# Structural elucidation and antimicrobial activity of secondary metabolites from Streptomyces *albus.* CN-4

**Bijjam Chinna Narasaiah**[1]
G.N.V.Febulus
Department of Botany S.V Degree College,
Giddaluru. India.

## ABSTRACT:

Laterite soil sample collected from Kandukuru, Prakasam (Dist) Andhra pradesh, India. A depth of 5 – 8 cm was pretreated with calcium carbonate (1:1 w/w) and dried at $45^{O}$C for 1 h in order to reduce the aboundence of bacteria and fungi. Both streptomycin (50µg/ml) and nystatin (50 µg/ml) were added to the media just before pouring into Petri plate. Soil dilution plate technique was employed for isolation of actinomycete strains. Out of 10 actinomycete strains isolated from two different media, one predominant strain designated as CN-4 was chosen for bioactive metabolite production. The secondary metabolites produced by actinomycete strain were extracted. The antimicrobial activity of the strain was determined by agar well diffusion method. As the strain CN-4 exhibited high antimicrobial activity against bacteria and fungi tested.

**Keywords:** Actinomycetes, Antimicrobial, Bioactive compounds, Antibiotics, Bacteria,

**Introduction:** Microorganisms from environments have gained considerable attention in recent years. This is mainly due to the fact that they hold about the molecular evolution of life. Natural products are the leading sources of novel biomolecules that have been used in the pharmaceutical industries since their inception. The majority of the natural products currently used in the market as therapeutic agents or as health supplements are derived from the terrestrial organisms including plants, animals and microorganisms. Microbial secondary metabolites are the important sources of natural compounds with potential bioactivities. In general, natural products including the microbial metabolites may be practically utilized in different ways including in the fields of medicine and agriculture industries, material for subsequent chemical or microbiological modification (derivatization), lead compounds in the synthesis of new analogs or as templates in Rational Drug Design studies. Soil is the perfect laboratory for the creation of natural medicines. It holds a wide array of tiny microhabitats that creates a vast variation in the appearance and survival strategies of soil microbes. The actinomycetes which represent a transitional form between the bacteria and fungi compose a large proportion of the soil flora. They are Gram positive bacteria, filamentous and sporulating with DNA rich in G+C ranging from 55-75% [1]. They are generally considered as terrigenous bacteria due to their wide distribution and abundance in soil. The name actinomycetes derived from Greek aktis (a ray) and mykes (fungus) was given to these organisms from initial observation of their morphology. These members are grouped into the class Actinobacteria belonging to the division of Fermicutes of the domain Bacteria. The class Actinobacteria is categorized into five subclasses namely Actinobacteridae, cidimicrobidae, Coriobacteridae, Sphaerobacteridae and Rubrobacteridae. Except Actinobacteridae, the other four subclasses contain only one order subsequently delineated into one family each. On

the other hand, subclass Actinobacteridae consists of two orders, the Actinomycetales and Bifidobacteriales. The order Actinomycetales has ten suborders, each sub order again categorized into a total of about 38 families.The order Bifidobacteriales constitute only one family namely Bifidobacteriaceae. Classification of actinomycetes proposed [2]. By the end of 2002 about 22500 bioactive secondary metabolites (including antibiotics) were published in the scientific and patent literature and actinomycetes constitute about 45% of the microbial products [3]. Actinomycetes have been especially useful to the pharmaceutical industry for their unlimited capacity to produce secondary metabolites with diverse chemical structures and biological activities. The practical importance of antibiotics and other secondary metabolites is tremendous. They are broadly used in the human therapy, veterinary, agriculture, scientific research and in countless other areas. Remarkably, the vast majority of these compounds are derived from the single actinomycete genus *Streptomyces*, raising the intriguing possibility that additional chemically prolific taxa await their discovery. Discoveries of important, novel bioactive compounds depend upon the development of objective strategies for the isolation and characterization of novel and rare micro-organisms for existing and new screens [4]. Although soils have been screened by the pharmaceutical industry for about 50 years, only a miniscule fraction of the surface of the globe has been sampled, and only a small fraction of actinomycete taxa have been discovered[5,6]. A very frightening consequence of indiscriminate use, over prescription of antibiotics is the development of antibiotic resistant bacteria. According to the World Health Organization, the emergence rate of drug resistant strains is quicker than the rate of discovery of new drugs and antibiotics. The multiple drug resistance problem demands that a renewed effort be made to seek antimicrobial agents effective against these MDROs. Polyenes like amphoterecin B, nystatin and natamycin are widely used for the treatment of candidiasis, coccidiodal meningitis, cutaneous dermatophytes and histoplasmosis. Wide spread use of the limited numbers of antifungal agents for control of mycotic diseases also led to the development of drug resistance. The life saving drugs used for treating the bacterial, fungal and candidal infections at present mostly are the products of terrestrial actinomycetes. By this it is apparent that continuous research on terrestrial actinomycetes may result in the isolation of novel actinobacterial species as well as bioactive metabolites active against MDROs. Proving their excellence in the field of medicine, actinomycetes extend their contribution towards agriculture. Fungal diseases in agriculture crops pose a great challenge. Fungicides are extensively used to ensure crop quality and production. The currently used fungicides have lethal effects that are not restricted to their target species and their application may have negative impact on organisms that benefit the wider agroeco system. Hence, it is necessary to develop environmentally safer and potent fungicides of natural origin. Microbial metabolites have been expected to minimize the deleterious side effects caused by synthetic fungicides. These metabolites are degraded within a month or even in a few days when exposed to natural environmental conditions, thus leading to low residual levels. The biological control of fungal pathogens causing plan diseases using extracellular metabolites of *Streptomyces* was previously reported [7-11]. Numerous environmental factors including nutrients (nitrogen, phosphorous and carbon sources), growth rate, enzyme inactivation and other physical conditions (oxygen supply, temperature and pH) are require to biosynthesis of the secondary metabolites [12]. An increasing demand for low-input agriculture has also resulted in a greater attention towards soil microorganisms that are able to enhance plant nutrition and growth [13]. Actinomycetes have the ability to produce secondary metabolites with biological activities such as antibiotic, antifungal, antiviral, anticancer, enzyme, immunosuppressant and other industrially useful

