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Research Article

Methanol Crude Extract Derived from *Streptomyces* sp. PFK4: Antimicrobial, Antioxidant Properties, Potential Modes of Action, and Chemical Profile of Bioactive Compounds

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Abstract

Bacteria of the *Streptomyces* genus are a promising source of bioactive products, with several applications. This study aimed to evaluate the antimicrobial and antioxidant properties of the methanol crude extract of *Streptomyces* sp. PFK4, its modes of action, and bioactive metabolites. Zone of inhibition (ZI), minimum inhibition concentration (MIC), and minimum bactericidal concentration (MBC) were conducted to determine antibacterial activity. Bacterial cell and protein synthesis were assayed as potential modes of action of extract. The antioxidant properties were determined by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging and Ferric reducing assays (FRAP). The gas chromatography-mass spectrometry (GC-MS) was used to identify bioactive compounds. The mean ZI ranged from 11.00±0.33 to 20.60±0.88 mm with the highest ZI observed against *Salmonella typhi* and *Staphylococcus aureus*. The MIC ranged from 0.25 to 0.5 mg/mL and MBC from 0.25 to 2 mg/mL against bacterial pathogens. The crude methanol extract of *Streptomyces* sp. PFK4 showed the ability to inhibit Escherichia coli cell from 100 to 60.17% while protein synthesis was from 5.45 to 5.17 µg Eq BSA/g. It also exhibited DPPH and ABTS scavenging activities at concentrations of 50 µg/mL with the highest inhibition concentration of 83.74 ± 2.10% and 72.97 ± 2.67%, IC50 values of 100.8 µg/mL and 15.69 µg/mL respectively. The highest FRAP was 8.04 ± 0.85 µg/mL at a 200 µg/mL concentration. From the GC-MS analysis, twenty-three biological compounds primarily fatty acids or their esters were identified. This study demonstrates the methanol crude extract of *Streptomyces* sp. PFK4 possesses antibacterial and antioxidant activities; inhibits bacterial cell and protein synthesis ability; and contains mostly fatty acids and their derivatives recognized as therapeutic agents.

Keywords: *Streptomyces* sp. PFK4; Crude Methanol Extract; Antimicrobial; Modes of Action; Antioxidant Properties; GC-MS

Introduction

Pathogenic microorganisms are resistant to the available drugs. It is therefore an emergency to find new drugs. Research is now focused on searching for natural products as alternative agents in drug formulation. Microbial natural products are a source of several important drugs of high therapeutic value. The majority of commercially available pharmaceutical products are secondary metabolites or their derivatives produced by bacteria, fungi, and actinobacteria [1]. Among all living organisms, the actinobacteria phylum currently represents the most prospective group of microorganisms for the discovery of bioactive compounds such as antimicrobials, antitumor agents, antiparasitics, anticancer agents, enzymes, and some other endogenous metabolites with free radical scavenging activities properties [2-4].

Actinobacteria are a large group of high G+C Gram-positive bacteria [5]. They are reported to be a dominant microbial population in several ecosystems [6,7]. They are regarded as the most prolific source of bioactive compounds of several commercialized antibiotics. Approximately, 22 500 biologically active substances compounds are obtained from microorganisms, 45% of which are produced by Actinobacteria, mostly the *Streptomyces* genus [5,8]. *Streptomyces* is an important industrial group of microorganisms that is highly explored for the wide range of biologically active compounds [9]. Nearly seventy-five percent of all the known industrial antibiotics and numerous economically important compounds were obtained from *Streptomyces* [10].

Most *Streptomyces* are isolated from the rhizosphere, a unique biological niche with a diverse microflora comprising bacteria, actinobacteria, fungi, protozoa, and algae [11]. Our previous study showed that, *Streptomyces* sp. PFK4 isolated from the rhizosphere of cocoyam (*Xanthosoma sagittifolium* L. Schott) exhibited strong antagonistic activity against *Pythium myriotylum* (the causal agent of cocoyam root rot disease), and other pathogenic fungi and bacteria [12]. In the current study, we evaluated the antimicrobial and antioxidant properties of the crude methanol extract of *Streptomyces* sp. PFK4. Additionally, we determined his potential modes of action and its bioactive metabolites.

Material and Methods

Streptomyces sp. strain PFK4

Streptomyces sp. PFK4 (KY400017) used in this study was isolated from the rhizosphere of cocoyam in the Kumba locality around Mount Cameroon and characterized in our previous study [12]. Pure cultures were kept in 20% glycerol at -80°C for storage.

Crude extract preparation

Solid-state fermentation was adopted for the production of crude extract [13]. For the preparation of inoculum, *Streptomyces* sp. PFK4 was streaked on the Yeast Extract Malt Extract Agar (ISP2 medium) plates and incubated at 28°C for 7 days. The spores were scraped from the plate, inoculated into 25 mL of Yeast Extract Malt Extract Broth, and incubated in a rotary shaker for 48 h at 150 rpm at 28°C. After incubation, the inoculum was prepared by transferring the 2 days' cultures in 250 mL of ISP2 broth contained in a 1000 mL conical flask and incubated at 28°C for 7 days. About 50 g of wheat bran was added into a 1000 mL conical flask with 50 mL of distilled water and sterilized. Then 10% of inoculum was added into a conical flask containing sterile wheat bran. The flasks were incubated at 28±2°C for 30 days. After incubation, the fermented biomass of *Streptomyces* sp. PFK4 strain was mixed with methanol and macerated (3×24 h). The crude extract was collected and concentrated by evaporation. The quantity of crude extract was measured by adding the crude into the dried 100 mL pre-weighed beaker. After evaporation of the solvent, the weight of the crude extract was measured and stored in sterile vials.

