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# Isolation, characterization, antimicrobial and other bioactivity profiles of three *Streptomyces* strains isolated from Lake Gerio, Yola, Adamawa State, Nigeria



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## **Abstract**

**Background:** Actinomycetes are prolific producers of bioactive compounds which can be used to combat microbial infections. Microbial infections have resulted to increase in mortality and morbidity rates world-wide, especially in developing nations. This study was carried out to isolate and identify actinomycetes with potentials of synthesizing bioactive compounds, to produce and partially purify antimicrobial extracts, to characterize bioactive compounds in the extracts, to assess their antibacterial activity and to profile other biological properties of the bioactive compounds synthesized by the actinomycetes.

**Results:** Streptomyces sp. SUI (MT584797), Streptomyces sp. SW72IV (MT584818) and Streptomyces sp. SW72VII (MT584816) were isolated and identified. Ultraviolet–visible spectra absorption ranged between 241 and 251 nm wavelengths showing the polyene or chromone and unsaturation nature of the natural compounds. Infrared (IR) spectra indicated functional groups such as hydroxyl, aliphatic bromo, carbonyls, esters, carboxylic acids and silicon oxy compounds in the compounds produced by the three strains. Gas Chromatography-Mass Spectrum (GC–MS) identified elaidic acid isopropyl ester (32.11%), Octadec-9-enoic acid (17.44%) and 2, 3-dihydroxyl elaidate (10%) to be mostly produced by Streptomyces sp. SUI, Streptomyces sp. SW72IV and Streptomyces sp. SW72VII respectively. The three strains exhibited antimicrobial activity against Bacillus sp. Pseudomonas aeruginosa ATCC 9077, Staphylococcus aureus ATCC 700699, Candida albicans and Asperailus flavus.

**Conclusion:** The results showed that the three strains of *Streptomyces* could be sources of antimicrobial bioactive compounds and other secondary metabolites that can be used in the production of pharmaceutical bioactive agents that are effective against pathogens, and production of biological materials that can be used in cosmetics and food industries.

**Keywords:** Batch fermentation, Biologically active compounds, Natural products, *Streptomyces* strains, 16S rRNA

## **Background**

Actinobacteria are a diverse group of Gram positive filamentous bacteria. The members of this group consist of most important microflora found in most places such as

soil, fresh water, marine, lakes as well as dumping sites. Extreme and unexplored areas in the environment could be home to antimicrobial sources (Qinn et al. 2020). They are characteristically known to produce distinctive odor from a chemical compound called geosmin usually perceived when soil is tilled. Actinomycetes play important roles in recycling of organic materials in the environment (Malibari 1991).

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The most studied genus has been Streptomyces for its bioactive compounds. They are endowed with secondary metabolites that are bioactive in nature. Most of these metabolites are antimicrobials, enzymes, enzyme inhibitor, antioxidants, anticancer and so on (Barka et al. 2016; Sharma and Thakur 2020). They are prominent producers of so many natural products that have found applications in pharmaceutical, food and chemical industries (Berdy 2005). Natural products obtained from actinobacteria contain bioactive compounds that are diverse in structures and functions. Most drugs primarily originated from these actinobacteria and are active against many emerged and re-emerging pathogens (Tripathi et al. 2004; Singh et al. 2012). Most known antimicrobial drugs applied in agriculture and medicines have been sourced from natural bioactive compounds synthesized by actinobacteria (Sanglier et al. 1996). About seven thousand compounds documented in the compendium of natural products are from the actinobacteria (Berdy 2005). Hence, their ability to produce these compounds is very essential to pharmaceutical and chemical industries (Berdy 2005). It is because of this that the research efforts at sourcing for novel strains of actinobacteria that possess the ability to synthesize bioactive compounds have been intensified especially in recent times (Singh et al. 2008). The last few years have witnessed a surge in the number of Streptomyces isolated and identified (Nandhini et al. 2015). Abd-Ellatif et al. (2019) isolated a marine strain that was capable of producing linoleic acid. Linoleic acids and its derivatives have useful applications in the treatment of cancer, depression and high blood pressure. Pheromone for instance has been identified among metabolites synthesized by Streptomyces lavendulocolor UHB-9 that has the potential of producing cishecadecenal. Cis-hexadecenal is an antimicrobial and a pheromone. It is a world-wide research activity to search for strains of actinobacteria with the potential to produce secondary metabolites with active biological properties that can have wide applications in pharmaceutical, industrial and agricultural sectors (Siddharth et al. 2020).

Resistance to antibiotics is spreading rapidly and this has attracted the attention of researchers to shift their focus to the discovery of novel and potent bioactive compounds of actinobacteria origin that can be used to treat infections caused by microbes (Sebak et al. 2021). Microbial infection is responsible for high mortality and morbidity rates especially in poor and developing countries of the world. According to Centre for Disease and Control, (CDC), resistance to antimicrobial drugs is responsible for nearly a million deaths annually (CDC 2019). It has debilitating effects on the economic power of patients and generally lowers productivity generally (Siddharth et al. 2020).

The objectives of this study were to isolate and identify Actinobacteria isolates from Lake Gerio, Yola, Adamawa State, Nigeria, to characterize secondary metabolites synthesized by the Actinobacteria and to assess their antibacterial activity as well as to profile the biological properties of different secondary metabolites produced by the identified strains.