compounds [14]. Among the soil microbes, actinomycetes are known to promote activities which can improve agricultural developments thus appear as research targets with regard to sustainability purposes. *Streptomyces* spp. found in the rhizosphere of plants can enhance plant growth by producing plant growth promoter substances like auxins or gibberellins [15,16].

## Meterials and Methodos

### Isolation and screening of actinomycete strains from laterite soil sample

Laterite soil sample collected for the isolation of potent actinomycete strains was initially analysed for physico-chemical properties such as moisture content (%), pH, organic carbon (%) and total nitrogen content (%).

### Isolation of actinomycete strains

Laterite soil sample collected from Kandukuru, Prakasam (Dist) a depth of 5 – 8 cm was pretreated with calcium carbonate (1:1 w/w) and dried at $45^{O}$C for 1 h in order to reduce the aboundence of bacteria and fungi [17] Yeast extract malt extract dextrose agar (YMD) and starch casein salts agar media were prepared, sterilized at 15 lbs pressure (120°C) for 15 min and poured into Petri plates under aseptic conditions. Both streptomycin (50 µg/ml) and nystatin (50 µg/ml) were added to the media just before pouring into Petri plate. Soil dilution plate technique was employed for isolation of actinomycete strains [18]. The pretreated soil (1 g) sample was suspended in 100 ml of sterile distilled water. Serial dilutions were prepared and 0.1ml of $10^{-3}$ and $10^{-4}$ dilutions were plated on media with the help of a spreader. The inoculated plates were incubated at 30°C for 7-14 days. After incubation, actinomycete colonies were isolated from soil. Streak plate method was used to purify the cultures of actinomycete strains. The colonies were picked with the loop according to the condition. The picked up specks of the colonies were streaked over YMD agar medium followed by incubation at $30^{O}$C for 7 days. Further, pure cultures were maintained on YMD agar slants and stored at $4^{O}$C for further study [18].

### Screening of predominant actinomycete strain for bioactive metabolites

Out of 10 actinomycete strains isolated from two different media, one predominant strain designated as CN-4 was chosen for bioactive metabolite production. The secondary metabolites produced by actinomycete strain were extracted by the method of [19]. The pure culture of the strain was transferred aseptically into the seed medium (ISP-2 broth). After 24 h of incubation, the seed culture at a rate of 10% was inoculated into the production medium of ISP-2 broth. Fermentation was carried out at 30°C for 1 week under agitation at 180 rpm. At every 24 h interval, the flasks were harvested and the biomass was separated from the broth. The dry weight of the biomass was recorded and expressed as g/100ml. The culture filtrate was extracted twice with ethyl acetate and the pooled solvent extracts were concentrated under vacuum to yield a crude extract. The residue dissolved in methanol was used for testing antimicrobial activity.

