Asian Journal of Medicine and Health
2(1): 1-10, 2017; Article no.AJMAH.30407

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Antibacterial Activities of *Streptomyces collinus* and *Streptomyces refuineus* from Cocoa, Orange and Timber Plantation Soils in Akure Metropolis

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Authors' contributions

This work was carried out in collaboration between both authors. Author FOE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author ASF managed the analyses of the study. Author ASF managed the literature searches. Both authors read and approved the final manuscript

Article Information

DOI: 10.9734/AJMAH/2017/30407
Editor(s):
(1) Domenico Lapenna, Associate Professor of Internal Medicine, Department of Medicine and Aging Sciences, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy.
Reviewers:
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(3) Jeffrey Lim Seng Heng, Malaysian Agriculture Research and Development Institute, Malaysia.
(4) Desak Gede Sri Andayani, Indonesian Institute of Sciences, Indonesia.
Complete Peer review History: http://www.sciencedomain.org/review-history/17436

Original Research Article

Received 9th November 2016
Accepted 28th December 2016
Published 5th January 2017

ABSTRACT

Study was carried out to determine the antibacterial activities of *Streptomyces* from two plantation soils in Akure metropolis against some pathogenic bacteria. Purification of the *Streptomyces* filtrates was done by column chromatography using chloroform and methanol in the ratio of 2:1 (v/v). The antibacterial activities of crude and partially purified metabolites were evaluated against typed and clinical cultures (human and plant) of some pathogenic bacteria. For control experiment, antibiotic sensitivity pattern of the pathogenic organisms was determined by using sensitivity disc. *Streptomyces collinus* ORFUTA was the most effective in inhibition of pathogenic bacteria for both crude and purified metabolite. The functional groups present in the bioactive compound of *S. collinus* ORFUTA revealed structure resembling hydroxyl group, alkane group and carbonyl groups. Partially purified *S. collinus* ORFUTA showed the highest zone of inhibition (21.31 mm)

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against *Staphylococcus aureus* (ATCC 25923) while the least zone of inhibition (3.77 mm) was observed against *Pseudomonas aeruginosa.* The minimum inhibitory concentration (MIC) of the test isolates ranges from 3.75 to 15 mg/ml. Ofloxacin had the highest activity 28.00 mm against *S. aureus*. *Streptomyces collinus* from orange plantation soil from Federal university of Technology Akure can be exploited in antibiotics production to treat staphylococcal infection.

Keywords: Streptomyces; antibacterial; pathogenic organisms; antagonistic activity.

1. INTRODUCTION

Actinomycetes are the most abundant organisms that form thread-like filaments in the soil. They grow as hyphae like fungi responsible for the characteristically “earthy” smell of freshly turned healthy soil [1]. George et al. [2] reported that the actinomycetes exist in various habitats in nature and represent a ubiquitous group of microbes widely distributed in natural ecosystems. Among actinomycetes, the *Streptomycetes* are the dominant [3]. *Streptomyces* is the largest prokaryotic genus of family Streptomycetacea [4], with 576 valid species recently reported by Euzéby and Labeda [5,6] and with increasing number every year. They are representative antibiotic producing prokaryotic group, while their morphological differentiation and genetic properties (linear chromosomes and genomic instability) have basic science interest [7]. *Streptomyces* species are widely recognized as industrially important organisms for their ability to elaborate different kinds of novel secondary metabolites [8]. The most interesting property of *Streptomyces* is the ability to produce bioactive secondary metabolites, such as antifungals, antivirals, antitumorals, anti-hypertensives, immunosuppressive, and especially antibiotics. The production of most antibiotics is species specific, and these secondary metabolites are important for *Streptomyces* species in order to compete with other microorganisms that come in contact, even within the same genre [9]. More than 500 species of the genus *Streptomyces* have been described and nearly two third of the naturally occurring antibiotics are produced by *Streptomycetes* [10]. There is need for current discovering of novel antibiotics of high quality and better yield.

2. MATERIALS AND METHODS

2.1 Sample Collection

Streptomyces species and clinical isolates of human pathogenic bacteria used were obtained from the Department of Microbiology, Federal University of Technology, Akure (FUTA). Typed cultures of the pathogens were collected from Nigeria Institute of Medical Research Laboratory Yaba in Lagos State of Nigeria. Also, the plant pathogenic organisms used were obtained from Department of crop soil and pest management, FUTA.