### Methods

# **Sampling location**

Lake Gerio is located within 9° 16′N–9° 21′N and 12° 24′E–12° 27′E of Yola, Adamawa State, North eastern part of Nigeria. It was naturally formed from cut off creek of River Benue that runs in between Girei and Yola North Local Government Area Councils of the State. It is about 2,500,000 m² in size and it is mostly used for fishing and farm irrigation (Abiodun and Miller 2007). Water samples were collected in June 2019. The pH of the water sample collected was between 6.8 and 9.0 while the temperature was 33 °C.

# Isolation of actinomycetes from collected Lake Gerio samples

Ten samples each of water were collected randomly from different points within Lake Gerio using sterile sampling bottles. The samples were immediately transported in a sterile container to the Industrial Biotechnology laboratory, Department of Biotechnology, Modibbo Adama University of Technology, Yola, Nigeria.

Actinobacteria were isolated using pour-plate technique after each sample was serially diluted according to previously described method of Harrigan and McCance (1976). 1.0 mL of serially was pour-plated in starch casein agar. Incubation was done at  $28\pm2$  °C for 5–7 days. Different formed colonies were sub-cultured on starch casein agar and incubated for another 5–7 days at  $28\pm2$  °C to obtain pure cultures which were preserved on starch casein agar slants at 4 °C in a refrigerator (Hisense). The pure cultures were used for further analyses.

# Screening for antibacterial activity in isolated actinobacteria

Antibacterial activity of the isolated actinomycetes was assessed by perpendicular streaking method described by Oskay (2009). Screening for antibacterial activity was done on Mueller Hinton agar with *P. aeruginosa* ATCC 9077 and *Bacillus* sp. *P. aeruginosa* ATCC 9077 was obtained from Biological Science Department, Redeemers University, Ede, Osun State, Nigeria while *Bacillus* sp. was a local isolate from obtained from Microbiology department, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria. Those that exhibited

inhibitory activity against the two test bacteria were selected for cultural, biochemical characteristics study and molecular identification using 16S rRNA gene.

# Cultural and biochemical characteristics and molecular identification of the selected isolates

The cultural and morphological study of the three selected actinobacteria isolates SUI, SW72IV and SW72VII were done using the description in International *Streptomyces* Project with five ISP media, starch casein agar and nutrient agar (Shirling and Gottlieb 1966a, b; Küster and Williams 1964; Atta and Ahmad 2009) while pigment production was observed on three ISP media (Shirling and Gottlieb 1966a, b).

The abilities of the selected isolates to utilize different sugars and to produce enzymes were carried out using the methods described by Fawole and Oso (2004) and Collins et al. (1995). Sugars that included glucose (control), galactose, arabinose, glycerol, xylose, mesoinositol, rhamnose, D-mannitol starch, lactose, maltose, sucrose and fructose were used to test for sugar utilization. The potential of *Streptomyces* sp. SUI, *Streptomyces* sp. SW72IV and *Streptomyces* sp. SW72VII to produce oxidase, catalase, urease, gelatinase was done using the method of Collins et al. (1995). Methyl red and Voges Proskaeur tests were carried with the method described by Prescott et al. (1991).

# Molecular identification of the selected actinomycetes using 16S rRNA gene

# Extraction of genomic DNA

Pure cultures of *Streptomyces* isolates SUI, SW72IV and SW72VII were grown in starch casein broth and incubated on a shaker at 250 rpm and at a temperature of  $28\pm2$  °C for 5 days. The suspension was cold centrifuged at 10,000 rpm for 10 min. The mycelium was then used for genomic DNA extraction using Norgen DNA extraction kit (Model 24700 Norgen, Canada) according to the instructions of the manufacturer. The extracted DNA was purified and stored on ice pack for PCR amplification.

# Identification of the actinomycetes isolates *PCR amplification*

The 16S rRNA gene of actinomycetes isolates SUI, SW72IV and SW72VII were amplified using forward primer 243F 5'GGATGAGCCCGCGGCCTA3' and reverse primer A3 5'CCAGCCCCACCTTCGAC-3' (Monciardini et al. 2002). The PCR reaction was carried out with a final volume of 50  $\mu$ L that contained 1.0  $\mu$ L of template DNA, 10 mM Tris–HCl 1.5 U of Taq DNA polymerase (Applied Biosystem), 1.5 mM MgCl<sub>2</sub>, each primer was 500 nM while for each dNTP 0.2 mM was added (Inqaba Biotechnological, Pretoria, South Africa). The

PCR reactions were carried out in PCR system (Applied Biosystem) with initial denaturation at 95 °C for five minutes. This was concluded with an extension at 72 °C with lasted for 10 min. The amplified product of 2  $\mu$ L was confirmed on agarose gel electrophoresis of 1.0% (w/v) agarose powder dissolved in TBE buffer of pH 8.2 at 100 V for 90 min done using Bio Rad set. A molecular ladder of 100 bp (NEB's fast DNA ladder) was added to serve as standard. After this, the gel was tracked with xylene cyanol FF and the gel was viewed under the illumination of UV light (Sanger and Coulson 1975).