### Antimicrobial assay

Bacteria employed for testing include *Bacillus megaterium,B. subtilis ATCC 6633 Escherichia coli, ATCC 35218,Pseudomonas aeruginosa, Staphylococcus aureus* and *Xanthomonas campestris* while *Alternaria* sp., *Asperigillus niger, Botrytis cinerea, Candida albicans ATCC 10231, Fusarium solani,* and *F. oxysporum* were used as test fungi. The antimicrobial activity of metabolites produced by the strain was determined by agar well diffusion method [20]. Nutrient agar (NA) and Czapek-Dox (CD) agar media were used for culturing the test bacteria and fungi respectively. NA medium (100 ml) was sterilized at 15 lbs pressure (121°C) for 15 min, cooled and inoculated with 0.1 ml of test bacterial suspension. After through mixing, the inoculated medium was poured into Petri plates under aseptic conditions. After solidification of agar medium, wells of about 6 mm diameter were punched with sterilized cork borer. In case of antifungal assay, test fungus ($10^5$ spores/ml) was plated onto the solidified CD agar plate. Ethyl acetate extract (50 ppm) was added to each well while the addition of only Ethyl acetate served as control. The inoculated plates were incubated at 30°C and the diameter of the inhibition zone was measured after 24 h of incubation for bacteria and 24-72 h for yeast and filamentous fungi. As the strain CN-4 exhibited high antimicrobial activity against bacteria and fungi tested, attempts were made for further study.

### Isolation and identification of bioactive compounds

The bioactive compounds from the fermented broth was harvested by filtration of biomass through Whatman filter paper no. 42 (Merck, Mumbai, India). The culture filtrate (30L) was extracted twice with an equal volume of ethyl acetate, pooled and the organic layer was concentrated in a Rotavac. The deep brown semi solid compound was extracted for structural elucidation.

## Results and Discussion

### Isolation of actinomycete strains

For the isolation of actinomycete strains, the soil sample amended with calcium carbonate was air - dried at $45^{O}$C for 1 h. Serial dilution plate technique was employed for the isolation of actinomycetes. Serially diluted soil sample (0.1 ml) was plated on YMD agar (ISP-2) and starch casein agar media amended with streptomycin (50 µg/ml) and nystatin (50 µg/ml) and the Petri dishes were incubated at 28±2ºC for 7-14 days. After incubation, the plates were observed for the growth of tough, leathery actinomycete colonies. Treatment of soil samples with calcium carbonate was reported to be the most efficient technique for the preferential isolation of actinomycetes [21]. A total of ten actinomycete strains were isolated from laterite soil. One of the strains designated as CN-4 was found to be more active when compared to the other isolates; hence the strain was maintained as pure culture on yeast extract, malt extract, dextrose (YMD) agar slants and preserved at 4ºC.

**Screening of predominant actinomycete strain for bioactive metabolites**

The predominant strain CN-4 was screened for its growth pattern and antimicrobial potential. The strain was initially cultured on ISP-2 broth for 48 h to obtain seed culture. It was transferred aseptically into the production medium (ISP-2) @ 10%. Fermentation was allowed to run for 8 days at 30ºC as shake cultures. The culture was harvested at every 24 h interval and the growth of strain was measured in terms of dry weight of biomass (g/100 ml). The strain entered log phase after 24 h of incubation and exhibited exponential growth followed by stationary phase extended up to 120 h. For determination of antimicrobial activity, the bioactive compounds produced by the strain in the production medium during the stationary phase were extracted with ethyl acetate and concentrated. The residue obtained served as crude bioactive extract for antimicrobial testing on test microorganisms. Maximum antimicrobial activity was observed with the crude extract obtained from 120 h old culture. Similarly, crude extracts of five day old cultures of *S. purpeofuscus* and *S. albidoflavus* were active against Gram positive as well as Gram negative bacteria and fungi [22,23]. While extracts of four day old cultures of *S. griseus* and *S. psammoticus* exhibited good antimicrobial activity [24,25]. A number of secondary metabolites with a wide spectrum of biological activities were reported from microorganisms.Antibiotics are the secondary metabolites majorly produced by actinomycetes. In particular several antibiotics have been reported from *Streptomyces* spp. The dramatic increase of infectious, opportunistic diseases as well as MDROs and decrease of novel antibiotic production resulted in the search of newer antibiotics as well as their sources. Discoveries of important, novel bioactive compounds depend upon the development of objective strategies for the isolation and characterization of novel microorganisms for existing and new screens. Hence it is expected that the present strain CN-4 which exhibited good antimicrobial activity was selected for further study of the cultural, morphological, physiological and biochemical characteristics molecular characterization and bioactive compounds production.

