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GC- MS ANALYSIS OF ETHYL ACETATE EXTRACT OF *STERPTOMYCES* SPECIES ISOLATED FROM VERMICAST

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ABSTRACT

In this study, the bioactive compounds present in the ethyl acetate extracts of two different *Streptomyces* strains isolated from vermicast (earthworm fecal pellets) were investigated using Gas Chromatography and Mass Spectrometry (GC-MS). GC-MS analysis showed the presence of 12 and 9 bioactive compounds in AS1 and AS3 respectively. GC-MS analysis of AS1extract revealed the presence of major bioactive compounds such as n-hexadecanoic acid, 6-hexadecanoic acid, methyl ester, 9-octadeconic acid, methyl ester, 2-4, cycloheadien-1-one, 3,5-bis (1,1-dimethyl ethyl) 4-hydroxy and AS3 extract contains hexadecanoic acid, 9-octadecanic acid-E, oleic acid, 6-ocatadecanic acid, 2-4, cycloheadien-1-one, 3,5-bis (1,1-dimethyl ethyl) 4-hydroxy, 1,2-Benzisothiazol-3- amine tbdms, hexamethyl cyclotrisiloxane and 1,2-Bis (trimethylsilyl) benzene.

KEYWORDS: **GC-MS analysis, bioactive compounds, *Streptomyces* species, ethyl acetate extract, vermicast.**

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INTRODUCTION

Actinomycetes produce many important bioactive compounds that have high commercial value. Their ability to produce variety of bioactive compounds has been utilized in a comprehensive series of researches in numerous industries. Actinomycetes are universal occurrence in nature and are widely distributed in natural and man-made environments.[1] The rhizosphere soil is an ecological niche in which develop microbial communities. Hatzinger and Alexander have shown that bacteria that attained the highest densities in rhizosphere survived successfully compared with those at the lowest densities.[1] *Streptomyces* are largely isolated from soil and marine sediments. Although soils has been screened by the pharmaceutical industry from about 50yrs only a miniscule fraction of the surface of the globe has been sampled, and only a small fraction of *Streptomyces* has been discovered.[2] Isolation of *Streptomyces* from soil source may be valuable for the production of antibiotics. *Streptomyces* species syntheses many different biologically active secondary metabolites such as antibiotics, herbicides, pesticides, anti parasitic and enzyme inhibitors, Of which antibiotics are more important therapeutically. Approximately one third of known antibiotics have been isolated from streptomyces.[3] *Streptomyces* produces of many secondary metabolites, which have different biological activities, such as antibacterial, antifungal, antiparasitic, antitumor, anticancer and immunosuppressive actions.[4] In particular the genus *Streptomyces* is an important group of actinomycetes because of its ability to produce many types of secondary metabolites. *Streptomyces* are the group of Gram positive filamentous bacteria which are ubiquitous various natural environments. *Streptomyces* are the most economically valuable to produce chemically diverse metabolites with wide range of biological activity.[5] The purpose of this study is to analyze bioactive compounds present in the ethyl acetate extract of S*treptomyces* species isolated from vermicast.

MATERIALS AND METHODS

Six Vemicast samples were collected from the Shevaroy hills, Eastern Ghats, Tamil Nadu and were transported aseptically in sterile plastic containers to the laboratory. The samples were air dried one week and kept at 45°C for 1 hr to prevent bacterial and fungal contamination. One gram of each vermicast sample was subjected to serial dilution method and they were cultured in by spread plate technique on starch casein agar and incubated at 37°C for 2 weeks. Selected colonies were subjected to starch casein nitrate broth for further growth.

CULTURE CHARACTERISTICS

Isolated *Streptomyces* species were used to study the morphology of spore bearing hyphae with the entire spore chain. This is was done by using cover slip method in which individual cultures were transferred to the base of cover slips buried in starch casein agar medium.

