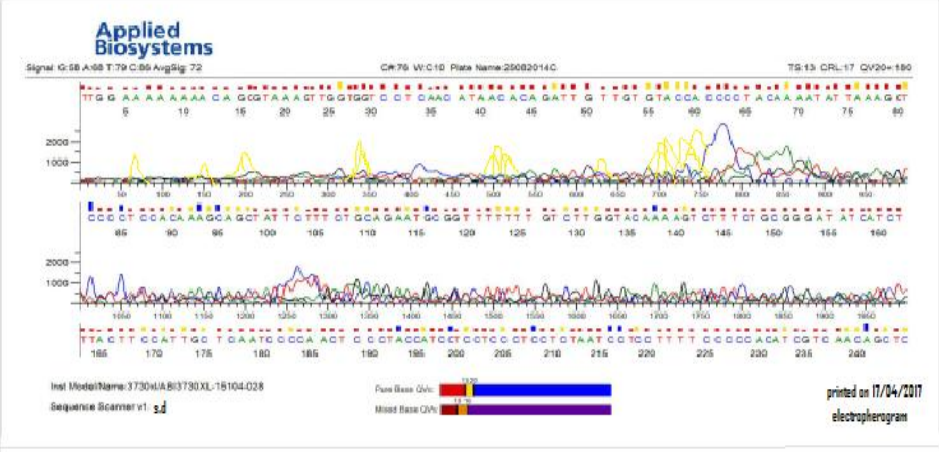
AGTCGAACGATGAACCACTTCGGTGGGGATTAGTG
GCGAACGGGTGAGTAACACGTGGGCAATCTGCCCT
GCACTCTGGGACAAGCCCTGGAAACGGGGTCTAAT
ACCGGATACTGATCATCTTGGGCATCCTTGGTGAT
CGAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGC
CTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAG
GCGACGACGGGTAGCCGGCCTGAGAGGGCGACCG
GCCACACTGGGACTGAGACACGGCCCAGACTCCTA
CGGGAGGCAGCAGTGGGGAATATTGCACAATGGG
CGAAAGCCTGATGCAGCGACGCCGCGTGAGGGAT
GACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGG
AAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGC
GCCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGT
AAAGAGCTCGTAGGCGGCTTGTCGCGTCGGTTGTG
AAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGAT
ACGGGCAGGCTAGAGTTCGGTAGGGGAGATCGGA
ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAG
GAGGAACACCGGTGGCGAAGGCGGATCTCTGGGC
CGATACTGACGCTGAGGAGCGAAAGCGTGGGGAG
CGAACAGGATTAGATACCCTGGTAGTCCACGCCGT
AAACGGTGGGCACTAGGTGTGGGCGACATTCCACG
TCGTCCGTGCCGCAGCTAACGCATTAAGTGCCCCG
CCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAA
GGAATTGACGGGGGCCCGCACAAGCGGCGGAGCA
TGTGGCTTAATTCGACGCAACGCGAAGAACCTTAC
CAAGGCTTGACATACACCGGAAAGCATTAGAGATA
GTGCCCCCCTTGTGGTCGGTGTACAGGTGGTGCAT
GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTT
GCCAGCAAGCTCCTTCGGGGGTGTTGGGGACTCAC
GGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGG
GGACGACGTCAAGTCATCATGCCCCTTATGTCTTG
GGCTGCACACGTGCTACAATGGCCGGTACAATGAG
CTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAG
CCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGAC
CCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCA
GCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCACGTCACGAAAGTCGGTAACAC
CCGAAGCCGGTGGCCCAACCCCTTGTGGGAGGGAG
CTGTCGAAGGTGGGACTGGCGATTGG