# Sequencing of amplified 16S rRNA gene and phylogenetic analysis

The amplified DNA was purified by PCR clean up and then it was sequenced in an automated DNA sequencer (BigDye Terminator Kit in a 3510 ABI Sequencer, Ingaba Biotechnological, Pretoria, South Africa) using the dideoxy chain termination method (Sanger and Coulson 1975). The 16S rRNA gene sequences of isolates SUI, SW72IV and SW72VII were analyzed and comparison with other deposited sequences was done using Entrez search engine at NCBI. The similarity between each isolate and typed Streptomyces strains at GenBank was done using BLAST program. Furthermore, multiple sequence alignment as well as the construction of the phylogenetic trees was done with molecular evolutionary genetics analysis (MEGA X) (Kumar et al. 2018). The gene sequences for Streptomyces sp. SUI, Streptomyces sp. SW72IV and Streptomyces sp. SW72VII were deposited at National Center for Biotechnology Information (NCBI) and they were given accession numbers.

# Production, extraction and partial purification of the bioactive compounds

Production of the secondary metabolites was carried out by submerged fermentation. A three days old seed inoculum of 100 mL was used to inoculate 5000 mL of sterile starch casein broth and was incubated at  $28\pm2\,^{\circ}\mathrm{C}$  for 10 days. The fermentation was halted on the tenth day and the broth was filtered using Whatman No1 filter paper. The filtrate was then mixed with ammonium sulphate at 70% (w/v) to precipitate out proteinous materials. The resulted suspension was centrifuged at 10,000 rpm for 10 min.

Furthermore, the solvent extraction of the bioactive compounds was carried out by mixing equal volume of graded ethyl acetate and the filtrate in a separating funnel. The suspension was thoroughly mixed together for about 30 min and allowed to settle down for 20 min. The extract recovered from the ethyl acetate was partially purified using a column chromatography. The column was packed with solid phase silica gel of 100–200

mesh while ethyl acetate was used as mobile phase. 3.0 mL fractions were collected from the column till the introduced crude extract was completely eluted. Each fraction was tested for antibacterial activity and those that exhibited activity were pooled together and concentrated in rotatory evaporator at 60 °C.

#### **Antimicrobial determination**

Determination of antimicrobial activity of the actino-bacteria strains was determined with *Bacillus* sp., *P. aeruginosa* ATCC 9077, *S. aureus* ATCC 700,699, *C. albicans* and *A. flavus* using agar well diffusion and disc diffusion methods (Wu 1984; Bauer et al. 1959). A well of 6 mm was made at the center of the petri dishes where the partially purified extract of 40 μL of 1 mg/mL concentration was introduced. Ethyl acetate (99.5%) was use as the negative control while both gentamicin (Oxoid, 10 μg) and fluconazole (Oxoid 25 μg) were used as positive controls. The diameter of the zone of inhibition was measured with a metric ruler and recorded in millimeter. All data were recorded in triplicates and mean values with standard deviation were determined.

# Characterization of chemical compounds in the partially purified extracts

## The UV-Vis, IR and GC-MS spectra

The UV-Vis spectra of the partially purified extracts were determined between the range 200-900 nm wavelengths using a UV-Jenway Model 6705 (California, USA) while the IR spectrum was determined with Buck Scientific Model 530 spectrophotometer (East Norwalk, USA) in scanned wavelengths that were between 4000 and 400 cm<sup>-1</sup>. Characterization of the bioactive compounds present in the extract was carried out with gas chromatography coupled to a mass spectrometer in Shidmazu GC 2010 (Japan) coupled with slit injection detector system as described by Yakubu et al. (2018). Briefly, 1.5 µL of the partially purified extract was injected into a capillary column of dimension while helium gas was used as carrier which flowed at the rate of 1.5 mL/min. The temperature of the column was initially set at 60 °C and lasted for 3 min. The temperature was raised to 250 °C and maintained throughout the running of the sample. The mass spectrometer was operated in the electron ionization mode at 70 eV while scanning was between 45 and 600 m/z. The identification of the compounds in the extract was done by comparing the spectral analyses of the extract with those at National Institute for Standard and Technology (NIST, USA) library.

## **Results**

#### Isolation and screening for bioactive actinobacteria

In this study three *Streptomyces* isolates SUI, SW72IV and SW72VII isolated from Lake Gerio were observed to demonstrate inhibitory activity against test strains such as *Bacillus* sp., *P. aeruginosa* ATCC 9077 and *S. aureus* ATCC 700699.

# Cultural, morphological and biochemical characteristics and molecular identification of the actinomycetes

The cultural, morphological and biochemical characteristics of the three selected isolates were carried out on five different International *Streptomyces* Program (ISP) media (ISP1, ISP2, ISP4, ISP5 and ISP7), starch casein agar and nutrient agar as presented in Table 1.

Abundant growth by *Streptomyces* sp. SUI was recorded on all the ISP media used as well as on starch casein and nutrient agars. *Streptomyces* sp. SW72IV had abundant growth on ISP1, ISP5, Starch casein and nutrient agar but poor growth was observed on ISP2, ISP4 and ISP7 media while *Streptomyces* sp. SW72VII showed abundant growth on ISP1, ISP2, ISP5, ISP7, Starch casein and nutrient agars but poor on ISP4 only. The three isolates were rough in appearance on the media used to study their cultural and morphological characteristics except in some few cases where they appeared smooth and shiny. Additionally, isolate SUI, SW72IV and SW72VII had different diffusible pigments on ISP2, ISP5 and ISP7 media.

