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ORIGINAL ARTICLE **OPEN ACCESS**

Analysis of Active Antibacterial Compound by GC and FTIR from *Nocardiopsis Prasina* ACT24 Against VRE

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ABSTRACT
The last decade, a spectacular raise in the incidence of Vancomycin Resistant Enterococci (VRE) has been renowned in health sector in the widespread general predicament. Consequently, we will perpetually need innovative antibiotics to stay treating broadly resistant forms but key growth in recognition of novel mechanism of act. Ethyl acetate extract of Nocardiopsis prasina ACT 24 was produced zone of inhibition around 30mm against VRE was equal to control chloramphenicol. The culture filtrate were further purified by column chromatography with eluting solvent was composed of ethyl acetate and hexane (8:2) fifty fractions were collected and tested for their activities. On TLC plate develop the antibiotic as single active spot on the solvent system Rf value 0.678. IR spectrum had hydroxyl group. GC-MS analysis and the chromatogram patterns revealed 9 peaks, indicating the presence of bioactive constituents, which included several important organic compounds namely Oxime-, methoxy-phenyl (28.0), 1-Heptacosanol (21.95), 1-Heptacosanol (42.50), (S,S*)-2-HYDROXY(4-METHOXY) (0.86), Tetracosanoic acid, methyl ester (1.20). In the present study, examined Nocardiopsis prasina ACT 24 strength encompass most elevated biological properties.*
Keywords*: Actinomycetes, Biological activity, Antibacterial, Taxonomy, Spectral activity*

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INTRODUCTION

Resistance of bacteria to the chattels of antibiotics has been a foremost difficulty in the heal of diseases. Contagious diseases are static and subsequent primary origin of demise worldwide [22, 5, 6]. Studies conducted in India by Karmarkar *et al*. [9] have reported multi drug resistant incident to be sky-scraping as more than 70 percent. These destructive health trends label for a novel notice in transferable disease as well as efficient strategies for management and anticipation [11, 12]. Microbial secondary metabolites like antibiotics are a unique class of chemotherapeutic agents' secreted by actinomycetes. Actinomycetes are good source of antibacterial amalgam gather by pharmaceutical industry about half a century [1]. Requirement of novel secondary agents is greater than forever, since sprouting of multidrug resistance in familiar pathogens, the speedy surfacing of new infections and the use of multidrug resistant pathogens in bioterrorism [18, 19]. In the present study describe the biological activities of antibacterial compound from *Nocardiopsis prasina ACT24* against vancomycin resistant *Enterococcus* sp.

MATERIAL AND METHODS

Taxonomy of actinomycete isolate

Actinomycete isolates were inoculated on diverse ISP media (Actinomycetes isolation agar, Starch casein agar) and incubated for 7 days at 30°C. The colonies were pragmatic under a high-power magnifying lens and colony morphology was renowned with respect to color, aerial and substrate mycelium, branching, and the nature of colony [2].

Production of antimicrobial compound

The active isolates resulted by primary screening, promising isolates were tested for their extra cellular antibiotic production capabilities under submerged fermentation conditions. A loopful inoculum of potential actinomycetes strain (ACT 24) was inoculated in to 100ml of Actinomycetes isolation broth and kept at 28°C for 72hrs with continuous shaking. After incubation 20ml of aliquots transferred to 1000ml of Starch Casein broth and incubated at 28°C and 250rpm for 7 to 10 days [21]. After incubation the crude

antibiotic extract was recovered from the broth culture filtrate by solvent extraction method using Ethyl acetate in accordance with the description of Liu *et al.,* [10].

Partial purification of antimicrobial compound

Column chromatography

The purification of antimicrobial substance was carried out using silica gel (2.5x50) chromatography ethyl acetate and hexane 8:2 (v/v) mixtures were used as an eluting solvent. The column was left overnight until the silica gel 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. 50 fractions were collected (each of 5 ml) and tested for their anti microbial activity [7].

Thin layer chromatography

Silica gel plates, 10cm x 20cm x1mm thickness were prepared. They were activated at 150°C for half an hour. Ten micro liters of the ethyl acetate fractions and reference antibiotic chloramphenicol were applied on the plates and the chromatogram was developed using ethylacetate: hexane (2:8) as solvent system. The TLC plates were exposed to iodine vapour chamber or UV chamber to develop the antibiotic. The fractions having same Rf value were mixed together and the solvent evaporated at 40°C in a vacuum oven. These fractions were tested for their antibacterial activity by using the agar diffusion assay method. The fractions showing antibacterial activity were again purified by Thin Layer Chromatography technique. The powder obtained was stored in an ampoule at 4°C [7].