The *Streptomyces* collected were *Streptomyces collinus* ORFUTA and *S. refuineus* COCADO while the clinical human pathogens obtained were *Salmonella typhi* and *Enterococcus faecalis.* Type cultures of pathogenic bacteria collected were *Pseudomonas aeruginosa* (ATCC 25923), *Escherichia coli* (ATCC 35218) and *Staphylococcus aureus* (ATCC 24162) while plant pathogenic bacteria obtained are *Serratia marcescens, Xanthomonas axonopodis, Erwinia corotovora* and *Pseudomonas aeruginosa.* The purity of the isolates was ascertained using some biochemical tests.

2.2 Screening of *Streptomyces* for Antimicrobial Metabolite Production

Fresh culture of *Streptomyces* was inoculated in starch casein broth and incubated at 28℃ for 7 days in water bath with shaking. Growth of the organism in the flask was confirmed by turbidity in the broth. The broth culture was centrifuged at 5000 rpm for 20 min and the supernatant was filtered through No 1 Whatman filter paper. The culture filtrate of the *Streptomyces* species was used for the determination of antimicrobial activity against the test organisms [11]. Molten agar was aseptically dispensed into Petri dishes containing 1 ml of each test organisms and allowed to gel. The seeded plates were allowed to set and wells at equidistant from each other were made in the agar plates with the aid of a cork borer (diameter 10 mm).

Each well was then filled with 0.1ml of the culture filtrate. The plates were allowed to stand for one hour to allow diffusion of the metabolite in the filtrate, then incubated at 37℃ for 24 h and observed for zones of inhibition. Three replicates of the experiment were performed and the diameters of the inhibition zones were measured and recorded.

2.3 Extraction and Bioassay of Antimicrobial Metabolite: Extraction of Crude Metabolite

Antimicrobial compound was recovered from the culture filtrate of *Streptomyces* (*Streptomyces* whose filtrate gave the highest zone of inhibition value) by solvent extraction with chloroform. Chloroform was added to the filtrate in the ratio 1:1 (v/v) and shaken vigorously; the organic phase containing the metabolite was separated from the aqueous phase. The extract obtained was evaporated to dryness using rotary evaporator and the crude extract was then used for bio assay [12].

2.4 Antimicrobial Assay of the Crude Metabolite

Antimicrobial activity of the crude metabolite was determined by agar well diffusion method as described by Olutiola et al. [13]. The test organisms were standardized to 0.5 McFarland standards as described by Oyeleke et al. [14]. Sterile Petri dishes were seeded aseptically with 1 ml each of the standardized broth cultures and 20 ml of sterilized Mueller- Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile cork borer, wells were created on the solidified agar medium. Exactly 0.5 mL of 50 mg/mL of the metabolite was then introduced into the wells and approximately labeled. The plates were incubated for 24 h at 37℃ in case of bacterial species and 28℃ for three days in case of fungal species. The plates were observed for zones of inhibition [15].

2.5 Purification by Column Chromatography

The purification of the antimicrobial compound was carried out using silica gel column chromatography as described by Atta [16] and Usha et al. [17]. Chloroform and methanol in the ratio 2:1 v/v was used as eluting solvent. The column was packed with silica gel (60-120 mesh). The sample to be separated was then added on the top of the packed column and eluted with the solvent at the flow rate of a drop per 3 sec. A collecting conical flask was placed at the bottom of the column to collect the eluted fractions. The collected elute was distilled, leaving the purified fractions. The fractions obtained were spotted unto TLC plates. Fractions with the same retention factor (Rf) were pooled together.

2.6 Thin Layer Chromatography (TLC)

The crude extract was subjected to TLC analysis on 2.6 × 8 cm silica gel plate. This was spotted onto a TLC plate 2 cm above the base. After thorough drying, the plate was placed in a solvent system (chloroform: methanol in the ratio 2:1) in a chromatography tank to develop. The plates were removed when the solvent front approached the top of the plates and air-dried. Spots on the plates were visualized in an iodine chamber. The distances travelled by the constituents were measured and used to determine their Rf values [16].

