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Isolation and Characterisation of a Soil Microbial *Streptomyces* Strain that Produces Membrane-Active Antimicrobial Metabolites

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Authors' contributions

This work was carried out in collaboration between all authors. Author PA performed the literature search the protocols and wrote the first draft of the manuscript. Authors PO, VK and FK helped in the protocols of the study. Author SS designed the study, managed the literature search, analysis of the results, revised the manuscript and supervised the experiments. All authors read and approved the final manuscript.

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ABSTRACT

In continuation of our previous research, more than 60 microbial strains that were isolated from soil samples collected from desert, farming and mineral regions of Iran and sixteen isolated strains that produced antimicrobial metabolites were found to be effective against *Escherichia coli, Candida albicans and Saccharomyces cerevisiae and Staphylococcus aureus* microorganisms. A total of 3 out of 16 strains produced membrane-active ethyl acetate fractions based on artificial vesicle

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assay. The antimicrobial and membrane interfering activities of crude extract and fractions from *Actinomycetes* strain were investigated in the current study. We report the isolation two compounds from biologically active fractions obtained from *Streptomyces* sp 0811 culture. Fractionation of *Streptomyces* sp 0811 demonstrated antimicrobial activities against bacteria and yeast strains. Vesicle assay showed membrane activity for the mentioned fraction. This suggests the presence of an antimicrobial membrane active agent. Further fractionation and purification strategies were applied to finally identify the isolated moleculesby using appropriate spectroscopic methods, such as ^{1}HNMR, COSY, and LC-MS. The two identified structures are described to be indole-type and ether amino derivative.

Keywords: Drug development; membrane active; ethyl acetate fraction; actinomycetes; antimicrobial.

1. INTRODUCTION

There has been an alarming increase in the number of people with health problems due to various drug-resistant bacteria, parasitic protozoans, and fungi [1]. Drug-resistant bacteria have become a global concern, and the search for new antibacterial agents is therefore urgent and ongoing [2]. Increase in the types of drug-resistant bacteria has raised the need for new and novel antimicrobials drugs to treat human diseases [3]. Research for innovative and more effective agents to deal with these diseases are now underway [4,5].

Microorganisms are an important source in the production of bioactive natural products with an enormous application potential in the discovery of new molecules for drug discovery and in industrial and agricultural applications [6-8]. Compared to other natural product sources such as plants, microorganisms are highly diverse but hardly explored. The ecosystem of microorganisms enables them to produce various substrates so that they can adapt to their biological environment [9].

Actinomycetes are soil-dwelling and Gram-positive bacteria with industrial applications originating from their wide range of bioactive metabolites, including many antibiotics. They are also pharmacologically important with commercial interest because they are rich in secondary metabolites. More than 7,000 compounds are produced by the actinomycetes–*Streptomyces* species [10]. Streptomycetes are known to produce many secondary metabolites with antibacterial, anti-parasitic, antifungal, immunosuppressive, and antitumor properties [11,12].In addition, more than 50% of anticancer and 60%–70% of antimicrobial medicines currently in clinical use are natural products or natural product derivatives [13]. Screening of antibiotics-producing soil streptomycetes has been reported in several studies [14-16].

Drugs have various modes of action to inhibit the growth of microorganisms, such as inhibition of protein synthesis, cell wall formation, and nucleic and ribonucleic acid production [17,18]. Membranes are a new and potential target for antibiotics action. Because the probability to developing resistance against membranes is extremely low, medicines that anchor in the microorganism membrane seem to have a high chance of success in combating resistance potential [19].

New microorganisms and products have been derived from different areas of the world, such as China, Australia, Antarctica, India, and Jordan. Thakur et al. studied the antimicrobial activity of *Actinomycete* strains isolated from the soil of Indian protected areas and fungal strains [20]. In the mentioned surveys, the antimicrobial activity of metabolites produced by the soil microorganisms has not been simultaneously studied with their membrane activity [21,22]. Various membrane-active compounds released by microorganisms to their environments can strongly interact with membrane components of the host cell [23].

The aim of this work is to evaluate the antimicrobial membrane activity using a colorimetric assay with a polydiacetylene (PDA) vesicle test, as reported in our previous studies [24]. In addition, the detection and purification of antimicrobial metabolite present in membrane-active fraction were carried out.

2. MATERIALS AND METHODS

2.1 Chemicals

All organic solvents were of analytic grade and HPLC grade obtained from Merck, Germany. Ethyl acetate was obtained from Chem lab, Belgium. Silica gel TLC sheets were obtained from Merck (Darmstadt, Germany). Silica gel (200–400 mesh size) was obtained from Merck,

Darmstadt, Germany. Sabouraud maltose broth was obtained from DIFCO, Becton, Dickinson, USA. Brain heart infusion broth was also used (BHI; Merck, Germany). Phospholipid, dimyristoyl phosphatidyl choline (DMPC), and diacetylenic monomer, 10, 12-tricocosadiynoic acid (TCDA), were both purchased from Sigma, USA.