Biochemical characteristics of the three isolates as presented in Table 2 showed that isolate SUI metabolized glucose, galactose, fructose, glycerol, arabinose, rhamnose, D-mannitol, meso-inositol, starch and xylose while isolate SW72IV used only five of the sugars that included glucose, glycerol, D-mannitol, meso-inositol and xylose. However, isolate SW72VII did not ferment any of the sugars used. Streptomyces sp. SUI was positive to catalase, urease, gelatinase, oxidase and Gram's reaction but negative to Voges-Proskauer. However, Streptomyces sp. SW72IV showed reactions to catalase, Voges Proskauer, gelatinase and Gram staining but negative to methyl red, urease and oxidase. Streptomyces sp. SW72VII was only positive to catalase, gelatinase and Gram reaction but negative to methyl red, Voges Proskauer, urease and oxidase.

The 16S rRNA gene nucleotide sequences were used to identify the three actinobacteria isolates. Isolates SUI, SW72IV and SW72VII showed similarity indices of 97%, 99% and 99% making them to be closely related to *S. acrimycini* strain NBRC 12736, *S. pseudogriseolus* strain NRRL B-3288 and *S. fradiae* strain

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Table 1 Cultural characteristics of Streptomyces sp. SUI, Streptomyces sp. SW72IV and Streptomyces sp. SW72VII

Characteristics	Streptomyces sp. SUI	Streptomyces sp. SW72IV	Streptomyces sp.SW72 VII
ISP1			
Growth	Abundant	Abundant	Abundant
Aerial color	metallic brass	Lovry	Metallic brass
Reverse color	metallic brass	Lovry	Metallic brass
Texture	Smooth and shiny	Smooth and shiny	Rough and dry
ISP2			
Growth	Abundant	Poor	Abundant
Aerial color	Metallic brass	Metallic brass	Metallic brass
Reverse color	metallic brass	Metallic brass	Metallic brass
Texture	Smooth and shiny	Smooth and shiny	Rough and dry
ISP4			
Growth	Abundant	Poor	Poor
Aerial color	Cream	Cream	Cream
Reverse color	Cream	Cream	Cream
Texture	Smooth and shiny	Smooth and shiny	Smooth and shiny
ISP5			
Growth	Abundant	Abundant	Abundant
Aerial color	Tan	Lovry	Cream
Reverse color	Whitish	Lovry	Cream
Texture	Rough and dry	Smooth and shiny	Rough and dry
ISP7			
Growth	Abundant	Poor	Abundant
Aerial color	Cream	Silver	Cream
Reverse color	Whitish	Silver	Cream
Texture	Smooth and shiny	Smooth and shiny	Rough and dry
Starch Casein agar			
Growth	Abundant	Abundant	Abundant
Aerial color	lvory	Cream	Ivory
Reverse color	lvory	Cream	Ivory
Texture	Smooth and shiny	Smooth and shiny	Rough and dry
Nutrient agar			
Growth	Abundant	Abundant	Abundant
Aerial color	lvory	Lovry	Ivory
Reverse color	lvory	Lovry	lvory
Texture	Smooth and shiny	Smooth and shiny	Smooth and shiny

ISP1 = Tryptone yeast extract agar, ISP2 = Yeast extract malt extract agar, ISP4 = Inorganic starch salt agar, ISP5 = Glycerol asparagine agar, ISP7 = Tyrosine agar

NBRC 12773 respectively. The three isolates have resemblances to other strains of *Streptomyces* strains at Genbank (Fig. 1). The 16S rRNA gene sequences of isolate SUI (Accession no MT584797), isolate SW72IV (Accession no MT584818) and isolate SW 7VII (Accession no MT584816) were deposited at NCBI GenBank. The results of the gel electrophoresis of the 16S rRNA gene were positive for the three strains of *Streptomyces* under study. The bands were estimated to be between 1.3 and 1.4 kb in size as shown in Fig. 2.

# Antimicrobial activity of *Streptomyces* sp. SUI, Streptomyces sp. SW72IV and *Streptomyces* sp. SW72sVII

The antimicrobial activity of the three strains presented in Table 3 showed that all the strains possessed antimicrobial activity against both Gram positive and Gram negative bacteria, *C. albicans* and *A flavus* making them to be broad spectrum in nature. The data presented in Table 3 indicated that *Streptomyces* sp. SW72VII was more active against the test strains with zones of inhibition of 20.4 mm against *Bacillus* sp. and 46.3 mm against *P. aeruginosa* ATCC 9077. These were quite higher than

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**Table 2** Pigment production, sugar utilization and biochemical characteristics of *Streptomyces* sp. SUI, *Streptomyces* sp. SW72IV and *Streptomyces* sp. SW72VII