GRAM STAINING

Smear of cultures were taken in clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1min and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 1min and washed with tap water. Alcohol was used to decolorize the smear until the violet colour ceased to flow away. The side was washed with water and counter stain safranine was added over the smear for 2min, then the slide was washed, drained, air dried, and viewed under microscope. The culture retaining the violet colour indicated that it was Gram positive organism.[5]

EXTRACTION OF BIOACTIVE COMPOUNDS

Based on the preliminary screening, the most potent isolates was grown in starch casein nitrate broth as a production medium for the extraction of crude compounds. The active strains were inoculated individually in starch casein nitrate broth and incubated for 5-7 days in shaker incubator at 28°C. Then it was centrifuged for 20 mins at 10,000rpm and the supernatant collected was mixed with an equal volume of ethyl acetate. Ethyl acetate was added to the filtrate in the ratio of 1:1(v/v) and incubated in shaker for 1 hr for complete extraction. The ethyl acetate phase contains bioactive compounds was separated from the aqueous phase. The extracted crude compounds were dried to get dry powder by using heating mantle at 40^{o}C.[6]

GC-MS ANALYSIS

Ethyl acetate extract of *Streptomyces* species were analyzed by GC-MS method. GC-MS technique was performed by using GC Shimadzu QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1.51ml/min and an injection volume of 2µl was employed (split ratio: 20). Injector temperature was 200°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (Isothermal for 2 min.) with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 -1000 m/z. Total GC running time was 35 min.[7]

IDENTIFICATION OF COMPOUNDS

Identification of components Interpretation of mass spectra of GC-MS was done using the database of National Institute of Standards and Technology (NIST) having over 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST 08 library8. The name, mass and structure of the components of the test materials were ascertained.[8]

RESULTS AND DISCUSSION

Five isolates were collected based on their different colony morphology and colour variations. Among these two isolates were selected and purified by repeated streak on starch casein nitrate agar medium sub-culture plates for active strain. The selected species were

inoculated in starch casein nitrate broth and cultured and were tested for GC-MS analysis to find out bioactive compounds.[9] GC-MS chromatogram of the ethyl acetate extract of S*treptomyces* AS 1(Figure 1) showed 12 bioactive compounds and AS3 (Figure 2) showed 9 bioactive compounds. Table 1 shows the presence of bioactive compounds in AS1 and the major compounds were n- hexadecanoic acid[10], hexadecanoic acid, methyl ester[11], 1,2-Bis(trimethylsilyl)benzene[12], 9-octadeconic acid, methyl ester[13], 9-12 octadeconic acid (z,z)[13] , 2-4,cyclohexadien 1-one, 1,2-bis (methyl ethyl) 4-hydroxy.[14] AS3 also contains major compounds n-hexadecanoic acid[10], hexadecanoic acid, methyl ester[11], 9-octadecanic acid-(E)[15], oleic acid [16,17], 6-ocatadecanic acid[18], 2-4,cyclohexadien-1-one, 3,5-bis (1,1-dimethyl ethyl)4-hydroxy[14], 1,2-Benzisothiazol -3- amine tbdm[19], cyclotrisiloxane, hexamethyl.[20] The earlier studies by other researchers clearly shows that the above mentioned bioactive compounds possess antimicrobial activity.