2.7 Antimicrobial Assay of Column Fractions of Crude Metabolite

Antimicrobial activity of the column fraction was determined by paper disc diffusion method of Aida et al. [18]. Sterile Petri disks were seeded aseptically with 0.1 ml of the standardized test organisms while about 20 ml of sterile Mueller Hinton agar was poured aseptically on the seeded plates. Sterile Whatman filter paper disks (6.00 mm in diameter) were impregnated with 30 mg/ml of the purified fraction reconstituted with 30% dimethyl sulfoxide (DMSO). The impregnated paper disks were allowed to dry and applied with the aid of sterile forceps on the seeded plates. Filter paper disks dipped into DMSO and allowed to dry served as control. The plates were incubated at 37℃ for 24 h. Antimicrobial activities were determined by the measurement of zone of inhibition around each paper disk.

2.8 Antibiotics Sensitivity Test

The antibacterial sensitivity test was carried out according to CLSI [19] in order to compare the sensitivity of the organisms to ten selected commercial antibacterial drugs. The commercial antibacterial drugs which included Amoxycillin disk (AMX), Ofloxacin disk (OFL), Streptomycin disk (STR), Chloramphenicol disk (CHL), Ceftriazone disk (CEF), Gentamycin disk (GEN), Pefloxacin disk (PEF), Cotrimoxazole disk (COT), Ciprofloxacin disk (CPX) and Erythromycin disk (ERY) were used for bacterial isolates.

The Kirby-Bauer test, also known as disk diffusion method described by [20], was used to determine the effect of standard antimicrobial drugs on the test organisms. Sterile Petri disks were seeded aseptically with 1 mL each of the

standardized broth cultures of the test organisms while about 15 mL of sterilized Mueller-Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution of the inoculum and allowed to gel. With the aid of sterile forceps, the antibiotic disks were carefully but firmly placed on the solidified plates and incubated for 24 h at 37℃. Seeded agar plates with wells containing sterile distilled water served as the control. After incubation, clear areas around the disks/wells, representing the zones of inhibition, were measured in milliliter (mm). The experiment was carried out in triplicate.

2.9 Determination of Minimum Inhibitory Concentration (MIC) of the Metabolite

The determination of Minimum Inhibitory Concentration (MIC) was carried out using agar well diffusion method as described by [21]. Varying concentrations of the metabolite (30 mg/mL, 15 mg/mL, 7.5 mg/mL, 3.75 mg/mL and 1.8 mg/mL) were reconstituted in 30% DMSO. Sterile Petri diskes were seeded aseptically with 0.1 mL of the standardized test organisms while about 20 mL of sterile Mueller Hinton agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. Five wells were created on the agar and the metabolite was introduced at different concentrations into the wells. The plates were incubated and the lowest concentration that inhibited the growth in a well was recorded. A well containing only 30% DMSO served as control

2.10 Spectroscopic Analysis of the Metabolite Produced by *Streptomyces collinus*

Infra-red (IR) analysis was performed with the aid of infra-red spectrophotometer (Perkin-Elmer spectrum bx). A drop of purified extract was placed on fused sodium chloride (NaCl) cell. It was carefully placed on cell loosely clamped and fixed on the IR beam. The IR data was compared to the table of IR frequencies using the methods of [22].

2.11 Statistical Analysis

Data are presented as mean ± standard error (SEM). Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 8 version 20 software. For all the tests, the significance was determined at the level of $P < 0.05$.

3. RESULTS

The screening of *Streptomyces* for antibiotics production using the culture filtrate revealed that *Streptomyces collinus* ORFUTA was most active against *S. aureus* (ATCC 25923). It also inhibited other pathogenic typed cultures *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 21332) and clinical pathogenic isolates namely *Enterococcus faecalis* and *Salmonella typhi* respectively (Table 1). The plant pathogenic bacterium, *Erwinia carotovora* was inhibited by *S. collinus* ORFUTA. Also, *Serratia marcescens* and *Pseudomonas aeruginosa* were slightly susceptible to crude metabolite of *S. refuineus* COCADO. *Xanthomonas axonopodis* was resistant to all crude metabolites of *Streptomyces* (Table 2).