**Table: 1 Antimicrobial Activity of Bioactive Metabolite Produced by strain-4 Under Optimized Condition**.

| S. No | **Test organism** | **Zone Represented in mm** |
|---|---|---|
| 1 | *Bacillus megaterium* | 20 |
| 2 | *B. subtilis* | 22 |
| 3 | *Escherichia coli* | 14 |
| 4 | *Pseudomonas aeruginosa* | 13 |
| 5 | *Proteus vulgaris* | 15 |
| 6 | *Staphylococcus aureus* | 15 |
| 7 | *Xanthomonas campestris* | 14 |
| **Fungi** | | |
| | *Asperigillus niger* | 15 |
| 2 | *Botrytis cinerea* | 10 |
| 3 | *Candida albicans* | 24 |
| 4 | *Helminthosporium* | 10 |
| 5 | *Fusarium solani* | 10 |

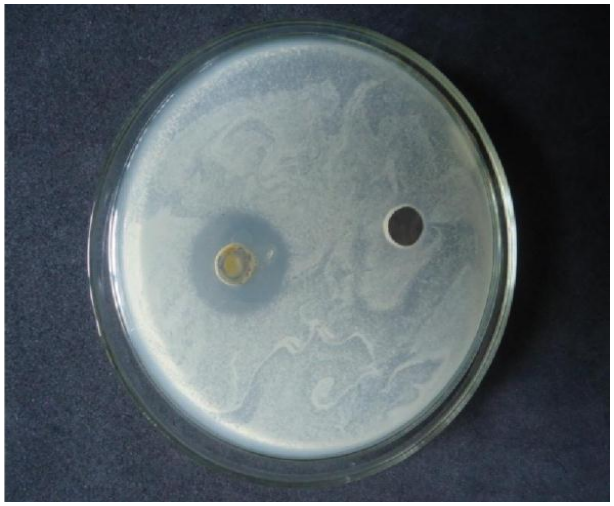


**Fig: 1 Antibacterial activity of *streptomyces albus* CN-4**

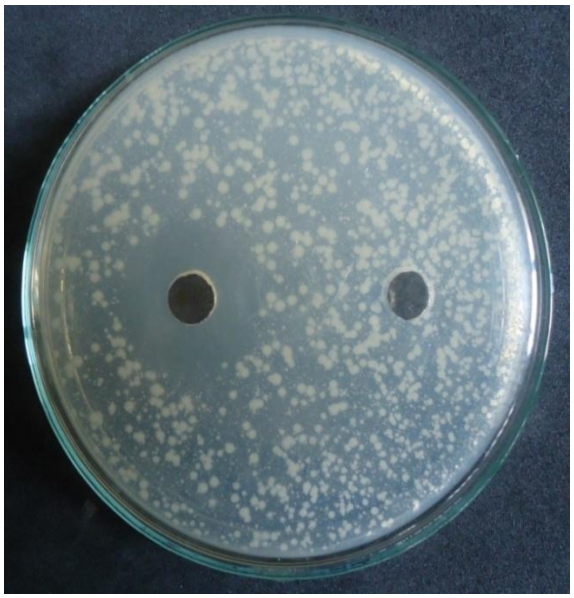


**Fig: 2 Antifungal activity of *streptomyces albus* CN-4**

**Extraction, structural elucidation and biological evaluation of bioactive metabolites produced by *Streptomyces* sp. CN-4**

For the production of bioactive compounds, seed broth (10%) was inoculated into the optimized production medium. The fermentation was carried out in 1L Roux bottles for 120 h at 30°C. The harvested broth (30L) extracted with ethyl acetate was concentrated in a Rotavac. The deep brown concentrated semi solid compound (4g) obtained served as the crude antimicrobial compound.

### Isolation of bioactive compounds from crude extract

The most important task of the present study is to extract, isolate and elucidate the structures of the bioactive compounds produced by the strain *Streptomyces albus* CN-4. Seed broth (10%) was inoculated into the optimized production medium for the production of bioactive compounds. The fermentation was carried out in 1L Roux bottles incubated at 30°C and terminated after 120 h. The harvested broth (30L) extracted with ethyl acetate was concentrated in a Rotavac. The deep brown concentrated semi solid compound (4 g) obtained served as the crude antimicrobial compound. Structural elucidation was done in the LUCID 84aboratories Pvt. Ltd. Balanagar, Hyderabad. Structural elucidation was done in the LUCID laboratories Pvt. Ltd. Balanagar, Hyderabad. GC-MSD Analysis revealed that strain CN-4 was produce 14 fractions. The structure of all these fractions was analyzed on the basis of GC-MSD analysis. Compounds such as Dichloroacetic acid,4 hexadecylester,Phenol,4(1,1,3,3,tetramethylbutyl),1-Nonadecene, yrrolo[1,2-a]pyrazine-1,4-dione, Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl),hexahydro-3-(2-methylpropyl), 2,5-Piperazinedione, 3,6-bis(2-methylpropyl), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl), 1,2- Benzenedicarboxylic acid, diisooctyl ester.