	Streptomyces. sp. SUI	Streptomyces. sp. SW7IV	Streptomyces. sp. SW72VII
Pigment production	า		
ISP2	Tan	Stone	Gold
ISP5	Lovry	Lovry	Celadon
ISP7	Tan	Grey	Grey
Sugar utilization			
Glucose	+++	++	_
Maltose	+	_	_
Lactose	+	_	_
Sucrose	++	_	_
Galactose	+++	_	_
Fructose	+++	+	_
Glycerol	++	+++	_
Arabinose	++	_	_
Rhamnose	+++	_	_
D-mannitol	+++	++	_
Meso-Inositol	+++	++	_
Starch	+++	_	_
Xylose	+++	++	_
Other biochemical	tests		
Catalase	+ve	+ve	+ve
Methyl red	+ve	-ve	-ve
Voges Proskauer	-ve	+ve	-ve
Urease	+ve	-ve	-ve
Gelatinase	-ve	+ve	+ve
Oxidase	+ve	-ve	-ve
Gram's reaction	+ve	+ve	+ve
-			

-ve = Negative, +ve = Positive, - = not utilized, + = fairly utilized, ++ = utilized, +++ = Highly utilized

22.3–25.1 mm recorded with Gentamicin, the positive control. The antifungal activity was highest against *C. albicans* and *A. flavus* with extract of *Streptomyces* sp. SW72IV with zones of inhibition of 42.2 mm and 29.4 mm respectively while the lowest zone of inhibition were 20.3 mm and 24.2 mm. The negative control, ethyl acetate had no effect on the test strains.

# The UV-Vis, IR and GC-MS spectra

The UV–Vis spectra of the bioactive compounds synthesized by the three strains of actinobacteria showed the highest peaks of UV–Vis absorption between 241 and 251 nm (Fig. 3). However, a smaller noticeable absorption was observed at wavelength 287 nm for *Streptomyces* sp. SW72IV.

IR spectra of the compounds present in the partially purified ethyl acetate extracts of the three strains showed absorption bands of wave numbers that ranged

between 604 and 633 cm<sup>-1</sup>. These wave numbers indicated the presence of aliphatic bromo compounds, 760–999 cm<sup>-1</sup> showed the presence of aromatic ring and plane compounds and peaks that ranged between 3412 and 3685 cm<sup>-1</sup> pointed to the presence of alcohol and hydroxyl functional groups. Similarly, wave bands that were between 1714 and 1722 cm<sup>-1</sup> were observed in the spectra of the ethyl acetate extracts of the three actinobacteria strains studied (Fig. 4).

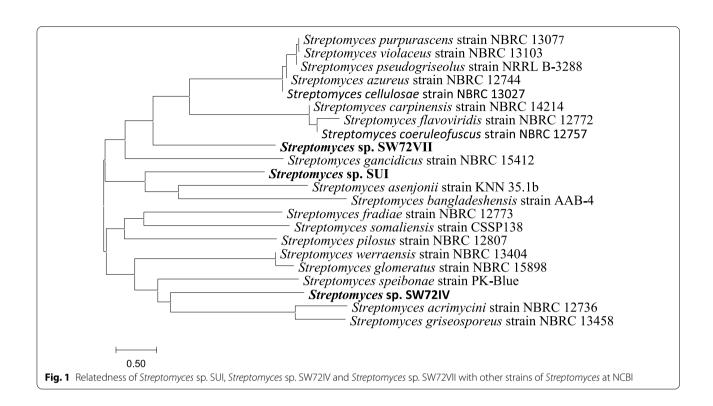
The GC-MS spectra of the partially purified extract characterized and identified thirteen, thirty four and thirty two compounds in extracts of Streptomyces sp. SUI, Streptomyces sp. SW72 IV and Streptomyces sp. SW72 VII respectively (Additional file 1: Tables S1-S3). The abundance of each compounds showed that Elaidic acid, isopropyl ester, C21H42O2 was mostly produced by Streptomyces sp. SUI. Its percentage abundance was 32% while 11-Hexadecenoic acid-15-methyl ester with 0.93%. Furthermore, Octadec-9-noic acid, C<sub>18</sub>H<sub>34</sub>O<sub>2</sub> was abundantly synthesized by Streptomyces sp. S72IV with 17.44% while 9-cycloheptadecen-1-one, (Z),  $C_{17}H_{30}$  and 3-Eicosene, (E) both with 0.02% were least produced by the strain. Streptomyces sp. S72VII produced more of 2, 3-dihydroxy propyl elaidate C<sub>21</sub>H<sub>40</sub>O<sub>4</sub>. It had 10.0% abundance and the least synthesized was Cyclopentacontane acetic acid, C<sub>7</sub>H<sub>12</sub>O<sub>2</sub>. These three major compounds have been reported to be biologically active exerting different functions as presented in Additional file 1: Tables S1-S3. The chemical characteristics as well as the biological properties of most of the identified compounds are presented in (Additional file 1: Tables S1-S3) for Streptomyces sp. SUI, Streptomyces sp. SW72 IV and Streptomyces sp. SW72 VII respectively.