2.2 Isolation of *Streptomyces* sp

The *Streptomyces* sp 0811 strain was isolated from one of the soil samples collected from desert, farming, and mineral regions of Iran. All isolates of the samples were screened and tested for their ability to produce antimicrobial substances against *Escherichia (E.) coli*, *Candida (C.) albicans*, and *Saccharomyces (S.) cerevisiae* microorganisms. Sixteen strains were isolated, which produced antimicrobial metabolites and were effective against test microorganisms. All the isolates grew on an ISSA (Inorganic Salt Starch Agar) medium showing morphology characteristics of *Actinomycetes*.

2.3 Preparation of Inoculum

The isolated *Streptomyces* sp 0811 was grown on an ISP4 (International Streptomyces Project Medium 4) plate and kept in an incubator at a temperature of 28ºC for 7 days. A loop of spores was scraped from the plate and inoculated into 10 ml Tryptic soy broth (TSB). It was kept in a rotatory shaker incubator at 150 rpm for a period of 7–10 days at 28ºC. Five millilitres of the *Streptomyces* sp 0811 metabolites culture inoculates was transferred into 1000 mL Erlenmeyer flasks containing 500 mL of TSB, and then incubated at 25ºC in a shaker at 200 rpm for 14 days. After the 14th day, the culture broth was centrifuged at 2000 g for 30 min to remove the biomass.

2.4 Extraction of Antimicrobial Metabolites with Ethyl Acetate Solvent

The cultured broth was filtered, and ethyl acetate was added to the broth in a ratio of 1:1 (V/V). The mixture was shaken for complete extraction and separated for further processing. This step was repeated three times. The ethyl acetate fractions, which contain the bioactive metabolites, was separated from the aqueous phase and pooled, and then evaporated with rotary evaporator to dryness. The residue obtained was used to determine the antimicrobial activity.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

MIC assay of the extracts towards one Gram positive and one Gram negative bacteria and one fungus was performed to determine the antimicrobial activity of the isolated *Actinomycetes* sp 0811. *E. coli* ATCC 25922, *C. albicans* ATCC 10231, and *S. aureus* ATCC 25923 were used as the test strains. The ethyl acetate fraction of extracts was dissolved in dimethyl sulfoxide (DMSO) to reach a concentration of 10 mg/ml. The broth microdilution method was employed for antifungal and antimicrobial activity tests. Sabouraud maltose broth (SMB) was used as the growth medium and *E. coli and S. aureus*were cultured in brain heart infusion (BHI) broth at 37ºC for 24 h. Modified antimicrobial susceptibility testing was done based on the NCCLS M27-A method. Broth media (100 ml) was added to each well of a 96-well micro plate, and then, 40 ml of the compounds and 60 µl of broth were added to a well (A). Then, 100 µl of the culture medium was added to the first well, followed by serial dilution from well (A) by taking 100 µl into (B). This dilution continued down the plate, and 100 µl from the last well (H) was discarded. Then, all the wells were filled with 100 µl of working fungal culture. AmB were used as a reference in the antifungal test, and streptomycin was used for the antimicrobial test. The plate was covered and incubated at 37ºC for 24 to 48 h. The minimal inhibitory concentration (MIC) values were obtained by reading the lowest concentration of compound in the well showing no growth [25].

2.6 Colorimetric Assay by Phospholipid/ PDA Vesicle

For detection of membrane active fractions, polydiacetylene (PDA) was used in conjunction with phospholipids as a membrane model vesicle. To prepare polymerized vesicles, dimyristoyl phosphatidyl choline (DMPC), and diacetylenic monomer, 10,12-tricocosadiynoic acid (TCDA) were separately dissolved in dichloromethane 1 mg/ml, mixed at a 2:3 molar ratio at a concentration of 1 mM. The lipid constituents were dried together in vacuum, followed by addition of deionized water and sonication. The vesicle solution is cooled, kept at 4ºC overnight, and then polymerized by irradiation at 254 nm for 1 h. The resulting vesicle solution had an intense blue colour owing to polymerisation of the diacetylene [26,27].

Amphotericin B was used as a positive control, and tetracycline was used as the negative control.

2.7 Circular Dichroism Spectroscopy

The circular dichroism (CD) experiment was performed using a Jasco spectropolarimeter model 810. The different spectra were measured in 1 mm length cell at room temperature from 190 to 260 nm. Data were recorded at 1 nm points with a scan rate of 100 nm/min and a time constant of 4 s. The average of three separate recordings with different concentrations 1X, 1:5X, and 1:250X were taken and then analysed [28].