Pefloxacin can better inhibit *S. typhi* and *Pseudomonas aeruginosa* (ATCC 27853) than Ciproxacin, Gentamycin, Cotrimoxazole, and Ceftriazone while, Ciproxacin is more active against *Enterococcus feacalis* and *Escherichia coli* (Table 3a).

Partially purified metabolite (PPME) of *Streptomyces collinus* ORFUTA can inhibit *Staphylococcus aureus* (ATCC 25923) better than Ciproxacin, Pefloxacin, Gentamycin, Cotrimoxazole and Ceftriazone, inhibit *Enterococcus feacalis* than Pefloxacin, Gentamycin, Cotrimoxazole and Ceftriazone, inhibit *Pseudomonas aeruginosa* (ATCC 27853) than Ceftriazone, Cotrimoxazole, and Gentamycin, and nearly inhibit *Salmonella typhi* like Ceftriazone. Also, PPME of *S. refuineus* is more active against *Pseudomonas aeruginosa* (ATCC 27853) than Ceftriazone (Table 3a). Chloramphenicol was the most active against *S. typhi* among Streptomycin, Ofloxacin, Amoxycillin and Erythromycin. However, Ofloxacin can inhibit *E. faecalis* and *P. aeruginosa* (ATCC 27853) than others (Table 3b). Ciproxacin, Pefloxacin and Gentamycin was more active than the metabolites produced by *S. collinus* and *S. refuineus* expect for Cotrimoxazole and Ceftriazone against *Pseudomonas aeruginosa* and *Serratia marcescens* (Plant pathogen) (Table 3a).

Partially purified metabolite (PPME) of *Streptomyces collinus* ORFUTA is more active against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* than Chloramphenicol, Streptomycin, Ofloxacin, Amoxycillin and Erythromycin. Also PPME of *Streptomyces collinus* ORFUTA can inhibit *Enterococcus feacalis* and *Pseudomonas aeruginosa* (ATCC 27853) than Chloramphenicol, Streptomycin, Amoxycillin and Erythromycin. This PPME can nearly inhibit *S. typhi* like Amoxycillin (Table 3b). All the commercial drug were more active against *Pseudomonas aeruginosa* than the metabolite produced by *Streptomyces collinus* ORFUTA and *S. refuineus* ORFUTA (Table 3b).

The highest MIC value recorded from the purified metabolite produced by *Streptomyces collinus* was 15.00 mg/ml for *Staphylococcus aureus* (ATCC 25923) a human pathogen and *Erwinia carotovora* plant pathogen while the lowest MIC recorded was 3.75 mg/ml for *Enterococcus faealis*. The metabolite produced by *Streptomyces refuineus* also recorded the highest MIC of 15.00 mg/ml for *Erwinia carotovora* and the lowest MIC recorded was 3.75 mg/ml for *Serratia marcescens* another plant pathogen (Table 4).

The infra-red spectrum of the antimicrobial agent showed bands corresponding to four peaks and these four peaks also denote four important functional groups revealing the hydroxyl group at 3390. The peak at 2080 showed the existence of alkane, carbonyl group at peak 1680 and the aromatic group at peak 1000 (Plate 1).

4. DISCUSSION

Screening for antimicrobial activity of orange, cocoa and timber plantation soil sites in Adofure farmland and Federal university technology (FUTA) school farm in Akure, Ondo State Nigeria have not been reported yet. Results obtained from this study have however shown the presence of *Streptomyces* species capable of producing antibacterial metabolites in this location.

In this research work, it was observed that the metabolite produced by *Streptomyces collinus* ORFUTA had greater inhibitory effect on Gram-positive than Gram-negative bacteria; such finding, which is in agreement with the study by Ama [23], may be explained considering the differences in the sensitivities of Gram-positive and Gram-negative bacteria related to the structure and composition of their cell wall. Gram-negative bacteria are known to possess a thin peptidoglycan layer and a outer membrane that is unique consisting of lipopolysaccharide (LPS) components. This outer membrane makes the cell wall impermeable to lipophilic solutes thus blocking certain antibiotics such as penicillin, dyes, and detergents from penetrating the cell [23].