Determination of antimicrobial assay of the methanol crude extract of *Streptomyces* sp. PFK4

Antibacterial and antifungal assays

The efficacy of the methanol crude extract of *Streptomyces* sp. PFK4 to inhibit bacteria was tested against four Gram-positive bacteria named *Streptococcus pneumonia* (ATCC 6465), *Haemophilus influenza* (ATCC 49247), *Staphylococcus aureus* (ATCC 43300), and *Bacillus cereus* and four Gram-negative bacteria named *Salmonella typhi*, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa*, *Salmonella enteritidis* by disc diffusion assay [14]. The reference bacterial cultures pathogens strains were obtained from the Laboratory of Clinical Microbiology of the University of Yaoundé 1. Bac-

terial inocula were prepared by growing cells in Mueller Hinton broth (MHB) for 24h at 37°C. The fungal pathogens strains were obtained from the Laboratory of Phytoprotection and Valorization of Genetic Resources of the Biotechnology Center of the University of Yaoundé I. The filamentous fungi were grown on Potato Dextrose Agar (PDA) slants at 28 °C for 14 days and the spores were collected using sterile double distilled water and homogenized. These microorganisms were maintained on an agar slant in a refrigerator at 4 °C.

The 24h on Mueller-Hinton Agar (MHA) test bacteria were inoculated into tubes containing sterile distilled water. Using sterile swabs, 100 µL of broth cultures of test bacteria were calibrated at 1.0 ×10^5 CFU/mL using a Mc Farland standard [15], were swabbed on sterile Mueller Hinton agar plates followed by depositing the sterile discs (6 mm) impregnated with 30 µL of methanol extract (20 mg/mL of 10% DMSO (Dimethyl sulphoxide). Antibiotic (Streptomycin, 1mg/mL) along with bacteria cultures was used as the positive control, and DMSO (10%) containing bacterial cultures was used as the negative control. The plates were incubated at 37°C for 24 h. The zone of inhibition formed was measured using a ruler. The experiment was carried out in triplicate and the average values were recorded.

The antifungal activity of crude extract was determined using the agar well diffusion method [16]. Antifungal bioassay was done on a 9 cm Petri plate with 20 mL of PDA. A 6 mm fungal plug was cut from the leading edges of a seven-day-old pure culture of *Pythium myriotylum*, *Phytophtora megakarya*, *Fusarium solani*, and was put in the center of the plate. A 6 mm diameter well was made at 1.5 cm from the fungal plug and another well at the opposite side, followed by pipetting 30 µL aliquot of each crude extract (20 mg/mL) into the two wells on each plate. Control plates contained wells of DMSO 10% and nystatin (2 mg/mL). Radial growth was recorded after incubation for 10 days. The radial growth of the fungal colony was recorded with a meter ruler along two diagonal lines drawn on the reverse side of each plate. The experiment was carried out in triplicate and the average value was recorded. The Percent Inhibition of Radial Growth (PIRG) of each treatment compared to control was computed utilizing the formula below:

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MICs of the methanol extract of *Streptomyces* sp. PFK4 were determined by microbroth dilution methods according to NCCLS (National Committee for Clinical Laboratory Standards) [15]. Bacterial and fungal pathogens were grown in sterile broth and 10 µL of log phase culture was added into 96 well micro titre plates. The crude extract was dissolved in 10% DMSO and diluted in different concentrations (16.000, 8.000, 4.000, 2.000, 1.000, 500, 250, 125, 62.5, 31.25, 15.62.and 7.81 µg/mL). Then, 10 µL of the bacterial and fungal suspension (1.0 × 10^5 CFU/mL) was inoculated into each well of a 96-well microplate, each containing a different concentration of the test agents. Diluted extracts and sterile broth were added into pre-coated microbial cultures, making up a total volume of 200 µL. Streptomycin (200 µg/mL) and nystatin (400 µg/mL) were used as the positive control for bacteria and fungi respectively, and culture medium (200 µL) was used as the negative control. The plates were sealed and incubated at 37 °C during 24h for bacteria and at 30 °C during 48 h for fungi. After incubation, MIC of extracts was revealed with 10 µL of iodonitrotetrazolium (2 mg/mL) by adding in each well and incubated for 30 minutes at room temperature. The wells where there was no coloration are considered as concentrations that possess inhibitory activity against pathogens and wells containing the smallest concentration uncolored were considered as the MIC.

The Minimum Bactericidal Concentration (MBC) values of the methanol crude extract of *Streptomyces* sp. PFK4 were determined [16]. A volume of 25 µL presents in wells of 1 MIC, 2 MIC, and 4 MIC have been introduced in microplates containing 175 µL of Yeast Malt Extract Agar (YMEA) and Potatoes Dextrose Broth (PDB) plates control for bacteria and fungi, respectively. In the control well, 200 µL of culture media were introduced. The plates were incubated at 37°C for 36 h for bacteria and at 26°C for 72 h for fungi. The growth of pathogen microorganisms was revealed with 10 µL of iodonitrotetrazolium (2 mg/mL) added in each well and incubated for 30 minutes at room temperature. Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of the test agent at which no microbial growth was observed on the plates.