Elemental studies of antibacterial compound

FTIR

Infrared (IR) spectra of the crude extracts were measured (as KBr discs) between 400-4000cm-1 on Perkin Elmer 2000 FT-IR spectrophotometer. The important IR bands, symmetric and asymmetric stretching and stretching frequencies were studied to determine the presence of functional group in the ethyl acetate solvent extracts [7].

GC – MS Analysis

Gas chromatography-mass spectrometry analyses were performed using an Agilent GC- MS 5973 (Palo Alto, CA, USA) assembly equipped with a HP-5 cross-linked fused silica capillary column (25m \ 0.32 mm \ 0.25 μm). Helium was used as carrier gas at 38 cm/s. The column total flow rate was 1 ml/min. General temperature conditions were: split/splitless injector at 280 °C, transfer line at 280 °C, source 230 °C, and column temperature program of 80°CD310 °C at 10 °C/min. Mass detection limits were 50D700 Da. Samples were reacted with BSTFA-pyridine (1:1, v/v) at room temperature for 30 min before analyses [8].

RESULTS

Taxonomy of isolate

Due to the broad spectral activity the *Nocardiopsis prasina* ACT 24 strain was selected for further study. The isolated strain identified by scanning electron microscope. The aerial mycelium is white in colour with sparse substrate mycelium with reverse yellow pigment. The colour of aerial mycelium is considered to be an important character for grouping and identification of isolate. Mycelial color difference may be due to the synthesis of various secondary metabolites [3].

The bioactive compounds were extracted from natural sources through several techniques. Solvent extraction was usually employed for the extraction of secondary metabolites from the culture filtrates. Same tendency of experimental method was used in this study and it is also establish that ethyl acetate solvent was most appropriate for compound extraction. The ethyl acetate extract of *Nocardiopsis prasina* ACT 24 showed effective activity against VRE the zone of inhibition (30mm) was equal to chloramphenicol (30mm)

Antibacterial activity of *Nocardiopsis prasina ACT24* against vancomycin resistant *enterococcus*:

The antimicrobial activity of isolated *Nocardiopsis prasina* ACT24 beside a number of clinical strains indicated the broad-spectrum nature of the antibiotic, since it was active against a number of Gram-positive and Gram-negative bacteria. Ethyl acetate extract of *Nocardiopsis prasina* ACT24 was produced zone of inhibition around 30mm against VRE was equal to control Chloramphenicol (Figure 1).

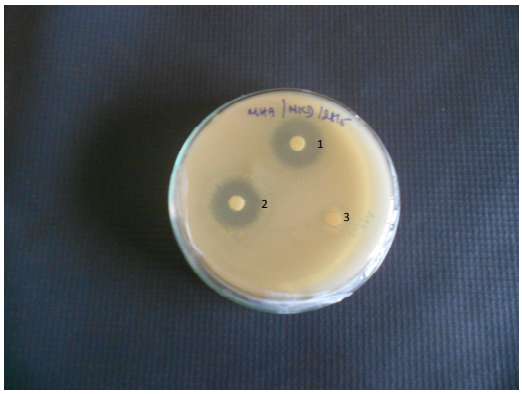


Figure 1 Antibacterial activity of *Nocardiopsis prasina ACT24* against vancomycin resistant *Enterococcus* Chloramphenicol (positive control 30mm) 2.*Nocardiopsis prasina* ACT 24 extract (30mm) 3.Negative control (methanol).

Partial purification of antibacterial compound

For the purpose of purification process, the antibacterial agent were allowed to pass through a column chromatography packed with silica gel and eluting solvent was composed of ethyl acetate and hexane (8:2) [7] fifty fractions were collected and tested for their activities. The maximum activity was recorded at fraction No. 20(34.4 mm). Similarly, many workers used a column chromatography packed with silica gel and eluting solvents composed of various ratios of chloroform and methanol.

The effective compound was extracted by solvents ethyl acetate and methanol at pH 7.0. The organic phase was collected and evaporated under reduced pressure using a rotary evaporator. Ten microliters of the fraction and reference antibiotics were applied on the plates and the chromatogram was developed using ethylacetate: hexane (2:8) as solvent system. The TLC plates were exposed to UV chamber to develop the antibiotic as single active spot on the solvent system Rf value 0.678 (Figure 2). The fractions having same Rf value were mixed together and the solvent evaporated at 40°C in a vacuum oven.