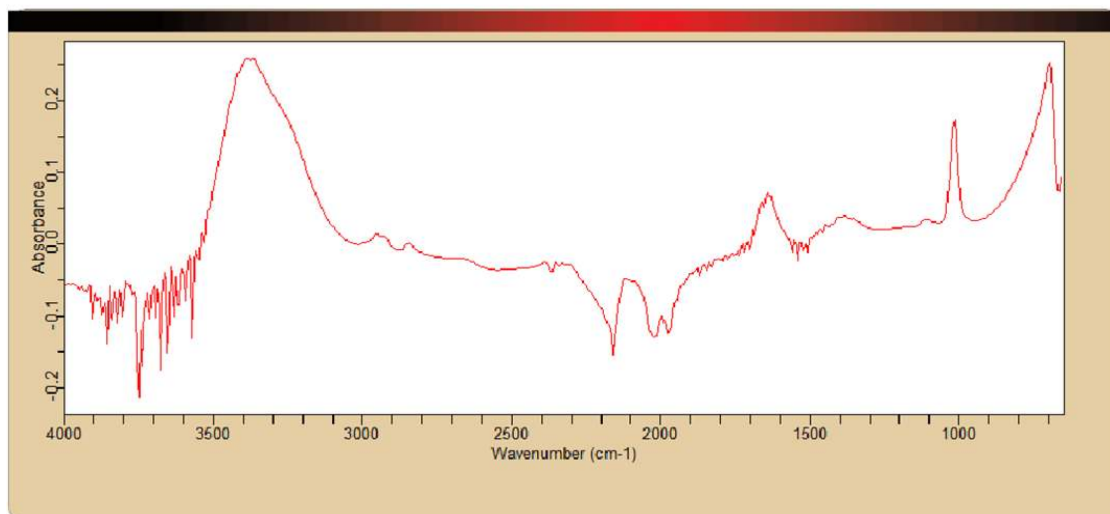

Plate 1. Infra-red Spectum of the metabolite from *Streptomyces collinus*

Table 1. Antibacterial activities of crude metabolite of *Streptomyces* species on human pathogenic bacteria

	Bacteria isolates/zones of inhibition (mm)			
Pathogenic bacteria	***Salmonella typhi***	***Enterococus faecalis***	***Escherichia coli* (ATCC 35218)**	***Staphylococus aureus* (ATCC 25923)**
A	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$6.03±0.03^b$
B	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
C	$7.23±0.15^b$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
D	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
E	$10.30±0.15^c$	$18.63±0.15^b$	$17.32±0.16^b$	$20.40±0.01^c$
F	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
G	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
H	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
I	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
J	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
K	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$

Data are presented as Mean ± SEM (n = 3). Values with the same superscript letter(s) along the same row are not significantly different (P < 0.05)

Key

A: Streptomyces albus ORFUTA
B: Streptomyce laurentii ORFUTA
C: Streptomyces refuineus COCADO
D: Streptomyces coelicolor ORFUTA
E: Streptomyces collinus ORFUTA
F: Streptomyces avermitilis COFUTA
G: Streptomyces griseoflavus COFUTA
H: Streptomyces fulvissium COFUTA
I: Streptomyces griseus TIFUTA
J: Streptomyces globisporus ORAADO
K: Streptomyces collinus ORAADO

ORFUTA= Isolate from rhizosphere of orange at FUTA
COCADO= Isolate from rhizosphere of cocoa at Adofure
COFUTA= Isolate from rhizosphere of cocoa at FUTA
TIFUTA= Isolate from rhizosphere of Timber (Teak) at FUTA
ORAADO= Isolate from rhizosphere of orange at Adofure

Table 2. Antibacterial activities of crude metabolite of *Streptomyces* species on selected plant pathogenic bacteria

	Bacteria isolates/zones of inhibition (mm)		
Name of isolates	***Pseudomonas aeruginosa***	***Serratia marcescens***	***Erwinia carotovora***
A	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
B	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
C	$5.20±0.11^c$	$8.57±0.18^b$	$6.13±0.09^b$
D	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
E	$3.77±0.02^b$	$0.00±0.00^a$	$8.30±0.17^c$
F	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
G	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
H	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
I	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
J	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$
K	$0.00±0.00^a$	$0.00±0.00^a$	$0.00±0.00^a$

Data are presented as Mean ± SEM (n = 3). Values with the same superscript letter(s) along the same row are not significantly different (P < 0.05)