Determination of the modes of action of the methanol crude extract of Streptomyces sp. PFK4

Bacterial cell lysis (Bacteriolysis)

The modified protocol of Limsuwan and Voravuthikunchai [17] was used to conduct this experiment. For this purpose, a standardized *Escherichia coli* (the most sensitive bacteria pathogen to the extract) suspension at the 0.5 McFarland scale in 0.9% NaCl was prepared. A volume of 100 µL of the methanol crude extract of *Streptomyces* sp. PFK4 was introduced into different tubes containing this suspension to have concentrations of the suspension equivalent to the Minimum Inhibitory Concentration (1 MIC) and half Minimum Inhibitory Concentration (0.5 MIC) in the medium. The suspensions obtained were incubated at 37°C while stirring. At times 0 h, 2 h, 4 h, and 6 h, the absorbance was measured at 620 nm. The absorbance at zero hours (0 h) was used to evaluate the relative absorbance (Ar) at different times to draw the Ar= f (t) curve.

Inhibition of bacterial protein synthesis

In five tubes each containing 9 mL of suspension Mueller Hinton, 0.5 mL of *Escherichia coli* suspension (standardized to McFarland's 0.5 scale) was added to each tube. The methanol crude extract of *Streptomyces* sp. PFK4 (100 µL) was then added to each preceding mixture to give the concentrations: 1 MIC and 0.5 MIC. The control tube was treated under the same conditions and received 0.5 mL of MHB instead of the extracts. The tubes were incubated at 37°C with a rotation of 80 rpm. After 24 h of incubation, centrifugation at 13.000 rpm for 2 minutes was used to recover the bacterial cells, which were weighed and mixed with the lysis buffer (tris-maléate 0.05 M pH= 10.2) at a rate of 40 mg of bacteria per 500 mL of buffer. After 1 h of incubation, centrifugation at 13.000 rpm for 3 minutes allowed the recovery of the supernatant containing the proteins, which were then assayed by the Bradford reagent (Sigma-Aldrich) using the microplate reader (FLUOstar Omega Microplate Reader). The lysis buffer constituted the blank [18].

Evaluation of antioxidant activities of methanol crude extract of *Streptomyces* sp. PFK4

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)

To evaluate the radical scavenging efficiency of the methanol crude extract of *Streptomyces* sp. PFK4, we have employed DPPH free radical scavenging assay [19]. Briefly, 500 µL of DPPH solution (0.0016% in methanol) was mixed with 500 µL of different concentrations of the crude methanol extract of *Streptomyces* sp. PFK4 (50, 100, 200, and 400 µg/mL), and reference standard (ascorbic acid) (0.5, 1, 2, and 4 µg/mL) in separate tubes. The tubes were incubated in the dark at room temperature for 20 minutes and the optical density was measured at 515 nm using a UV-Visible spectrophotometer. The absorbance of the DPPH control (without extract/standard) was noted. The scavenging activity (%) was calculated using equation 1:

DPPH scavenging activity (%) = (Absorbance of Control-Absorbance of sample)/(Absorbance control) ×100

2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay (ABTS)

The ABTS scavenging antioxidant assay was conducted to investigate ABTS scavenging potential of the methanol crude extract of *Streptomyces* sp. PFK4. The ABTS antioxidant assay was performed according to Tan., *et al*. [20]. In short, 100 µL of ABTS solution (0.0016% in methanol) was mixed with 500 µL of different concentrations of the methanol crude extract of *Streptomyces* sp. PFK4 (50, 100, 200, and 400 µg/mL), and reference standard (ascorbic acid) (0.5, 1, 2, and 4 µg/mL) in separate tubes. Tubes were incubated in the dark at room temperature for 30 minutes and the optical density was measured at 734 nm using a UV-Vis spectrophotometer. The absorbance of the ABTS control (without extract/standard) was noted. The scavenging activity (%) was calculated using the formula:

ABTS scavenging activity (%) = (Absorbance of control-Absorbance of sample)/(Absorbance control) × 100

Ferric reducing antioxidant power (FRAP) assay

The reducing potential of the methanol crude extract was determined by ferric reducing assay [21]. In this assay, 100 µL of different concentrations of the methanol crude extract of *Streptomyces* sp. PFK4 (50, 100, 200, and 400 µg/mL) and ascorbic acid (reference standard) (0.5, 1, 2, and 4 µg/mL) in 1 mL of methanol were mixed separately with 0.5 mL of phosphate buffer (200 mM, pH 6.6) and 0.5 mL of 1% potassium ferricyanide. The tubes were incubated at 50°C for 30 minutes in a water bath, cooled rapidly, and mixed with 0.5 mL of 10% trichloroacetic acid and 0.05 mL of 0.1% ferric chloride. After 30 minutes in the dark, the amount of iron (II)-ferricyanide formed was determined by measuring the formation of Perl's Prussian blue at 593 nm. An increase in absorbance on

an increase in concentration indicates increased reducing power. EDTA was used as a positive control.

Metal chelating activity (%) = (Absorbance of control-Absorbance of sample)/ (Absorbance control) × 100

Gas chromatography-mass spectrometry analysis (GC-MS) of the crude methanol extract of *Streptomyces* sp. PFK4

The methanol crude extract of *Streptomyces* sp. PFK4 (10 µL) was subjected to gas chromatography-mass spectrometry (GC-MS) analysis on GC-MS-5975 (Agilent Palo Alto, CA, United States), column DB 5MS Agilent, dimension length Z 30.0 m, Internal Diameter (ID) Z 0.2 mm, film thickness Z 0.25 mm, with a temperature program of 70-300 °C, 10°C/minute, injection temperature Z 240°C, carrier gas Z helium, flow rate Z 1.51 mL/minute, equipped with GC-MS NIST-II library. Each metabolite's relative amount was calculated by comparing its average peak area to the overall area. Identifying the isolated volatile metabolites was done using retention indices and mass spectrometry with the NIST library database [22].