Reverse Cycle

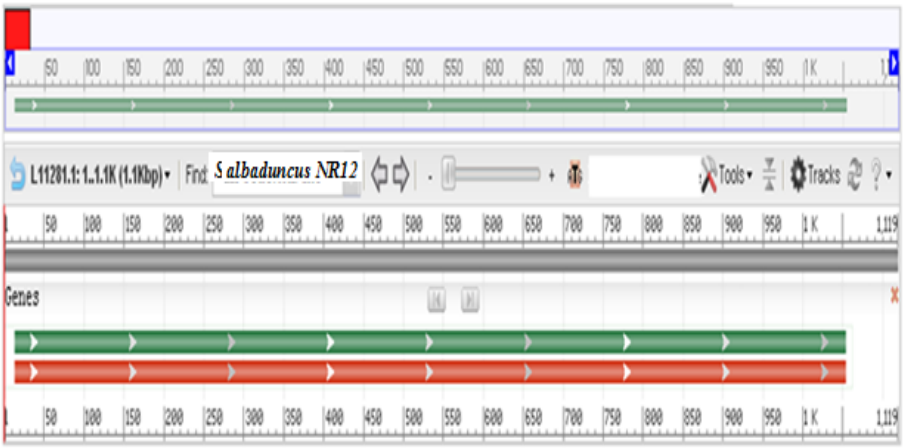
GAACGCTGGCGGCGTGCTTAACACATGCAAGTCGA
ACGATGAACCACTTCGGTGGGGATTAGTGGCGAAC
GGGTGAGTAACACGTGGGCAATCTGCCCTGCACTC
TGGGACAAGCCCTGGAAACGGGGTCTAATACCGG
ATACTGATCATCTTGGGCATCCTTGGTGATCGAAA
GCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATCA
GCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACG
ACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCGAAAG
CCTGATGCAGCGACGCCGCGTGAGGGATGACGGCC
TTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGC
GAAAGTGACGGTACCTGCAGAAGAAGCGCCGGCT
AACTACGTGCCAGCAGCCGCGGTAATACGTAGGGC
GCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGC
TCGTAGGCGGCTTGTCGCGTCGGTTGTGAAAGCCC
GGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCA
GGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGG
TGTAGCGGTGAAATGCGCAGATATCAGGAGGAAC
ACCGGTGGCGAAGGCGGATCTCTGGGCCGATACTG
ACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGGTG
GGCACTAGGTGTGGGCGACATTCCACGTCGTCCGT
GCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGA
GTACGGCCGCAAGGCTAAAACTCAAAGGAATTGA
CGGGGGCCCGCACAAGCGGCGGAGCATGTGGCTT
AATTCGACGCAACGCGAAGAACCTTACCAAGGCTT
GACATACACCGGAAAGCATTAGAGATAGTGCCCCC
CTTGTGGTCGGTGTACAGGTGGTGCATGGCTGTCG
TCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTTGTCCCGTGTTGCCAGCAA
GCTCCTTCGGGGGTGTTGGGGACTCACGGGAGACC
GCCGGGGTCAACTCGGAGGAAGGTGGGGACGACG
TCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACA
CGTGCTACAATGGCCGGTACAATGAGCTGCGATAC
CGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCA
GTTCGGATTGGGGTCTGCAACTCGACCCCATGAAG
TCGGAGTCGCTAGTAATCGCAGATCAGCATTGCTG
CGGTGAATACGTTCCCGGGCCTTGTACACACCGCC
CGTCACGTCACGAAAGTCGGTAACACCCGAAGCCG
GTGGCCCAACCCCTTGTGGGAGGGAGCTGTCGAAG
GTGGGACTGGCGATTGGGACGGAACGCGGCCGAT
ACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAA
CAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GGTGGGCACTAGGTGTGGGCGACATTCCACGTCGT
CCGTGCCGCAGCTAACGCATTAAGTGCCCCGCCTG
GGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAA
TTGACGGGGGCCCGCACAAGCGGCGGAGCATGTG
GCTTAATTCGACGCAACGCGAAGAACCTTACCAAG
GCTTGACATACACCGGAAAGCATTAGAGATAGTGC
CCCCCTTGTGGTCGGTGTACAGGTGGTGCATGGCT
GTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTTGTCCCGTGTTGCCA
GCAAGCTCCTTCGGGGGTGTTGGGGACTCACGGGA
GACCGCCGGGGTCAACTCGGAGGAAGGTGGGGAC
GACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTG
CACACGTGCTACAATGGCCGGTACAATGAGCTGCG
ATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGT
CTCAGTTCGGATTGGGGTCTGCAACTCGACCCCAT
GAAGTCGGAGTCGCTAGTAATCGCAGATCAGCATT
GCTGCGGTGAATACGTTCCCGGGCCTTGTACACAC
CGCCCGTCACGTCACGAAAGTCGGTAACACCCGAA