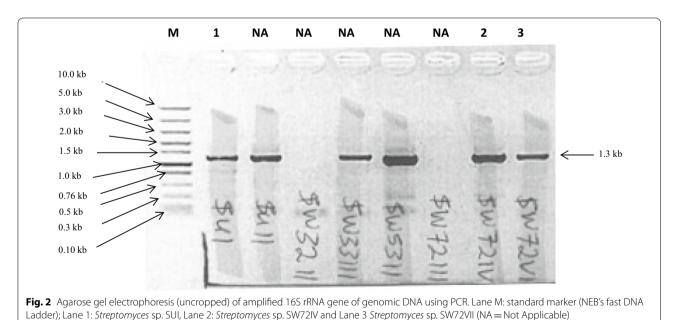
# Discussion

## Isolation and screening for bioactive actinobacteria

Several studies carried out on Lake Gerio are majorly on these two, fishing and dry season farming (Ekundayo et al. 2014; Sogbesan et al. 2018). However, no report has been presented in exploring the actinobacteria present and their bioactive compounds that could be of benefits to man. Different environment especially marine and desert have been reported to harbor actinomycetes that are sources of potent bioactive compounds (Valli et al. 2012; Nithya et al. 2018). The same could be said of Lakes which has been explored for its bioactive compounds from Actinomycetes (Gebreyohannes et al. 2013). The run off from surrounding farm areas during raining season could be a source of organic wastes which might have supported the proliferation of bacteria in the Lake. This was earlier mentioned by Achife et al. (2021). In this study, three strains of Streptomyces were observed to be

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active against both Gram positive and negative bacteria, which confer them of pharmaceutical importance. Gebreyohannes et al. (2013) had previously mentioned the antimicrobial activity of actinobacteria isolated from Lake Tana in Ethiopia against both Gram positive and Gram negative bacteria. Lakes being reservoir of

actinobacteria has been mentioned earlier by Gebreyohannes et al. (2013); Benhadj et al. (2019). Our findings were similar to the earlier reports by these authors who reported antibacterial activity of the *Streptomyces* species they worked with. Earlier submissions from Cho et al. (2012) and Sudha et al. (2018) showed that antibacterial

**Table 3** Antimicrobial activity of *Streptomyces* sp. SUI, *Streptomyces* sp. SW72IV and *Streptomyces* sp. SW72VII against some test strains

Actinomycetes isolate and controls	Indicator strains	Zone of inhibition (mm)
Streptomyces sp. SUI	S. aureus ATCC 700699	$18.4 \pm 0.2$
	P. aeruginosa ATCC 9077	$15.5 \pm 0.2$
	Bacillus sp.	$20.2 \pm 0.1$
	Candida albicans	$20.3 \pm 0.2$
	Aspergillus flavus	$28.2 \pm 0.1$
Streptomyces sp. SW72IV	S. aureus ATCC 700699	$18.5 \pm 0.3$
	P. aeruginosa ATCC 9077	$14.5 \pm 0.2$
	Bacillus sp.	$20.4 \pm 0.2$
	C. albicans	$42.2 \pm 0.1$
	A. flavus	$29.4 \pm 0.2$
Streptomyces sp. SW72VII	S. aureus ATCC 700699	$46.3 \pm 0.2$
	P. aeruginosa ATCC 9077	$27.5 \pm 0.2$
	Bacillus sp.	$20.3 \pm 0.2$
	C. albicans	$22.4 \pm 0.3$
	A. flavus	$24.2 \pm 0.2$
Gentamicin (10 μg) (+ve control)	S. aureus ATCC 700699	$25.1 \pm 0.1$
	P. aeruginosa ATCC 9077	$22.3 \pm 0.3$
Fluconazole (25 µg) +ve control	Bacillus sp.	$24.2 \pm 0.1$
	C. albicans	$22.1 \pm 0.1$
	A. flavus	$25.2 \pm 0.2$
Ethyl acetate (99.5%)	S. aureus ATCC 700699	0.00
(Negative control)	P. aeruginosa ATCC 9077	0.00
	Bacillus sp.	0.00
	C. albicans	0.00
	A. flavus	0.00

activities of *Streptomyces* species isolated from the environment were reported against pathogens such as *P. aeruginosa*, *B. cereus* and *Klebsiella pnuemoniae* as well as methicillin and vancomycin resistant *S. aureus*.

# Cultural, morphological and biochemical characteristics and molecular identification of the actinomycetes

Abundant growth of Streptomyces sp. SUI, Streptomyces sp. SW72IV and Streptomyces sp. SW72VII were observed in almost all the ISP and casein agar and nutrient agar used for cultural study. These observations were slightly different from the report of Singh et al. (2018) who reported moderate and slight growth on the same ISP media for a strain of Streptomyces. A similar pattern of growth of abundance and good was mentioned by Daigham and Mahfouz (2021) with the species of Streptomyces they studied. The observations being reported along with previous submissions could be due to ability of the strains to use the media for growth while some of those media with poor or moderate growth might not support the strains for growth. Different aerial and reverse side colors were observed in the three strains studied. Varying colors of the aerial and substrate hyphae as well as melanoid pigments such as pink, red, black, yellowing green have been mentioned by different authors have been reported by Gebreyohaness et al. (2013), Singh et al. (2018), Daigham and Mahfouz (2021). Different aerial and substrate mycelial such as lovry, cream, ivory and metallic brass were observed in this study. The different aerial, substrate and melanoid colors noticed could be attributed to the physiological status of the strains used for the research.