Figure 2 TLC showing the development of antibiotic as single spot

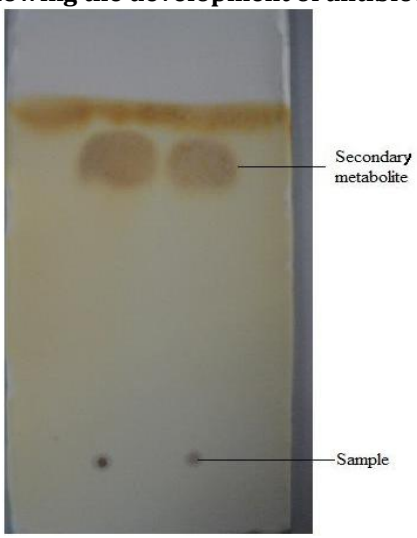


Figure showing single spot of active metabolite

Segregation of secondary metabolite into separate entity has been tried by thin-layer chromatography using a solvent system composed of ethyl acetate and hexane (2:8, v/v) as developing solvent [2].

Structure elucidation of antibiotic compound

Infrared Spectrum (IR):

The IR spectrum revealed the cycloaliphatic nature of the compound and the following signal were given: Cm-1; 3400 (OH) broad due to hydrogen bonding supporting the conjugated unsaturated nature; The IR spectrum (Figure 3) had OH group.

Figure 3 IR spectrum of ethyl acetate extract of the *Nocardiopsis prasina ACT 24*

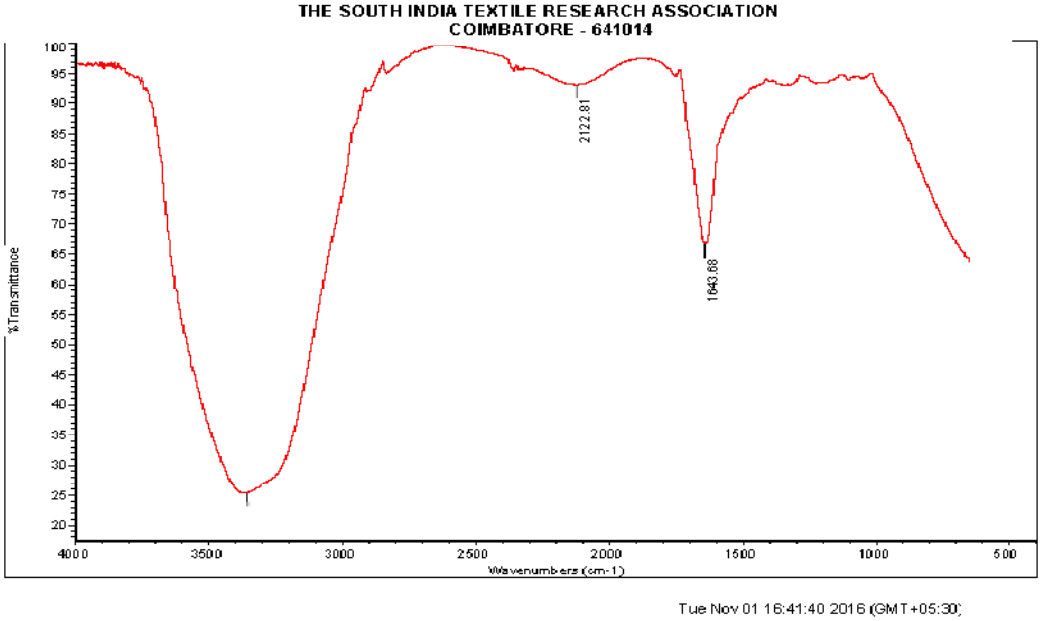
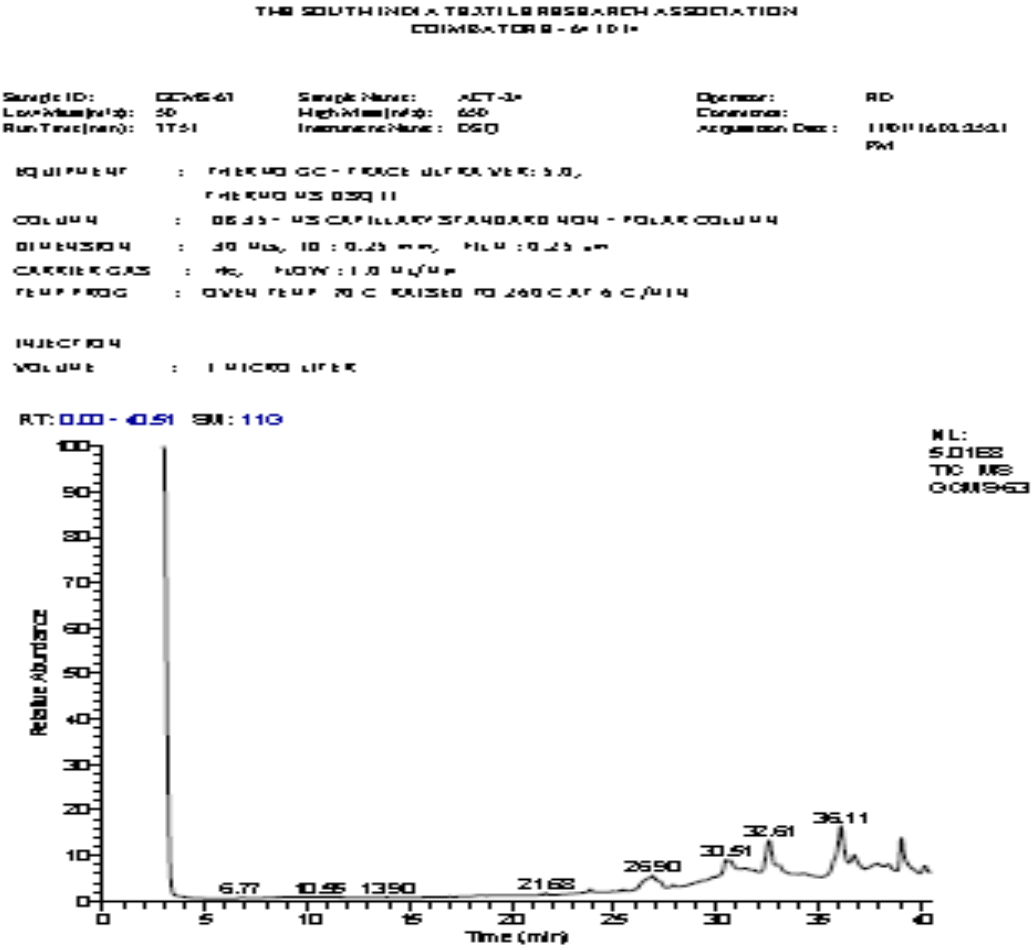