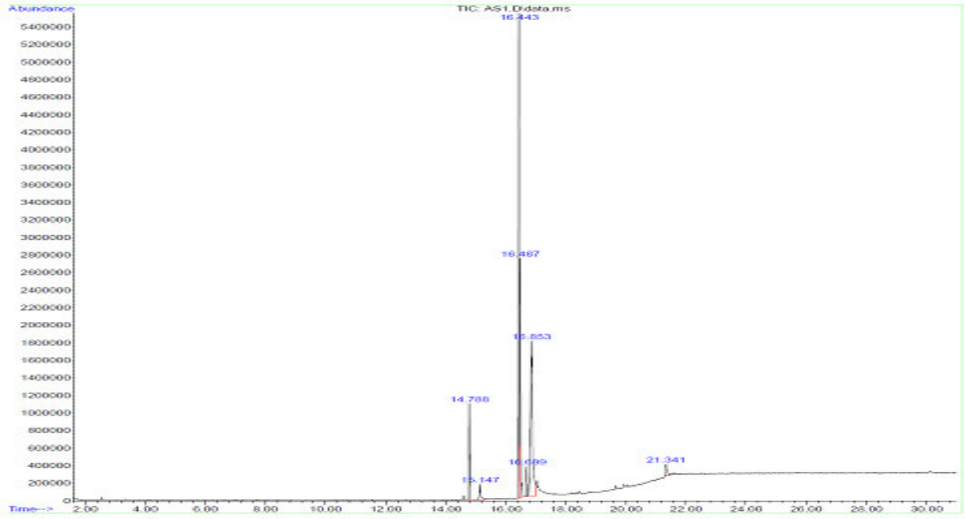

Figure 1
GC MS Chromatogram of AS1 Strain

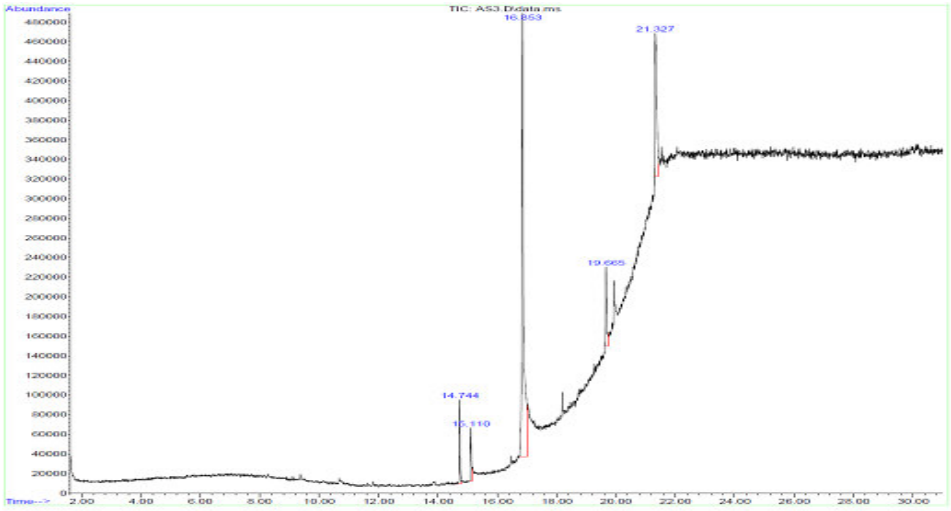

Figure 2
GC MS Chromatogram of AS3 Strain

TABLE 1
Bioactive compounds identified in the Ethyl Acetate Extract of Streptomyces isolates (AS1-AS3) by GC-MS.

S. No.	Retention Time	Compound Name	Molecular Formula	Mol. Weight
1	15.47	n- hexadecanoic acid	$C_{16}H_{32}O_2$	256
2	14.78	hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
3	16.48	9-octadeconic acid, methyl ester (E)	$C_{19}H_{36}O_2$	296
4	16.85	9-12 octadeconic acid (z,z)	$C_{18}H_{32}O_2$	280
5	21.34	2-4,cyclohexadien 1-one, 3,5-bis (1,1-dimethyl ethyl) 4-hydroxy	$C_{14}H_{22}O_2$	222
6	16.85	oleic acid	$C_{18}H_{34}O_2$	282
7	16.85	6-ocatadecanic acid	$C_{18}H_{34}O_2$	282
8	19.66	1,2-Benzisothiazol -3- amine tbdms	$C_{12}H_{22}Si_2$	222
9	21.32	cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222
10	21.34	1,2-Bis (trimethylsilyl) benzene	$C_{12}H_{22}Si_2$	222