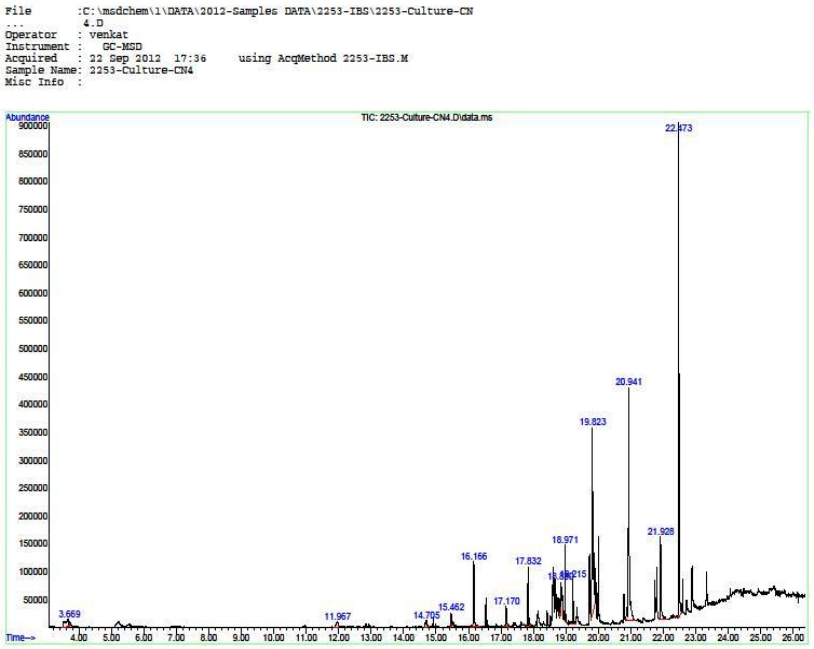
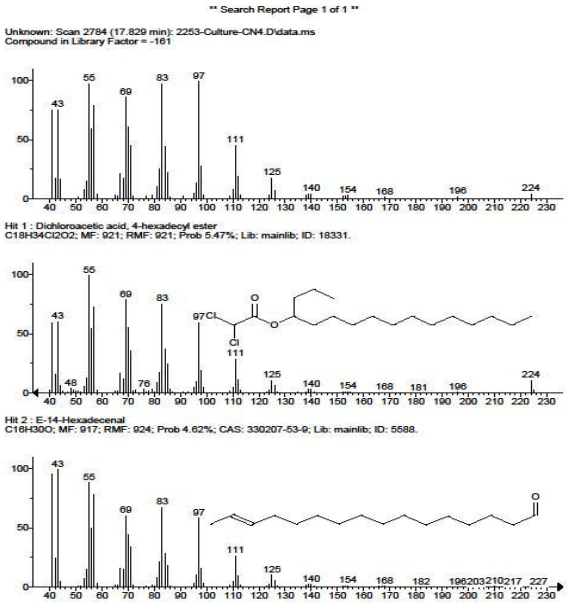



**Fig: 3 GC-MSD Spectrum of *Streptomyces albus* CN-4**



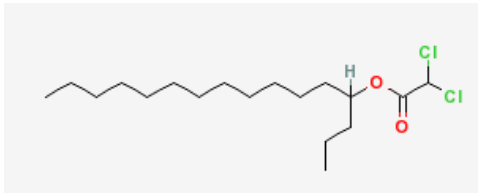
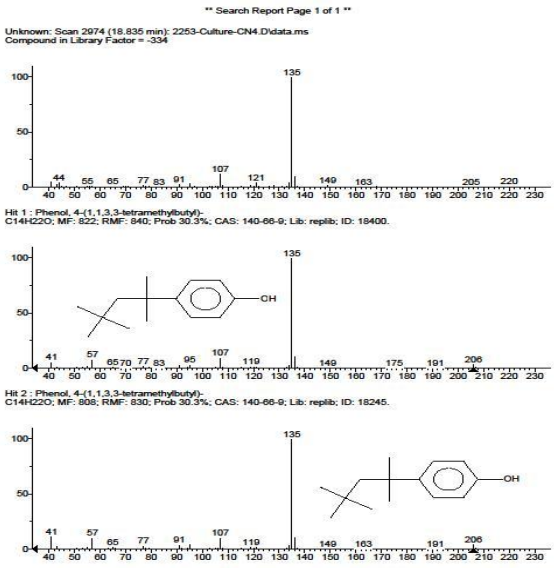