2.8 Identification of Metabolites by Thin Layer Chromatography

In the current study, we used thin layer chromatography (TLC) to screen the ethyl acetate extracts of *Streptomyces* strain for metabolites. The extract was checked by TLC on analytical silica gel plates (mesh 60, 20 x 20, Merck) with different mobile phases such as dichloromethane, chloroform, and ethyl acetate/n-hexane solvent system. The crude extract was spotted, and the solvent front was allowed to be developed. The solvent was then dried in order to separate the bioactive compounds.The different solvent systems were prepared, and TLC was carried out to select the capable solvent system for showing a better resolution. Elution of compounds was detected with UV at 254 nm and 366 nm in a UV TLC chamber. A TLC plate showing the number of bands (compounds) can be further purified using preparative TLC (PTLC) and high-performance liquid chromatography (HPLC) [29,30].

2.9 Purification by Preparative Thin-layer Chromatography

First, for slurry preparation to coat the plate, Silica gel 60, GF254 Merck, for PTLC was mixed with water, and then, this mixture spread as a thick slurry on a clean glass plate. The resulting plate was dried and activated by heating in an oven at 110ºC for 1 h. The size of the plate was 20 × 20 cm^2. The choice of eluent was determined by a preliminary analytical TLC investigation that included n-hexane–ethyl acetate with rate of 1:1. The bands, localized after elution in glass tanks, were scraped off the plate with a spatula. To remove the silica gel, it was extracted with suitable solvent (methanol), and then, the silica residue was removed by centrifugation and the supernatant was transferred to a vial. The individual metabolites were again spotted on a TLC plate for confirmation of metabolites.

2.10 Compounds Characterisation by HPLC, ^{1}HNMR, COSY, and LC-MS

2.10.1 HPLC analysis

The HPLC system was a Perkin Elmer model Flexar with a photodiode array detector (DAD). It consisted of an injection valve with a 20 µl loop, and C18 column (10 mm × 150 mm) Perkin Elmer packed with reverse phase silica gel particles. The column is equipped with a C18 (4.0 mm × 3.0 mm) guard column. The spectrophotometric detector was a DAD, which plotted the detector outputs.

The reagents for mobile phase preparation were of HPLC grade. A standard HPLC method was set up with HPLC mobile-phase consisting of acetonitrile: water. The gradient system was set as follows (with acetonitrile % water %). First, 8 min with 20% water, then 3 min with 50% water, followed by 5 min with 100% water, and the last 7 min with 20% water. This gave a total run time of 23 min. The flow rate was 1 ml/min, and the detection wavelength was 254 nm. The DAD provided all characteristic UV wavelengths. The pure fraction was carried out on the same HPLC. For pure fraction, a method was set up with water and methanol. The isocratic profile for pure fraction was set as follows: water 30% for 12–30 min, with an injection volume of 20 µL. Pure picks for each fraction were obtained, and then, we started analysis by ^{1}HNMR, ^{13}CNMR, COSY, and LC-MS [31,32].

2.10.2 ^{1}HNMR, COSY, and LC-MS spectroscopy

^{1}HNMR, COSY, and LC-MS spectra were performed for characterisation of compounds. ^{1}HNMR and COSY spectra were recorded on Bruker 300 MHz Fractions were dissolved in a MeOD-d4 solvent and poured in an NMR tube and observed with an applied magnetic field. All 1D and 2D spectra were obtained using the standard Bruker software. LC-MS was obtained using the Agilent Technologies 6410 Triple quad LC-MS, LC 1200 series. LC condition: mobile phase A; water, mobile phase B: acetonitrile. The program was as follows: first 8 min with 20% B, followed by 3 min with 50% B, 5 min with 100% B, and finally, 7 min with 20% B. The total run

time was 23 min. Other parameters were as follows: Column C18 flow rate: 1 ml/min; DAD detector: 254 nm; injection volume: 20 µl; mass condition ESI: positive mode [33].

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2.11 Cultivation of Yeast and Toxicity Testing

For the growth of the inoculum and for the toxicity testing, *S. cerevisiae* was grown at 30ºC in SMA for 48 h. The ethyl acetate fraction of extracts was dissolved in dimethyl DMSO to a reach concentration of 10 mg/ml.

For the toxicity testing, the malt extract medium was supplemented with a series of dilutions of studied fractions, ranging from 6.25–1000 µg/l. The broth microdilution method was performed, 100 µl of SMB media was added to each well of a 96-well microplate, and then, 40 µl of each fraction of extracts and 60 µl broth was added to the first well. Then, 100 µl of the solution was serially diluted from well A by taking 100 µl into B until the end (well H). This dilution was continued down the plate, and 100 µl from the last well (H) was discarded. Then, all the wells were filled with 100 µl of the working fungal inoculum (a value of 0.5 McFarland standards was used as a reference). The plate was covered and incubated at 37ºC for 24 to 48 h.