CONCLUSION

This study shows that AS1 and AS3 may possess antimicrobial activity against various microorganisms because of presence of potent antimicrobial compounds. Thus the organisms studied can be used as a potent tool for formulation of useful drugs. The ethyl acetate extracts of that organisms shows the presence of bioactive compounds that possess antimicrobial activity which can be eluted and purified and can be used as a drug that can be assessed in future studies.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Duraipandiyan V, Sasi AH, Islam VI, Valanarasu M, Ignacimuthu S. Antimicrobial properties of actinomycetes from the soil of Himalaya. Journal de Mycologie Médicale/Journal of Medical Mycology. 2010 Mar 31;20(1):15-20.
2. Gesheva V. Rhizoshere microflora of some citrus as a source of antagonistic actinomycetes. European Journal of Soil Biology. 2002 Feb 28;38(1):85-8.
3. Usha Nandhini S and Masilamani M, Bioactive Compounds Produced by *Streptomyces* Strain. International J of Pharmacy and Pharamaceutical Sciences.2013 Jan 5:176-178.
4. Bizuye A, Moges F, Andualem B. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pacific Journal of Tropical Disease. 2013 Oct 31;3(5):375-81.
5. Usha Nandhini S and Masilamani M, Bioactive Compounds Produced by *Streptomyces* Strain. International J of Pharmacy and Pharamaceutical Sciences.2013 Jan 5:176-178.
6. Lalitha P,Karikalan K. and Thirugnannsambantham K. Antimicrobial activity of crude ethyl acetate extract of marine Actinomycetes isolated form marine sediments. International J of Current Research. 2013 Dec.5(12) :4053-4056.
7. Rana S, Salam MD. Antimicrobial potential of actinomycetes isolated from soil samples of Punjab. India. J Microbiol Exp. 2014;1(2):00010.
8. Sudar S, Justin Koil Pillai, GC-MS Analysis Of Petroleum Ether And Methanol Extracts Of *Solanum Virginianum* L Leaves. Int J Pharm Bio Sci. 2014 Oct; 5(4): (P) 561 - 568.
9. Aruna S, Vijayalakshmi K, Shashikanth M, Surekha R, Jyothi K. First report of antimicrobial spectra of novel strain of *Streptomyces tritolerans* (Strain AS1) isolated from earthworm gut (*Eisenia foetida*) against plant pathogenic bacteria and fungi. Curr Res Bacteriol. 2008;1:46-55..
10. Kalaivani CS, Sathish SS, Janakiraman N, Johnson M. GC-MS studies on *Andrographis paniculata* (Burm. f.) Wall. ex Nees–A medicinally important plant. Int J Med Arom Plants. 2012 Feb;2(1):69-74.
11. Chandrasekaran M, Senthilkumar A, Venkatesalu V. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. European review for medical and pharmacological sciences. 2011 Jul;15(7):775-80.
12. Tyagi R and Sharma V.GC-MS LB. A comparison of volatile compounds in different genotypes of *sesamum indicum* L. BY GC-MS. International J of Pharma Sciences and Research.2014: 5(1): 249-258.
13. Wei LS, Wee W, Siong JY, Syamsumir DF. Characterization of anticancer, antimicrobial, antioxidant properties and chemical compositions of *Peperomia pellucida* leaf extract. Acta Medica Iranica. 2011 Oct 1;49(10):670
14. Wafaa H. B. Hassan, Ali A. El Gamal, Ebtesam El-Sheddy, Mai Al-Oquil and Nida Nayyar Farshori, The chemical composition and antimicrobial activity of the essential oil of *Lavandula coronopifolia* growing in Saudi Arabia. J of Chem. Pharma. Res.2014: 6(2): 604-615.
15. Seanego CT, Ndip RN. Identification and antibacterial evaluation of bioactive compounds from *Garcinia kola* (Heckel) Seeds. Molecules. 2012 May 31;17(6):6569-84.
16. Choi JS, Park NH, Hwang SY, Sohn JH, Kwak I, Cho KK, Choi IS. The antibacterial activity of various saturated and unsaturated fatty acids against several oral pathogens. Journal of Environmental Biology. 2013 Jul 1;34(4):673.
17. Awa EP, Ibrahim S, Ameh DA. GC/MS analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of *Annona senegalensis* pers. International Journal of Pharmaceutical Sciences and Research. 2012 Nov 1;3(11):4213.
18. Nahar N, Rahman MS, Rahman SM, Moniruzzaman M. GC-MS analysis and antibacterial activity of *Trigonella foenum-graecum* against bacterial pathogens. Free Radicals Antioxid. 2016;6:109-4.
19. Priyanka C, Kumar P, Bankar SP, Karthik L. In vitro antibacterial activity and gas chromatography–mass spectroscopy analysis of *Acacia karoo* and *Ziziphus mauritiana* extracts. Journal of Taibah University for Science. 2015 Jan 31;9(1):13-9.
20. Keskın D, Ceyhan N, Uğur A, Dbeys AD. Antimicrobial activity and chemical constitutions of West Anatolian olive (*Olea europaea* L.) leaves. Journal of Food, Agriculture & Environment. 2012 Apr;10(2):99-102.