**Fig: 5 Molecular structure of the Dichloroacetic acid, 4-hexadecyl ester**

**Fig: 4 Mass spectrum and chemical structure of Dichloroacetic acid,4-hexadecy ester(Hit-E-14) Hexadecenal(Hit-2) by *Streptomyces albus* CN-4**



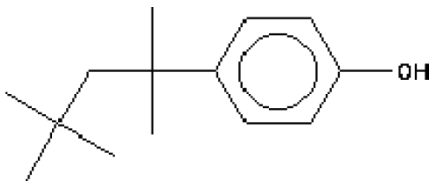
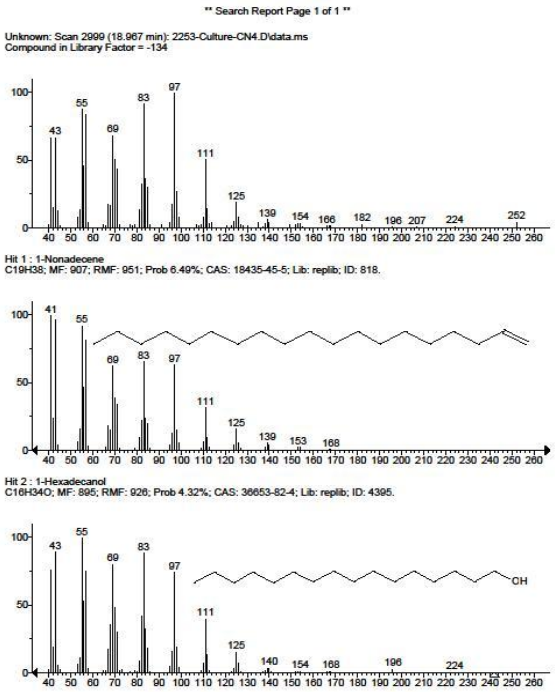




**Fig: 7 Molecular structure of the Phenol, 4-(1,1,3,3-tetramethylbutyl)-**

**Fig: 6 Mass spectrum and chemical structure of Phenol,4-(1,1,3,3-tetramethylbutyl) (Hit -1,Hit-2) by *Streptomyces albus* CN-4**



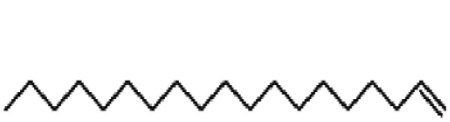
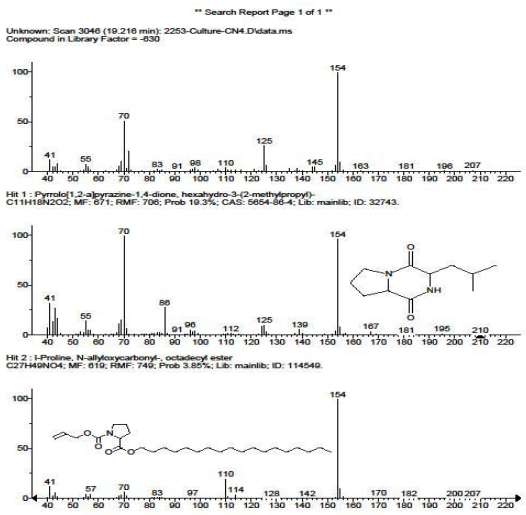



**Fig: 9 Molecular structure of the 1-Nonadecene**

**Fig:8 Mass spectrum and chemical structure of 1-Nonadecene (Hit -1) 1-Hexadeca (Hit-2) by *Streptomyces albus* CN-4**



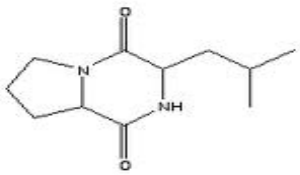
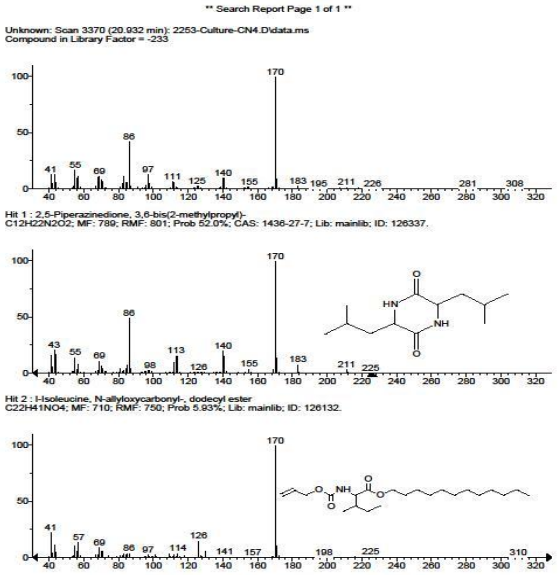