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E: Streptomyce collinus ORFUTA
F: Streptomyces avermitilis COFUTA
G: Streptomyces griseoflavus COFUTA
H: Streptomyces fulvissium COFUTA
I: Streptomyces griseus TIFUTA
J: Streptomyces globisporus ORAADO
K: Streptomyces collinus ORAADO

ORFUTA= Isolate from rhizosphere of orange at FUTA
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COFUTA= Isolate from rhizosphere of cocoa at FUTA
ORAADO= Isolate from rhizosphere of orange at Adofure

Table 3a. Antibacterial activities of selected commercial drugs and partially purified metabolites of *Streptomyces*

	Partially purified metabolites		Commercial antibacterial drugs			
Pathogenic bacteria	***Streptomyces collinus ORFUTA***	***Streptomyces refuineus COCADO***	**CPX**	**COT**	**PEF**	**GEN**
S. typhi	11.35±0.01	9.53±0.02	19.53±0.33^{c}	0.00±0.00	22.47±0.01^{f}	15.93±0.33^{e}
E. faecalis	19.69±0.01	0.00±0.00	20.93±0.12d	0.00±0.00	18.83±012^{e}	18.98±0.16^{f}
E. coli (ATCC 35218)	19.64±0.02	0.00±0.00	21.33±0.67^{e}	0.00±0.00	18.03±0.03^{d}	13.98±0.17^{d}
S. aureus (ATCC 25923)	21.31±0.01	0.00±0.00	19.77±0.12^{c}	0.00±0.00	18.90±0.10^{e}	18.90±0.10^{f}
P. aeruginosa (ATCC 27853)	17.21±0.02	11.61±0.03	20.17±0.17^{cd}	0.00±0.00	24.03±0.03^{g}	12.87±0.13^{bc}
E. carotovora	9.58±0.10	8.36±0.01	21.79±0.10^{e}	0.00±0.00	14.60±0.12^{b}	12.63±0.17^{b}
X. axonopodis	0.00±0.00	0.00±0.00	17.60±0.10^{b}	0.00±0.00	17.93±0.67^{d}	15.90±0.10^{e}
P. aeruginosa	3.67±0.02	6.47±0.02	19.90±0.10^{c}	0.00±0.00	14.87±0.13c	13.08±0.83^{c}
S. marcescens	0.00±0.00	8.46±0.01	10.63±0.67^{a}	0.00±0.00	10.07±0.67^{a}	10.93±0.67^{a}

Data are presented as Mean ± SEM (n =3). Values with the same superscript letter(s) along the same row are not significantly different (P < 0.05).
Key CPX = Ciprofloxacin, COT = Cotrimoxazole, PEF = Pefloxacin, GEN = Gentamycin, CEF = Ceftriazone, Test M.O = Test microorganisms
Zones of inhibition (in mm)

Table 3b. Antibacterial activities of selected commercial drugs and partially purified metabolites of *Streptomyces*

	Partially purified metabolites		Commercial antibacterial drugs			
Pathogenic bacteria	***Streptomyces collinus* ORFUTA**	***Streptomyces refuineus* COCADO**	**CHR**	**STR**	**OFL**	**AMX**
S. typhi	11.35±0.01	9.53±0.02	20.11±0.06^{h}	18.95±0.05^{g}	17.96±0.04^{d}	12.03±0.03^{f}
E. faecalis	19.69±0.01	0.00±0.00	16.83±0.17^{f}	14.04±0.04^{d}	28.03±0.03^{i}	15.20±0.20^{g}
E. coli (ATCC 35218)	19.64±0.02	0.00±0.00	18.68±0.09^{g}	12.95±0.05^{c}	13.03±0.03^{c}	4.73±0.07^{b}
S. aureus (ATCC 25923)	21.31±0.01	0.00±0.00	12.87±0.13^{c}	15.63±0.03^{f}	19.03±0.03^{e}	16.93±0.52^{h}
P. aeruginosa (ATCC 27853)	17.21±0.02	11.61±0.03	16.04±0.04^{e}	15.04±0.04^{e}	22.03±0.03^{g}	11.95±0.47^{f}
E. carotovora	9.58±0.10	8.36±0.01	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
X. axonopodis	0.00±0.00	0.00±0.00	0.00±0.00^{a}	19.04±0.04^{g}	19.93±0.03^{f}	6.93±0.67^{d}
P. aeruginosa	3.67±0.02	6.47±0.02	13.95±0.05^{d}	10.88±0.02^{b}	23.93±0.07^{h}	16.67±0.07^{e}
S. marcescens	0.00±0.00	8.46±0.01	11.87±0.13^{b}	0.00±0.00^{a}	11.87±0.13^{b}	6.04±0.40^{c}