Figure 4 MS chromatogram of ethyl acetate extract of the *Nocardiopsis prasina* ACT 24



GC- MS analysis:

The presence of volatile chemical constituents of ethyl acetate crude extract were analyzed by the GC-MS analysis and the chromatogram patterns revealed 9 peaks, indicating the presence of bioactive constituents, which included several important organic compounds namely Oxime-, methoxy-phenyl (28.0), 1-Heptacosanol (21.95), 1-Heptacosanol (42.50), (S*,S*)-2-HYDROXY(4-METHOXY) (0.86),

Tetracosanoic acid, methyl ester (1.20). Interpretation on mass spectrum of GC-MS was done using the database NIST08, WILEY8 (Figure 5).

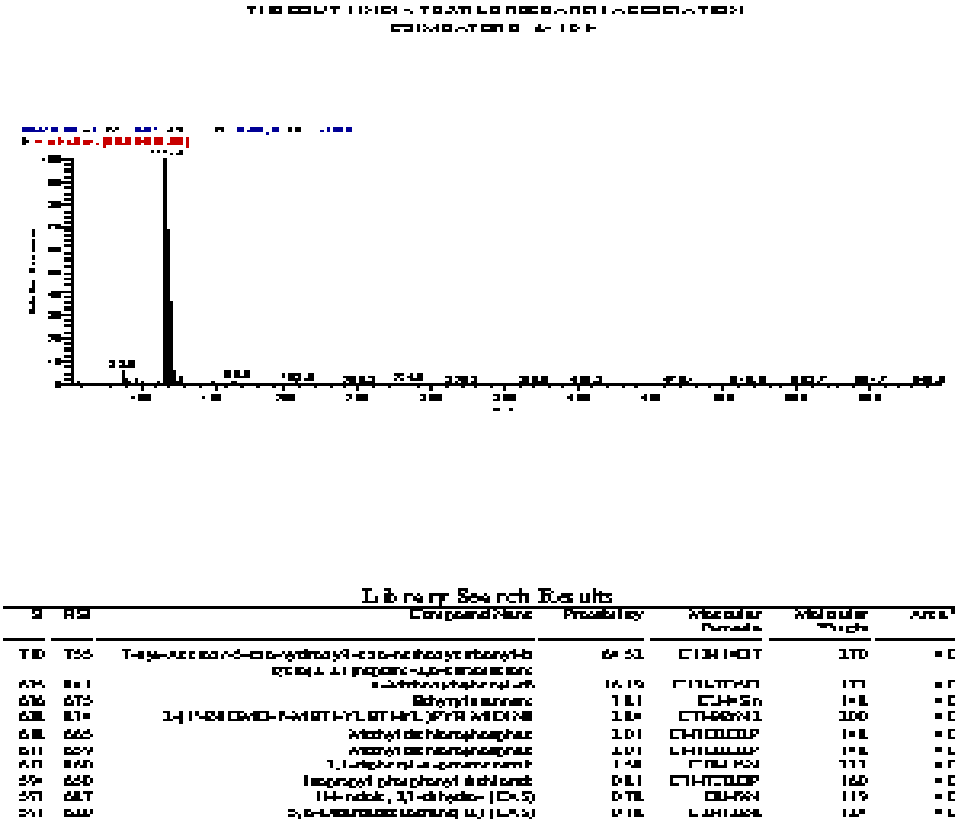


Figure 5: Interpretation on mass spectrum of GC-MS was done using the database NIST08, WILEY8

DISCUSSION

The main findings in this paper was antibacterial activity of *Nocardiopsis prasina* ACT24 against vancomycin resistant enterococcus sp.. The genetic engineering tool was used to predict the novel generation of drug candidate and to a great extent improved our understanding of microbial life. Comparatively polymerase chain reaction amplification suggests the specificity and accuracy of sequencing than culture method [16]. Similarly the author reported that more than one multi drug resistant species are inhibited by the wide spectrum of the isolate *Nocardiopsis* sp.TFS65 [5]. The concurrent studies of *Streptomyces sp* PM-32 isolated from off-shore sediments collected at the Bay of Bengal coast was reported to have antimicrobial activity against a group of bacterial and fungal pathogens [11]. Sujatha *et al.* [20] reported that the capacity and capability to produce extra cellular product of marine actinomycetes against clinical pathogen like methicillin resistant *Staphylococcus aureus* (MRSA). A *Streptomycete* BT-408 producing polyketide antibiotic SBR-22 effective against methicillin resistant *Staphylococcus aureus* was already reported. The compound 2-(2', -Dibromophenoxy)-4,6-dibromophenol isolated from the marine sponge *Dysidea granulosa* (Bergquist) has been reported to possess inhibitory activity against VRE strains [17]. Lynamicins A-E (1-5), was discovered from a novel marine actinomycete, NPS12745 and has been reported to possess inhibitory activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* [13].

Vijayakumar *et al.,* [21] suggested for large scale production of antibiotic compound highly depends upon screening and cost of solvents. Other sources like terrestrial and hotspot isolation of actinomycetes metabolites are less active than marine sediment actinomycetes against multi drug resistant pathogens.

Based on the structural lipopolysaccharide arrangements in outer polysaccharide membrane sensitivity pattern of gram positive and gram negative bacteria could be described. This makes the cell wall impermeable to lipophilic solutes, The gram positive should more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier [14] .

The peaks appearing at 3600-3200 cm-1 indicated the presence of hydroxyl group absorption [4]. Similarly Janardhan *et al*., [7] reported that IR signal at 3410 cm^{-1} represents the presence of aliphatic OH

group. IR signal at 3013 cm^{-1} represents the presence of aromatic protons. In the present study, the partial characteristics of the active molecules indicated that bioactivity was likely to be due to the production of potential organic substances. The chemical fingerprints of the crude extract was identified and further manifested in differentiation from the related chemical profiles. Hence, the poor availability of drugs and the increasing number of treatment failures have motivated current searches for therapeutic alternatives which can work effectively as potential antimicrobial agents [15].

CONCLUSION

In the study, examined *Nocardiopsis prasina* ACT 24 might have been found will bring most elevated antibacterial properties. Further FTIR and GC-MS examination about Nocardiopsis sp. uncovered the vicinity from claiming different mixes including Oxime-, methoxy-phenyl, 1-Heptacosanol, (S*,S*)-2-HYDROXY(4-METHOXY), Tetracosanoic acid, methyl ester. Hence, from the metabolite perspective view of *Nocardiopsis prasina* ACT 24 recognized to have exceedingly proficient antimicrobial components against VRE.

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