**Fig:11 Molecular structure of the Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3- (2-methylpropyl)-**

**Fig: 10 Mass spectrum and chemical structure of Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- (Hit -1) l-Proline, N-allyloxycarbonyl-, octadecyl este (Hit-2) by *Streptomyces albus* CN-4**



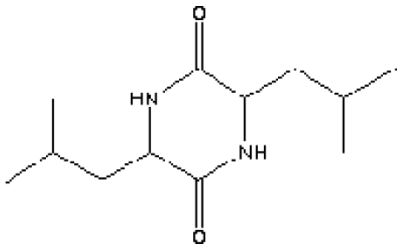
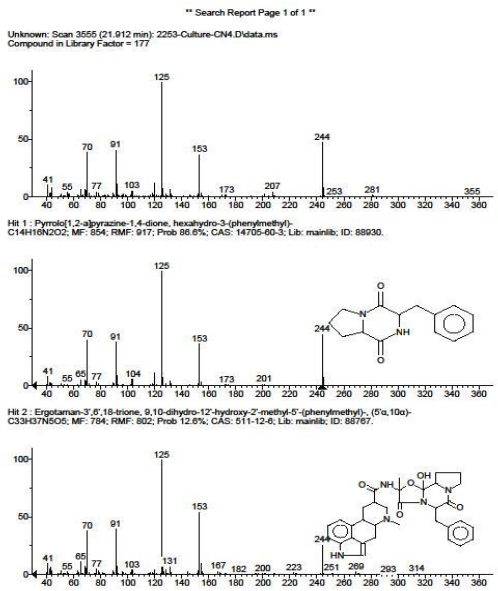



**Fig: 13 Molecular structure of the 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-**

**Fig: 12 Mass spectrum and chemical structure of 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-(Hit -1), l-Isoleucine, N-allyloxycarbonyl-,dodecyl ester (Hit-2) by *Streptomyces albus* CN-4**



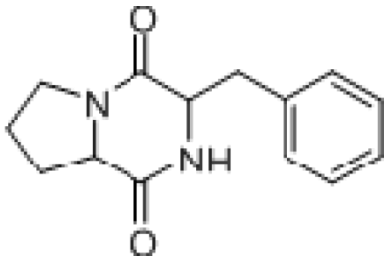



**Fig: 15Molecular structure of the Pyrrolo[1,2-a]pyrazine-1,4-dione, Hexahydro-3-(phenylmethyl)-**

**Fig: 14Mass spectrum and chemical structure of Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- (Hit -1), Ergotaman-3',6',18-trione, 9,10-dihydro-12 hydroxy-2'-methyl-5'-(phenylmethyl)-, (5'α,10α)-(Hit-2) by*Streptomyces albus* CN-4.**

## Conclusion:

In the present work demonstrated the production of bioactive compounds of a predominant actinomycete strain CN-4 isolated from laterite soil. Screening of the isolate for antimicrobial activity revealed that the strain is active against a number of Gram positive and Gram negative bacteria as well as fungi. The strain produced bioactive metabolites structurally confirmed on the basis of mass spectroscopy techniques with good antimicrobial activity proving their excellence for the production of natural products with wide pharmaceutical magnitude.