Statistical analysis

All the analysis was done in triplicate (n = 3) as well as all results were expressed as mean ± deviation. Statistical analysis of collected data was performed using GraphPath prism 9.00 software using Tukey's multiple comparison tests, the significance difference was chosen at $P<0.05$.

Results

Streptomyces sp. PFK4 extraction yield

The *Streptomyces* sp. PFK4 strain showed good growth in the wheat bran used as substrate. From 1150 g of wheat bran, is found the yield 24.11% with methanol used as an extracted solvent. Morphological characteristics of the crude methanol extract of *Streptomyces* sp. PFK4 is represented in Table 1.

Table 1: Characteristics and extraction yield of the methanol crude extract of *Streptomyces* sp. PFK4.

PFK4 extract	Color of extract	Aspects	Initial weight	Final weight	Extraction yield
	Brown	Oily	1150g	277.35g	24.11%

Antimicrobial activity of the methanol crude extract of *Streptomyces* sp. PFK4

Antibacterial and antifungal activities

The methanol crude extract of *Streptomyces* sp. PFK4 exhibited antibacterial and antifungal activities against Gram-positive, Gram-negative bacteria, and fungi tested. The zone of inhibition values of the methanol crude extract of *Streptomyces* sp. PFK4 against bacteria pathogens ranged between 12.00 ± 0.33 mm and 20.66 ± 0.67 mm, but these values were lower than those obtained with the standard Streptomycin (Table 2). Among the fungi pathogen tested, a reduction of mycelial growth was significantly observed against *Pythium myriotylum* (30.01%) (Table 2).

Table 2: Antibacterial and antifungal activities of the methanol crude extract of *Streptomyces* sp. PFK4.

Test microorganisms	Zone of inhibition(mm)		
Bacteria	**Methanol extract**	**Streptomycin**	**DMSO**
Salmonella typhi	20.66 ± 0.67a	23.30 ± 0.33a	0.00
Salmonella typhimurium	20.30 ± 0.88a	21.30 ± 0.88b	0.00
Escherichia coli ATCC 25922	12.00 ± 0.57d	21.00 ± 0.57b	0.00
Pseudomonas aeruginosa	14.00 ± 0.57c	20.00 ± 0.00b	0.00
Bacillus cereus	15.00 ± 0.57c	24.00 ± 1.52a	0.00
Streptococcus pneumoniae ATCC 6465	17.30 ± 0.33b	20.00 ± 0.00b	0.00
Haemophilus influenza ATCC 49247	15.60 ± 0.57c	23.60 ± 0.33a	0.00
Streptococcus aureus ATCC 43300	20.60 ± 0.33a	24.60 ± 0.33a	0.00
Fungi	**Percentage of inhibition (%)**		
	Methanol extract	**Nystatin**	**DMSO**
Pythium myriotylum	32.01 ± 0.5a	51.42 ± 1.2	0.00
Pytophthora megakarya	30.57 ± 0.4a	43.11 ± 0.3	0.00
Fusarium oxysporium	27.51 ± 0.2b	55.00 ± 0.9	0.00
ATCC: American Type Culture Selection; DMSO: Dimethylsulfoxide			

Minimum Inhibition Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The potential antibacterial action with a MIC ranging between 0.25 mg/mL and 0.5 mg/mL, and MBC of 0.25 mg/mL to 2.0 mg/mL against indicator bacteria strains, while the potential antifungal action with MIC was 0.725 mg/mL against both oomycetes *Pythium myriotylum* and *Pytophthora megakarya* and 1.45 mg/mL against *Fusarium oxysporium,* and MFC of 1.450 mg/mL against *Pyhythium myriotylum*, and *Pytophthora megakarya*. However, the MIC of streptomycin ranged between 0.0156 mg/mL to 0.0625 mg/mL and MBC of 0.0125 mg/mL to 0.0625 mg/mL against the same bacteria indicator strains, while MIC of nystatin ranged between 0.181 to 0.362 mg/mL and MFC of 0.362 to 0725 mg/mL against the same fungi indicator strains (Table 3).

Table 3: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimal Fungicidal Concentration (MFC) of the methanol crude extract of *Streptomyces* sp. PFK4.

Bacteria	MIC (mg/mL)		MBC (mg/mL)	
	Methanol extract	Streptomycin	Methanol extract	Streptomycin
Salmonella typhi	0.25	0.0312	1.00	0.0312
Salmonella typhimurium	0.50	0.0312	0.50	0.0625
Escherichia coli ATCC 25922	0.25	0.0156	0.25	0.0625
Bacillus cereus	0.25	0.0312	1.00	0.0125
Pseudomonas aeruginosa	0.50	0.0625	0.50	/
Streptococcus pneumoniae ATCC 6465	0.25	0.0156	0.25	0.0312
Haemophilus influenza ATCC 49247	0.50	0.0625	2.00	0.0625
Streptococcus aureus ATCC 43300	0.50	0.0312	1.00	0.0625
Fungi	**MIC (mg/mL)**		**MFC (mg/mL)**	
	Methanol extract	**Nystatin**	**Methanol extract**	**Nystatin**
Pythium myriotylum	0.725	0.362	1.450	0.725
Pytophthora megakarya	0.725	0.362	1.450	0.362
Fusarium oxysporium	1.450	0.181	/	0.362

ATCC: American Type Culture Selection; MIC: Minimum Inhibition Concentration, MBC: Minimum Bactericidal Concentration, MFC: Minimum Fungicidal Concentration

Potential modes of action of the crude methanol extract of Streptomyces sp. PFK4

- **Bacteriolysis:** The 0.5 MIC (0.125 mg/mL) and 1MIC (0.25 mg/mL) of the crude methanol extract of *Streptomyces* sp. PFK4 has significantly inhibited the *E.coli* cell compared to the negative control (without crude methanol extract) (Figure 1a).
- **Inhibition of bacterial protein synthesis:** The 0.5 MIC of the crude methanol extract of *Streptomyces* sp. PFK4 has significantly inhibited *E.coli* protein synthesis while 1 MIC has not significantly inhibited *E. coli* protein synthesis compared to the negative control (without crude methanol extract) (Figure 1b).