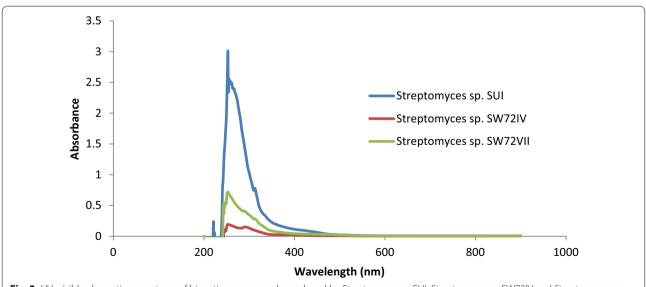
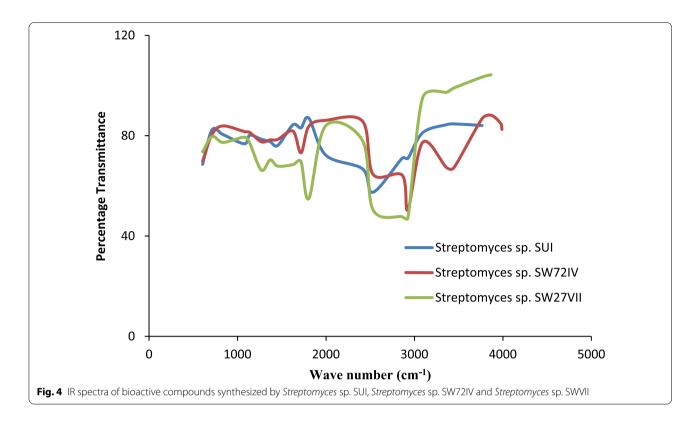


Fig. 3 UV-visible absorption spectrum of bioactive compounds produced by Streptomyces sp. SUI, Streptomyces sp. SW72IV and Streptomyces sp. SWVII

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In this study, it was observed that *Streptomyces* sp. SUI metabolized all carbon and energy sources used for study, Streptomyces sp. SW72IV utilized only seven out thirteen carbon and energy sources while Streptomyces sp. SW72VII did not metabolize any of the sugars as carbon and energy sources. These observations had been reported earlier (Salim et al. 2017; Ganesan et al. 2017; Aharonowitz and Demain 1978). The use of other media nutrients such as organic acids for energy and carbon requirement by actinomycetes was reported by Aharonowitz and Demain (1978) which could be responsible for the non-utilization of any of the sugars used in this study by Streptomyces sp. SWVII. The three strains studied exhibited different enzymatic properties such as catalase, urease oxidase and gelatinase activities. Varying enzyme activities are common to the actinobacteria. Enzymes are essential to the metabolic and physiological activities of these three strains, such as breaking down of macromolecules for growth and development. Sugars utilization and biochemical characteristics could be attributed to genes and physiological conditions of these isolates. The three strains of Streptomyces species SUI, SW72 IV and SW72 VII were filamentous in morphology and positive to Gram's reaction. These observations were similar to what was previously reported by Ganesan et al. (2017).

A distinct single ampilicon band of 1.3 kb and 1.4 kb of 16S rRNA gene was observed in the three strains studied. The 16S rRNA gene is the most studied gene used in the identification of bacteria up to species level. The sizes of the bands recorded in this study were within the range of 1.2 kb and 1.5 kb reported by Kumari et al. (2019) and Escalante-Rendiz et al. (2019). The slight difference noticed could be due to the level of purity of the extracted DNA.

# Antimicrobial activity of the three Streptomyces species

The three actinobacteria strains being reported exhibited antibacterial activity against both Gram positive and Gram negative bacteria, C. albicans and A. flavus making them to be broad spectrum in nature. However, Gram positive bacteria test strains were more sensitive to the inhibitory activity of the three Streptomyces studied. This could be attributed to the differences in the cell wall constituents. Gram negative test bacteria are less sensitive due to the presence of outer lipopolysaccharide layer, a hydrophobic material which renders the cell wall impermeable for lipophilic substances (Shirling and Gottlieb 1966a, b; Kim et al. 1994). Also the observed difference in the susceptibility of the Gram positive and Gram negative bacteria test organisms could due to secretions of different metabolites by the strains as an entity endowing them with broad spectrum activity (Benhadj et al.

2019). The findings in this work were similar to the previous reports of Ganesan et al. (2017) and Gebreyohaness et al. (2013) that stated the antimicrobial activity of isolated actinobacteria strains against different bacterial test strains. The synergistic antimicrobial activity of the bioactive compounds synthesized by the Streptomyces sp. SW72VII could be responsible for the higher inhibitory activity compared to gentamicin. Furthermore, the extract produced by the three strains exhibited inhibitory activity against C. albicans and A. flavus. Our findings are similar to previous report of Hadizadeh et al. (2015) who stated the inhibition of *A. fumigatus* by extract of *S.* rochei HF391. Sarika et al. (2021) reported the isolation of Streptomyces felleus BHPL-KSKU5 with anticandidal property. Additionally, the reported bioactivities of secondary metabolites synthesized by the three strains of Streptomyces used in this study showed that these strains possessed antifungal, antioxidant, anticancer, antiinflammatory properties.