## Reference

1. Ho, C., Lo, C., Lai, N., Cheah, H. and Wong, N. (2002). Actinomycetes isolated from soil samples from the cocker range sabah. **ASEAN Rev. Biodiversity Environ. Conserv., 9:** 1-7.
2. Stackebrandt, E., Rainey, F. A. and Rainey, N. L. W. (1997). Proposal for a New hierarchic classification system, *Actinobacteria classis* nov. Int. J. Syst. Bacteriol., 47: 479-491.
3. Berdy, J. (2005). Bioactive microbial metabolites. **J. Antibiot., 58:** 1-26.
4. Nolden, L., Beckers, G., Mockel, B., Pfefferle, W. Nampoothiri, K.M., Kramera R., Burkovskia, A.(2000). Urease of corynebacterium glutamicum: organization of corresponding genes and investigation of activity. **FEMS Microbiol Lett., 189:** 305-310.
5. Baltz, R. H. (2005). Antibiotic discovery from actinomycetes: will a renaissance follow the decline and fall? **SIM News, 55:** 186-196.
6. Baltz, R. H. (2007). Antimicrobials from actinomycetes: Back to the future. **Microbe, 2:** 125-131.
7. Chamberlain, K., and Crawford, D.L. (1999). *In vitro* and *in vivo* antagonism of pathogenic turf grass fungi by *Streptomyces hygroscopicus* strains YCED9 and WYE53. **J. Ind. Microbiol. Biotechnol., 23 :** 641-646.
8. Lee, J. Y. and Hwang, B. K. (2002). Diversity of antifungal actinomycetes in various vegetable soils of korea. **Can. J. Microbiol., 48:** 407-417.
9. Hoster, F., Schmitz, J. E. and Daniel, R. (2005). Enrichment of chitinolytic microorganisms: isolation and characterization of a chitinase exhibiting antifungal activity against phytopathogenic fungi from a novel *Streptomyces* strain. **Appl. Microbiol. Biotechnol., 66:** 434-442.
10. Joo, J. G. (2005). Purification and characterization of an extracellular chitinase from the antifungal biocontrol agent *Streptomyces halstedii.* **Biotechnol. Letters, 27:** 1483-1486.
11. Sharma, H. and Parihar, L. (2010). Antifungal activity of extracts obtained from actinomycetes. Journal of Yeast and Fungal Research, 1: 197-200.
12. Lin, J., Bai, L., Deng. Z. and Zhong. J. J. (2010). Effect of ammonium in medium on Ansamitocin P-3 Production by *Actinosynnema pretiosum.* **Biotechnology and Bioprocess Engineering, 15: 119-125.**
13. Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. and Barea J. M. (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. **Biol. Fertil. Soils 37:** 1–16.
14. Naine, J., Srinivasan, M.V. and Devi, S.C. (2011). Novel anticancer compounds from marine actinomycetes: **a review. J. Pharm. Res. 4:1285-1287.**
15. Brown, M. E. (1972). Plant growth microorganisms of soil and substances produced by rhizosphere. **J. Appl. Bacteriol., 35:** 443-451.

16. Merckx, R., Dijkra, A., Hartog, A. D. and Veen, J.A.V. (1987). Production of root-derived material and associated microbial growth in soil at different nutrient levels. **Biol. Fertil. Soils 5:** 126-132.
17. El-Nakeeb, M. A. and Lechevalier, H. A. (1963). Selective isolation of aerobic actinomycetes. **Appl. Microbiol., 11:** 75-77.
18. Williams, S. T. and Cross, T. (1971). Isolation,purification, cultivation and preservation of actinomycetes. Methods Microbiol., 4: 295-334.
19. Ellaiah, P., Adinarayana, G., Saisha, V. and Vasu, P. (2005). An oligoglycosidicantibiotic from a newly isolated *Streptomyces albovinaceus.* **Indian J. Microbiol., 45:** 33-36.
20. Cappuccino, J. G. and Sherman, N. (2004). **Microbiology, a laboratory manual.** Pearson Education, Inc., New Delhi, pp. 282-283.
21. Alferova, I. V., Terekhova, L. P. and Prauzer, K. H. (1989) Selective medium with nalidixic acid for isolating antibiotic producing actinomycetes. **Antibiot. Khimioter., 34:** 344-348.
22. Anupama, M., Narayana, K. J. P. and Vijayalakshmi, M. (2007). Screening of *Streptomyces purpeofuscus* for antimicrobial metabolites. **Res. J. Microbiol., 4:** 1-3.
23. Narayana, K.J.P., Prabhakar, P.,Vijayalakshmi,M., Venkateswarlu, Y. and Krishna, P.S.J. (2008). Study on Bioactive compounds from *Streptomyces* sp. ANU 6277. **Pol. J. Microbiol.,** 57: 35-39.
24. Otani, T., Sugimoto, Y., Aoyagi, Y., Igarashi, Y., Furumai, T., Saito, N., Yamada, Y., Asao, T. and Oki, T. (2000). New Cdc 25B tyrosine phosphate inhibitors, nocardiones A and B, produced by *Nocardia* sp. TP-A0248: Taxonomy, fermentation, isolation, structural elucidation and biological properties. **J. Antibiot., 53:** 337-344.
25. Sujatha, P., Bapiraju, K.V.V.S.N. and Ramana, T.(2005). Studies on a new marine streptomycete BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus.* Microbiologial Res., 160: 119-126.