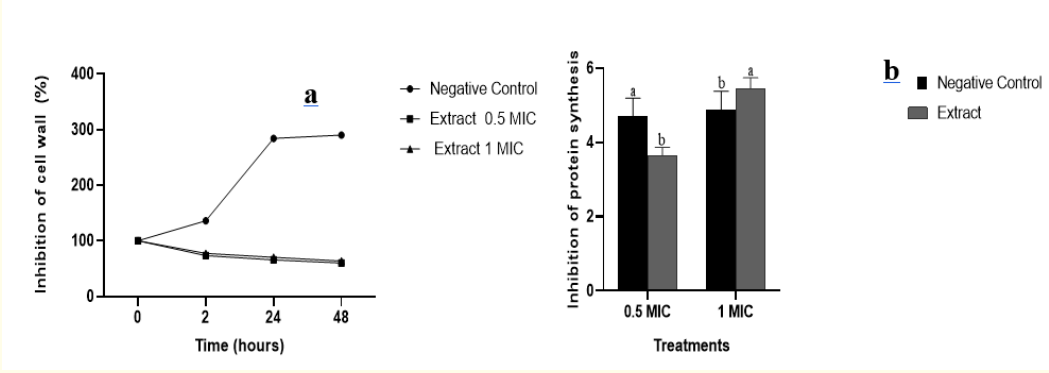

Figure 1: Effect of the methanol crude extract of *Streptomyces* sp. PFK4 on bacterial (*E. coli*) cell (a) and inhibition of protein synthesis (b). Each bar represents mean ± SEM, n = 3.[a, b] $P < 0.05$.

Antioxidant activities of methanol extract of *Streptomyces* sp. PFK4

2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH)

The highest inhibition concentration of DPPH radical scavenging activity of the methanol crude extract of *Streptomyces* sp. PFK4 was found to be 83.74 ± 2.10% at concentrate ion 50 µg/mL. The IC50 value for the DPPH radical scavenging activity of the extract was 100.8 µg/mL [Figure 2].

Ferric reduction antioxidant power (FRAP) activity and 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS)

The reducing power increase with increasing concentration of the crude methanol extract of *Streptomyces* sp. PFK4 [Figure 2]. The highest inhibition concentration of ABTS radical activity of the methanol crude extract of *Streptomyces* sp. PFK4 was found to be 72.97 ± 2.67% at a concentration of 50 µg/mL. The IC50 value for ABTS radical scavenging activity of the extract was 15.69 µg/mL [Figure 2].

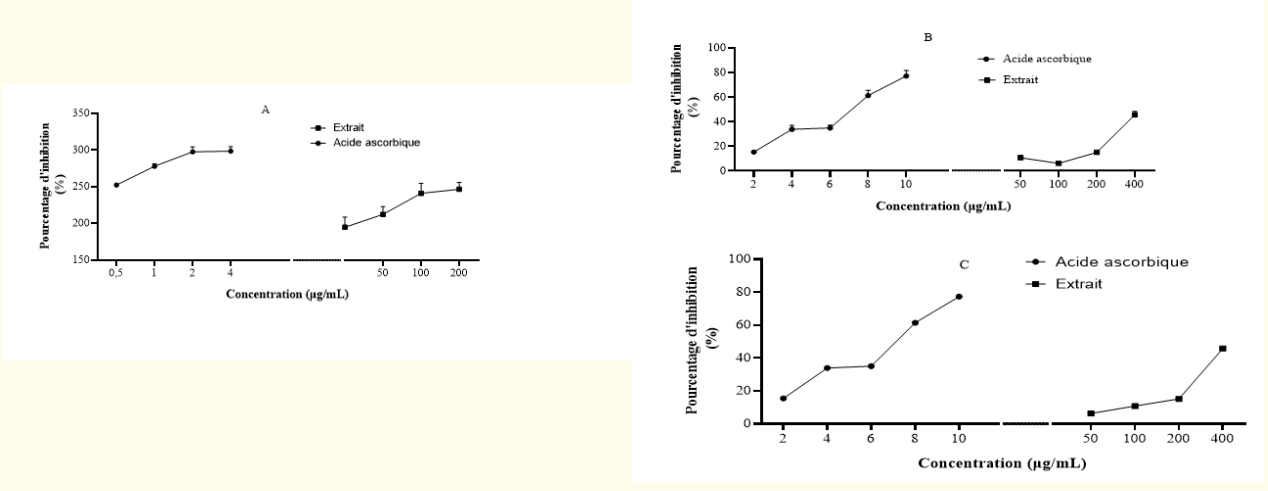

Figure 2: DPPH radical scavenging activity (A), Ferric reducing activity (B), 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity (ABTS) of the methanol crude extract of Streptomyces sp. PFK4, and ascorbic acid.

Gas chromatography-mass spectrometry analysis (GC-MS)

The GC-MS analysis of the methanol crude extract of *Streptomyces* sp. PFK4 extract revealed the presence of twenty-three biological compounds [Figure 3].