Data are presented as Mean ± SEM (n = 3). Values with the same superscript letter(s) along the same row are not significantly different (P < 0.05).
Key, CHL = Chloramphenicol, STR = Streptomycin, OFL= Ofloxacin, AMX = Amoxycillin and ERY = Erythromycin. Test M.O = Test microorganisms
Zones of inhibition (in mm)

Table 4. Minimum inhibitory concentration (MIC) values of the metabolite against selected test organisms

Test microorganisms	***Streptomyces collinus* (mg/ml)**	***Streptomyces refuineus* (mg/ml)**
Salmonella typhi	7.50	7.50
Enterococus faecalis	3.75	ND
Escherichia coli (ATCC 35218)	7.50	ND
Staphylococcus aureus (ATCC 25923)	15.00	ND
Pseudomonas aeruginosa (ATCC 27853)	7.50	7.50
Erwinia carotovora	15.00	15.00
Pseudomonas aeruginosa	7.50	7.50
Serratia marcescens	ND	3.75

Key ND = Not determined

Three (3) out of the 11 *Streptomyces* species (27.27%) showed antimicrobial activity against the test human and plant pathogenic microorganisms. Zheng et al. [24] reported that eight out of twenty-nine (29) strains, representing 28% of the microbes considered in their study were able to inhibit the growth of at least one of the target microorganisms. Also, Ivanova et al. [25] reported that out of the 491 bacteria isolated from different marine sources, 26% of the isolates were active. Mainly *Streptomyces collinus* ORFUTA has shown good inhibition zone against *S. aureus* (ATCC 25923) and *E. faecalis*.

The best strain in this study was found to be *S. collinus* ORFUTA as it showed broad-spectrum activity against the test human and plant pathogenic bacteria. *Streptomyces collinus* Tü 365 produces antibiotic kirromycin [26]. The mechanism of action may be due to the reason as described by Parmeggiani and Nissen [27] that, complex elongation factor Tu-GTP and acceptaminoacyl-tRNA can bind to ribosome therefore allowing the Codon-anticodon recognition to follow by GTP hydrolysis and the release of EFTu-GDP-kirromycin from ribosomes will be blocked, resulting in the inhibition of subsequent steps of peptide bond formation.

The commercial antibacterial drugs used in this research work were more effective in inhibiting the test organisms this may be as a result of high level of purification of the antibiotics. The reason might be due to the suggestion of Doughari et al. [28] who reported that the state of administration of an antimicrobial agent affect the effectiveness of such agent and the antimicrobial being in a refined state may record higher activities.

The purification process through a column chromatography packed with silica gel and an eluting solvent composed of chloroform-methanol (2:1) showed fractions in contrast with the results obtained by Hitchens and Kell, [29] and Atta et al. [15], who reported lesser values of retention factor.

The infra-red spectrum of the antimicrobial agent in this study showed Peak at 3390 indicating an hydroxyl group also peak at 2080 suggesting the presence of CH while the peak at 1010 indicated that there is carbonyl group. This finding agrees with the study by Ogundare et al. [30], who observed that the spectroscopic characteristics of the antimicrobial agent revealed peaks at 3421 - 3394, thereby suggesting a hydroxyl group. Gabriel et al. [31] revealed these functional groups in phenolic-type antimicrobial agents, which are used for antiseptic, disinfectant, or preservative properties, depending on the compound and also used in large quantities in the production of plastics, drugs, dyestuffs, explosives, pesticides, detergents, stabilizers and antioxidants.

5. CONCLUSION

The metabolite produced by *Streptomyces collinus* ORFUTA from Federal University of Technology Akure (FUTA) in Ondo State is hereby recommended for the treatment of *Staphylococcus aureus* (ATCC 25923), *Enterococus faecalis, Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Erwinia carotovora.* Furthermore, complete purification needs to carried out in order to improve the efficacy of the increase metabolite.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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