GCCGGTGGCCCAACCCCTTGTGGGAGGGAGCTGTC
GAAGGTGGGACTGGCGATTGGGACG

The Amplification of 16S rRNA Sequencing conformed by chromatogram



Graphical View of the Identified Organisms



CONCLUSION

From this study, it can be concluded that the actinomycetes *steptomyces albaduncus* showed the bioactivity compound were characterized from the actinomycetes and the extracts showed antimicrobial, Antiproliferative on A-375 cell line. The DNA of actinomycetes was amplified using universal eubacterial 16S rRNA gene primers and it was phenotypically identified as *steptomyces albaduncus.*Data from the literature as well as our results reveal the great potential of actinomycetes extracts showed various antimicrobial, cytotoxic activities and have not been completely investigated. Additional studies would be needed further to evaluae the potential of this extracts for the isolation of novel actibiotic.

ACKNOWLEDGEMENT

We are grateful to staff members of Biotechnology Department, Malankara catholic college for their encouragement throughout this work. We are also thankful to Fr. Jose Bright, correspondent and secretary and Dr. Thampi Thanka Kumaran, Principal, Malankara Catholic College, Mariagriri for constant encouragement and support. We are also grateful to Jayagenic biological, chennai, Biogenix Research center, Thiruvananthapuram, Biogenova scientific Innovation and sequence Analysis Bangaluru.

REFERENCES

1. Bergey, D.H., Harrison, F.C., Breed, R.S., Hammer, B.W., and Huntoon, F.C.(1994). Bergey's manual of determi edition. The native bacteriology, 9th Williams and Wilkins Co- Publishers, Baltimore.
2. Germana Esposito, Roberta Teta, Roberta Miceli, Luca S. Ceccarelli, Gerardo Della Sala, Rosa Camerlingo, Elena Irollo, Alfonso Mangoni, Giuseppe Pirozzi and Valeria Costantino. 2015. Isolation and Assessment of the in Vitro Anti-Tumor Activity of Smenothiazole A and B, Chlorinated Thiazole-Containing Peptide/Polyketides from the Caribbean Sponge, *Smenospongiaaurea.* Mar. Drugs 2015; 13: 444-459; doi: 10.3390/md13010444 marine drugs ISSN 1660-3397.

3. Jayasankar N.P., (1985) An appraisal on the biological extraction of coir, in proc workshop Res. Held on 2-7 February (Cochin, Kerala).
4. Kannan, N.1996. Laboratory manual in General Microbiology 1st Edtion paramount Publication, Palani, Tamilnadu.
5. Kutchma, Small –scale isolation of genomic DNA. 1998.
6. Laurence, M., Harwood, Christopher, j., and moody. (2008). Expermental organic chemistry: Principles and practice., M. Priya, B. Anandaraj and T. Ganesan. Antimicrobial Activity of Estuarine Actinobacteria against Selected Human Pathogens. ISSN: 2319-7706 Volume 4 Number 9 (2015) pp. 120-125. International journal of current microbiology and applied sciences. Int. J. Curr. Microbiol. App. Sci., 2015; 4(9): 120-125.
7. Nakuleshwar Dut Jasuja, Richa Saxena, Subhash Chandra and Suresh C. Joshi. Isolation and identification of microorganism from polyhouse agriculture soil of Rajasthan. 11 October, 2013; 7(41): 4886-4891, DOI: 10.5897/AJMR2012.2413.
8. NanjwadeBasavaraj K, S Chandrashekhara, Ali M Shamarez, PrakashS Goudanavar and Fakirappa V Manvi. Isolation and Morphological Characterization of Antibiotic Producing Actinomycetes. Tropical Journal of Pharmaceutical Research, June 2010; 9(3): 231-236 © Pharmacotherapy Group.
9. Pandey B, Ghimire P and Agrawal V.P., Internation conference on the Great Himalayas: Climate, Health, Ecology, Management and Conservation, Kathmandu, Organized by Kathmandu university and the Aquatic Ecosystem Health and Management Society, Canada: 2004.
10. Rohilla S.K.and Salar R.K., 2012; 2277-2502.
11. S. Silambarasan, E.praveen kumar, T. Murugan, D. Saravanan and R. Balagurunathan 2012; 2: 099-1Garvalov, B.K.; Acker, T. Cancer stem cells: A new framework for the design of tumor therapies. J. Mol. Med. (Berl.) 2011; 89: 95–10703.
12. Solomon Oluwole Oladeji and Kehinde Abraham Odelade 2016; pp 201-208, 180-185.