Table 4 showed the biological compounds' names, retention time, area, molecular weight, molecular formula, and bio-active uses of bioactive compounds identified from the methanol crude extract of *Streptomyces* sp. PFK4. The major compounds identified

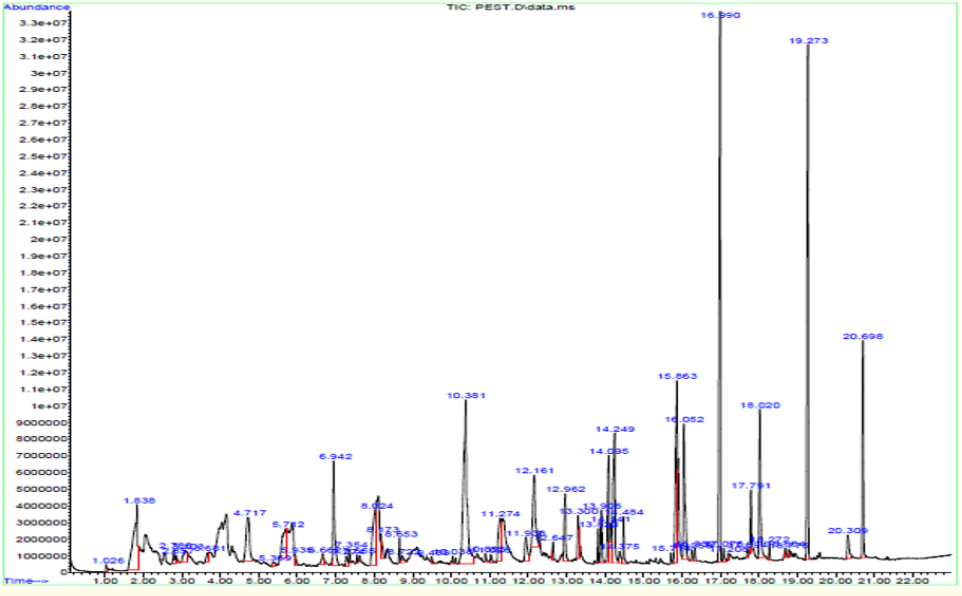

Figure 3: GC-MS chromatogram of the methanol crude extract of Streptomyces sp. PFK4. Each number on peaks represents the retention time of the compounds.

were linoleic acid ethyl ether (22.93%) with a retention time of 16.092 minutes, trans-13-Octadecenoic acid (11.23%) with a retention time of 15.846, n-hexadecanoic acid (10.32%) with a retention time of 14.181 minutes, butyl 9,12-octadecadienoate (8.70%) with retention time of 17.694 minutes, ethyl oleate (8.23%) with a retention time of 16.092 minutes, hexadecanoic acid, ethyl ester (7.97%) with a retention time of 14.478 minutes, Iron,-4-Cyclopentadien-1-YI)(2,3,3,4,4,5,5,6,6-Nonafluoro01-Cyclohexen-1-YI) (5.33%) with a retention time of 1.026 minutes, hexadecanoic acid, butyl ester (2.25%) with a retention time of 16.241 minutes and 2-methoxy-4-vinylphenol (2.13%) with a retention time of 7.0005 minutes [Table 4].

Discussion

Secondary metabolites produced by microbes continue to attract attention due to their sophisticated chemical structure and highly specific biological activities. Filamentous soil bacteria belonging to the genus of Streptomyces spp. are rich sources of a high number of natural products with biological activities; they are extensively used in pharmaceutical and agrochemical industries. These bacteria produced about 70% of commercially and medically useful antibiotics [23]. The optimized production of secondary metabolites generally involves the selection of a suitable fermentation process. In this study, solid-state fermentation was used for

Table 4: GC-MS of bioactive compounds present in the methanol crude extract of *Streptomyces* sp. PFK4**.**

Peak	Retention time (min)	Area (%)	Compound name	Molecular Formula	Molecular weight	Nature of compound	Biological activity
1	1.026	5.33	Iron,4-Cyclopentadien-1-Yl) (2,3,3,4,4,5,5,6,6-Nonafluoro-1-Cyclohexen-1-Yl)	$C_{13}H_5F_9FeO_2$	420	Phenolic acid	Anticancer [46]
2	1.615	0.5	Ethyl .alpha-d-glucopyranoside	$C_8H_{16}O_6$	208	Glucoside	Antituberculous, antioxidant, alpha amylase inhibitor, hypolipidemic, anticon-vulsant[8]; Anticancer [47]
3	1.650	0.45	beta-D-Ribopyranoside, methyl	$C_5H_{10}O_5$	160	Pentose	Antimicrobial, antioxidant [48]
4	2.027	1.09	2-Acetyl-2-Thiazoline	C_5H_7NOS	129	Pyrozine	Flavor, Aroma [49]
5	2.760	0.28	Octadecanoic acid, ethyl ester	$C_{10}H_{20}O_2$	172	Fatty acid ethyl ester	Antimicrobial [50]
6	7.005	2.13	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	Phenolic com-pound	Antioxidant, Antimicrobial and Anti-inflammatory [51,52]
7	7.412	0.78	Hydrocinnamic acid	$C_9H_{10}O_2$	150	Phenolic acid	Antiinflammatory, antioxidant and antimicrobial [53,54]
8	7.938	0.38	Ethyl alpha-d-glucopyranoside	$C_8H_{16}O_6$	208	Glucoside	Antituberculous, antioxidant, alpha amylase inhibitor, hypolipidemic, anticon-vulsant [8]; anticancer [48]
9	9.346	1.31	Unknown	Unknown	Unknown	Unknown	Unknown
10	13.214	0.23	Unknown	Unknown	Unknown	Unknown	Unknown
11	13.820	0.74	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	Fatty acid methyl ester	Antioxidant, antiinflammotory, hy-pocholesterolemic, antiandrogenic, flavor, nematicide [38].
12	14.038	0.48	2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	$C_9H_{14}O_2$	154	Ketone	Antioxidant [55]; anti-inflamma-tory, antiproliferative, an-timicrobial [56].
13	14.181	10.32	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Palmitic acid ester	Antiinflammatory [57]. antioxi-dants, hypocholesterolemic, nema-ticide, 5 alpha-reductase inhibitors, antiandrogenic, flavor, hemolytic [21,38]
14	14.478	7.97	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	Palmitic acid ester	Antioxidant, hemolytic, hypocho-lesterolemic, ne-maticide, antian-drogenic [38]
15	15.440	0.46	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	Fatty acid methyl ester	Antibacterial, antioxidant [48]
16	15.806	13.31	Unknown	Unknown	Unknown	Unknown	Unknown
17	15.846	11.23	Trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	Fatty acid ester	Acidifier, acidulant, arachidonic, acid-inhibitor,inhibit production of uric acid [43]
18	16.040	22.93	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	Fatty acid ethyl ester	Antiarthritic, antiandrogenic, 5 alpha-reductase inhibitor, hypocho-lesterolemic, antiacne, nematicide [38]
19	16.092	8.23	Ethyl Oleate	$C_{20}H_{38}O_2$	310	Fatty acid	Primer pheromone [44]