#### The UV-Vis, IR and GC-MS spectra

Though the UV absorption recorded ranged between 241 and 251 nm wavelengths, the maximum absorption was recorded at 251 nm for the partially purified ethyl acetate extract of the Streptomyces SUI, Streptomyces sp. SW72IV and Streptomyces sp. SW72VII which suggested the presence of chromone-like nucleus structure in the bioactive compounds produced by the three strains. This was close to the earlier report of Singh et al. (2012) on the metabolites obtained from S. levis RS25. The strains was said to inhibit bacteria. Also, a smaller but noticeable absorption at wavelength 287 nm in the partially purified ethyl acetate extract of Streptomyces sp. SW72 VI might indicate the presence of another major compound. This absorption value was similar to that of extract from the strain G614C1 reported by Maleki and Maschinchian (2011) as well as to the report on *Streptomyces* isolated from soil samples of South-Eastern Serbia by Ilić et al. (2005) which showed maximum absorbance peaks of UV-Vis spectral data ranged between 215 and 270 nm. The maximum UV peaks observed with the compounds present in the partially purified extracts indicate high polyene nature of the bioactive compounds synthesized by the strains. Different UV-Vis maximum absorption has been absorbed at wavelength such as 213.5 nm, 225 nm, 263.5 nm, 324 nm and 529 nm (Ignacimuthu et al. 2017) while Charousova et al. (2018) observed maximum absorption at wavelengths 268 nm and 364 nm. According to Sudha et al. (2018) several metabolites of microbial origin exhibit characteristics absorption spectra in the UV-Vis region which can be give an insight into the identification of the compounds when the spectra are compared with the spectra documented in the library.

The bands recorded based on their wavelength showed that carbonyl groups functional groups (ketone and aldehyde), esters and carboxylic acids were present in the extracted compounds (Coates 2000; Singh et al. 2018). Functional groups such as aliphatic, silicon oxy, carboxylic acid, hydroxyl and carbonyl were identified in the extract (Ignacimuthu et al. 2018; Adesalu et al. 2016; Cetinyaka et al. 2020). The observed similarities could be attributed to similar bioactive compounds synthesized by these strains of *Streptomyces*.

Fermentation conditions such as carbon, nitrogen sources, temperature and pH to a large extent influence the production of metabolites by different strains of actinomycetes. A wide range of secondary metabolites that possessed different biological activities were present in the ethyl acetate extract of the three strains of *Streptomyces* studied in this work. The report being presented in this study was in agreement with the report of Elbendary et al. (2018) who also mentioned that bioactivity of actinobacteria was due to the presence of different bioactive molecules.

## **Conclusion**

The three strains of Streptomyces isolated from Lake Gerio, in Yola, Adamawa State, Nigeria Streptomyces sp. SUI, Streptomyces sp. SW72IV and Streptomyces sp. SW72VII exhibited broad spectrum antibacterial activity. The UV-Vis and IR spectra showed that the bioactive compounds produced by the three strains were unsaturated and polyene in nature. Elaidic acid isopropyl ester, Octadec-9-enoic acid and 2, 3-dihydoxypropyl elaidate was produced in abundance by Streptomyces sp. SUI, Streptomyces sp.SW72IV and Streptomyces sp. SW72VII respectively. These compounds have been reported to possess biological properties such as antimicrobial, antihypertensive, antioxidant and anticancer. The three strains Streptomyces sp. SUI, Streptomyces sp.SW72IV and Streptomyces sp. SW72VII can be used in the production of pharmaceuticals, particularly antibacterial and other bioactive compounds needed in cosmetics and food industries.

#### **Abbreviations**

NCBI: National center for biotechnology information; UV–Vis: Ultra violet–visible; IR: Infrared; GC–MS: Gas chromatography-mass spectrophotometer; ATCC: American type culture collection; RNA: Ribonucleic acid; DNA: Deoxyribonucleic acid; CDC: Center for diseases and control; g: Gram; mL: Milliliter; µL: Microliter; mg: Milligram; h: Hour; nm: Nanometer; cm<sup>-1</sup>: Reciprocal centimeter; Kb: Kilobase; PCR: Polymerase chain reaction; MEGA: Molecular evolutionary genetics analysis; dNTP: Deoxyribose nucleoside triphosphate; ISP: International Streptomyces program.

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# **Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s42269-021-00606-x.

**Additional file 1: Supplementary Table S1-S3.** Bioactive compounds identified by GC-MS produced by *Streptomyces* sp. SUI, *Streptomyces* sp. SW72IV and *Streptomyces* sp. SW72VII.

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#### Authors' contributions

Author OM conceived the idea, OM, MI and FV carried out the laboratory work, collection and analysis of data, OM drafted the original manuscript, proofread and corrected by MI and FV. All authors have read and approved the manuscript.

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## Availability of data and materials

The data that support the findings of this study are available at NCBI GenBank database. The 16S rRNA nucleotide sequences of the three strains used in this study can be accessed through the accession number MT584797 for *Streptomyces* sp. SUI, MT584818 for *Streptomyces* sp. SW72IV and MT584816 for *Streptomyces* sp. SW72VII.

#### **Declarations**

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing interest

The authors declare that they have no competing interest.

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