20	16.241	2.25	Hexadecanoic acid, butyl ester	$C_{20}H_{40}O_2$	312	Fatty acid butyl ester	Antimicrobial [58] antioxidant [59]
21	16.309	0.46	Octadecanoic acid, ethyl ester	$C_{20}H_{38}O_2$	284	Fatty acid ethyl ester	Antimicrobial [58] antioxidant [60]
22	17.694	8.70	Butyl 9,12-octadecadienoate	$C_{22}H_{40}O_2$	336	Fatty acid	Antimicrobial [45]
23	17.814	0.45	Unknown	Unknown	Unknown	Unknown	Unknown

the production of secondary metabolites on wheat bran substrate. The *Streptomyces* sp. PFK4 strain showed good growth in the wheat bran. This result can be explained by the fact that solid-state fermentation conditions more closely resemble native ones compared to artificial liquid-state fermentation. A recent study showed wheat bran to enhance the production of antibiotics and other bioactive secondary metabolites compared to another low-cost substrate rate [24]. Compared to the results obtained by Lima., *et al*. [25]. Using parboiled rice on fermentation in a solid medium for the production of bioactive metabolites of *Streptomyces* sp. PFK4, obtained a yield of 18.74%. The yield of the methanol crude extract of *Streptomyces* sp. PFK4 on wheat bran showed better productive efficiency since its percentage of yield is 24.11%.

The antibacterial activity of the methanol crude extract of *Streptomyces* sp. PFK4 and the control Streptomycin and DMSO (10%) were evaluated. Our results showed that the values of the zone of inhibition obtained from antibacterial activity were highest than those obtained from the methanol crude extract of Streptomyces sp. SA32 against Gram-positive and Gram-negative bacteria pathogens which ranged from 0 to 12.5 mm [26]. These results demonstrated that the methanol crude extract of *Streptomyces* sp. PFK4 contained particular molecules that inhibit the growth of the Gram-positive and Gram-negative microbial pathogens.

The efficacy of the methanol crude extract of Streptomyces sp. PFK4 was generally evaluated in terms of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The results of this study showed that potent antimicrobial action with a MIC ranging between 0.25mg/mL and 0.5mg/mL and a MBC of 0.25mg/mL to 2.0 mg/mL against indicator strains. Similar results were reported by Arayes., *et al*. [27] with the crude methanol extract of Streptomyces sp. EMSM31 against indicator bacteria. Interestingly the standard streptomycin showed MIC and MBC values lower than the crude extract. Our finding is similar to the results of Adeyemo., *et al*. [28] and Al-Dhabi., *et al*. [29] who found that the crude extracts produced from actinobacteria showed significantly higher MIC and MBC values against indicator strains comparable to standard antibiotics, streptomycin, or gentamycin. This may be due to the degree of purity of the antimicrobial substances and the different indicator strains used. Vijayakumar., *et al*. [30] reported that the MIC for a given agent is not constant because it is influenced by the type of organism used, inoculum size, extract concentration, and aeration.

Modes of action of the methanol crude extract of *Streptomyces* sp. PFK4 such as inhibition of bacterial cell and protein synthesis were evaluated against *E. coli*, the most sensitive bacteria pathogen. Bacteria cells are surrounded by a cell wall made of peptidoglycan, which consists of long sugar polymers. The peptidoglycan undergoes cross-linking of the glycan strands by the action of transglycosidases, peptide chains extend from the sugars in the polymers and form cross-links, one peptide to another [31]. This cross-linking strengthens the cell wall. Our results showed that the 0.5 MIC and 1MIC of the crude methanol extract of *Streptomyces* sp. PFK4 has significantly inhibited the *E. coli* cell compared to the negative control (without crude methanol extract). Based on this result, the methanol crude extract *Streptomyces* sp. PFK4 could contain glycopeptides which are recognized to inhibit bacterial cell wall synthesis. Furthermore, the 0.5 MIC of the methanol crude extract of *Streptomyces* sp. PFK4 has significantly inhibited *E.coli* protein synthesis while 1 MIC has not significantly inhibited E. coli protein synthesis compared to the negative control (without crude

methanol extract). Protein biosynthesis is catalyzed by ribosomes and cytoplasmic factors. The bacterial 70S ribosome is composed of two ribonucleoprotein subunits, the 30S and 50S subunits [32]. Antimicrobials inhibit protein biosynthesis by targeting the 30S or 50S subunit of the bacterial ribosome [33]. Among these antimicrobials, aminoglycosides, and tetracyclines are recognized to respectively interact with the 16S r-RNA of the 30S subunit near the A site through hydrogen bonds or act upon the conserved sequences of the 16S r-RNA of 30S ribosomal subunit to prevent binding of t-RNA to the A site [33]. Antimicrobials belonging to the class of macrolides affect the early stage of protein synthesis, namely translocation, by targeting the conserved sequences of the peptidyltransferase center of the 23S rRNA of the 50S ribosomal sub-unit [34]. Oxazolidinones interfere with protein synthesis at several stages such as (i) inhibition of protein synthesis by binding to 23Sr RNA of the 50S subunit and (ii) suppression of 70S inhibition and interaction with peptidyl-t-RNA [33].

In addition; the antioxidant capacity of the methanol crude extract of *Streptomyces* sp. PFK4 was evaluated using three complementary assays; DPPH, ABTS, and FRAP. The methanol crude extract of *Streptomyces* sp. PFK4 exhibited various free radical scavenging activities. DPPH is a useful reagent to evaluate the free radical scavenging of the hydrogen-donating oxidant, which can transfer hydrogen atoms or electrons to DPPH radicals [35]. The results showed that the highest inhibition concentration of DPPH radical scavenging activity of the methanol crude extract of *Streptomyces* sp. PFK4 was found to be 83.74 ± 2.10% at concentrate ion 50 µg/mL. The IC50 value for the DPPH radical scavenging activity of the extract was 100.8 µg/mL. Hydroxyl radical is one of the reactive oxygen species regenerated in the body, and removing hydroxyl radicals is important for antioxidant defense in living cell systems [36]. The methanol crude extract of *Streptomyces* sp. PFK4 exhibited ABTS radical activity. The highest inhibition concentration of ABTS radical activity of the crude methanol extract of Streptomyces sp. PFK4 was found to be 72.97 ± 2.67% at a concentration of 50 µg/mL. The IC50 value for ABTS radical scavenging activity of the extract was 15.69 µg/mL. From the FRAP assay, the highest FRAP reducing power was 8.04 ± 0.85 µg/mL at the concentration of 200 µg/mL. The reducing power increase with increasing concentration of the methanol crude extract of *Streptomyces* sp. PFK4. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [37].

The GC-MS analysis of the methanol crude extract of *Streptomyces* sp. PFK4 showed that fatty acids and fatty acid esters were the dominant compounds. Tanvir., *et al*. [38] reported fatty acids and their derivatives as therapeutic agents that cover a wide range of indications such as cancer, bacterial infections, parasitic infection, and inflammation to name a few. Tyagi and Agarwal [39] reported that linoleic acid ethyl ester possessed antiarthritic, antiandrogenic, 5 alpha-reductase inhibitor, hypocholesterolemic, antiacne, and nematicide activities. Fatty acids had been reported to inhibit bacterial growth by changing the permeability of bacterial membranes inhibiting fatty acid synthesis [40]. n-hexadecanoic acid detected in the crude methanol extract of Streptomyces sp. SCA3-4 by GC–MS analysis, possessed antimicrobial activity [41]. Narendhran., *et al*. [42] reported that the presence of palmitic acid and hexadecanoic acid in the fractions must also be responsible for the antimicrobial, antioxidant, and anticancer activity of *Streptomyces roxburghiana*. El-Naggar., *et al*. [43] detected octadecanoic acid and octadecanoic acid methyl ester from *Streptomyces anuannulus* AE-94 by GC–MS analysis. They act as antigens biotic against multidrug-resistant Staphylococcus aureus. Adegoke., *et al*. [44] reported that trans-13-octadecanoic acid belonging to fatty acid ester possessed several activities such as acidifier, acidulant, arachidonic, acid-inhibitor, and inhibits prod the reduction of uric acid. El-Naggar., *et al*. [43] detected octadecanoic acid and octadecanoic acid methyl ester from *Streptomyces annulus* NEAE-94 by GC–MS analysis. They act as an antibiotic against multidrug-resistant Staphylococcus aureus. Other authors also detected ethyl oleate in their extract by using GC-MS analysis and reported that it cans as a primer pheromone [45]. The antimicrobial activity of butyl 9,12-octadecadienoate was reported by Tleubayeva., *et al*. [46].

Conclusion

The methanol extract of *Streptomyces* sp. PFK4 displayed significant antimicrobial activities against Gram negative, Gram-positive bacterial pathogens, pathogenic fungi, and exhibited DPPH and ion-reducing power antioxidant activities in vitro. The methanol extract of PFK4 significantly showed a great effect on the inhibition of bacteria cell wall and protein synthesis. GC-MS showed the presence of twenty-three biological compounds, and the major compounds belong to fatty acids and fatty acid esters. The fatty acids are recognized to act as structural component of cell membranes, energy sources and storage, and signaling molecules (inflammation, metabolic regulation, neuroprotection); while fatty acid es-

ters are known for their significant physiological activities such as metabolic health, immune function and gut heath. Despite the biological functions of the major compounds identified in the methanol extract of Streptomyces sp. PFK4, the cytotoxicity essays and mechanisms tests on several microbial species must be done for a better valorization of Streptomyces sp. PFK4 as therapeutic agent.

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Conflicts of Interest

The authors declare that they have no known competing interests or personal relationships that could have appeared to influence the work reported in this paper.

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