After that, 10 µl of Alamar blue 0.1% (Himedia, India) was added to each test well. The plates were re-incubated at 37ºC for 30 min. Spectrophotometric analysis was performed in the microplate reader at 540–630 nm. The percentage of cytotoxicity was calculated using MS excel by adding trend line and obtaining the relevant equation. The values of cytotoxic concentration (CC_{50}) were established using the Wolfram Alpha website [34].

2.11.1 Determination of selectivity index

The selectivity index of the ethyl acetate fraction of extracts was calculated by evaluating the ratio between the CC50 of *S. cerevisiae* and MIC for each test microorganism. Values greater than 1 were considered as suitable selectivity.

3. RESULTS

Based on our previous study, among the isolates from the soil, 16 out of 60 isolated strains showed antimicrobial activity in the liquid medium MIC test [24]. Among the active isolates, 3 strains, namely 0811, 08317, and 08346, showed the highest antimicrobial effect against the tested microorganisms (*E. coli*, *C. albicans*, and *S. aureus*) (Table 1).

The results indicated that selected strains produced metabolites inhibiting the growth of bacteria and yeast used in the assay. According to the results shown in Table 1, the ethyl acetate fraction of the cultured strains showed no toxicity on the concentrations tested (7.4 to 1000 µg/ml) on the indicator cell culture (S. cerevisiae).

The colour changes were produced by interaction of the membrane model vesicles with the bioactive metabolites secreted by microorganisms. Colour transition of blue to red is interpreted as the positive result and was observed in a fraction of strain 0811 in TSB and ISP2 media. In addition, amphotericin B was used as the positive control, and tetracycline was used as the negative control (Fig. 1).

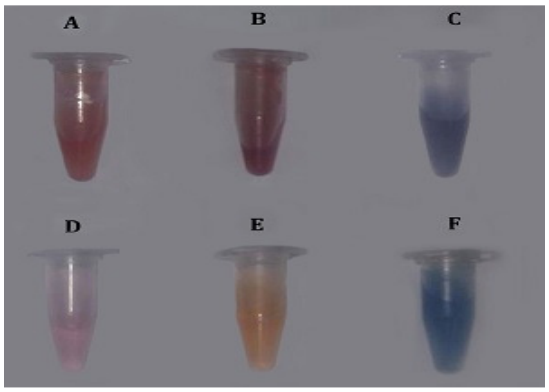


Fig. 1. Colorimetric assay by Lipid/PDA vesicle; red and orange (active), blue (inactive), pink (weak activity). First row: 0811 ISP2 (A), 0811 TSB (B), Vesicle only (C), DMSO (D), AmB (E), and tetracycline (F)

Using CD spectropolarimetry, the components of Lipid/PDA artificial membrane did not show desirable results with 1X, 1: 5X, and 1: 250X concentrations, and were finally tested with a concentration of 1: 2500X (Fig 2). The CD graph and absorbance profile generated by the instrument indicated a good degree of similarity between the fractions of 0811 TSB and amphotericin B. In the absorbance section of the CD graph (bottom graph), the difference between the control group (vesicle only) and the other groups is evident. These changes are consistent with colour change of the macroscopic vesicles.

Table 1. Antimicrobial activity of the ethyl acetate extract of actinomycete cultures against *E. coli*, *S. aureus*,and *C. albicans* expressed as (mg/ml), and evaluation of cytotoxicity and selectivity index

Strain/Medium		0811/ ISP2		0811/ TSB		08317/TSB		08317/ISP2		08346/TSB		08346/ISP2		Ketoconazole	AmB	Streptomycin	Kanamycin	DMSO
Microorganism		**24 h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24h**	**48h**	**24 h**	**48h**	**24 h**	**48h**					
C.albicas ATCC 10231	MIC (mg/ml)	0.5	0.5	0.5	1	0.25	0.25	0.5	1	0.125	1	0.25	1	0.0015	0.0075	-	-	10%v/v
	SI	1.24	1.24	1.78	0.893	5.64	5.64	2.1	1.06	10.26	1.28	5.08	1.27					
E. coli ATCC 29922	MIC (mg/ml)	0.5	1	0.5	0.5	>1	>1	1	>1	>1	>1	1	1	-	-	-	0.006	10%v/v
	SI	1.24	0.62	1.78	1.78	1.41	1.41	1.06	1.06	1.28	1.28	1.27	1.27					
S.aureus ATCC 25923	MIC (mg/ml)	0.5	N	0.25	N	0.0468	N	0.062	N	>1	N	>1	N	-	-	-	0.05	10%v/v
	SI	1.24	N	3.57	-	30.66	N	17.01	N	1.28	N	1.27	N				-	

MIC: Minimum Inhibitory Concentration; SI: The selectivity index was determined by dividing CC50 to MIC for each test microorganism; (-) Not tested ; N = no detected values

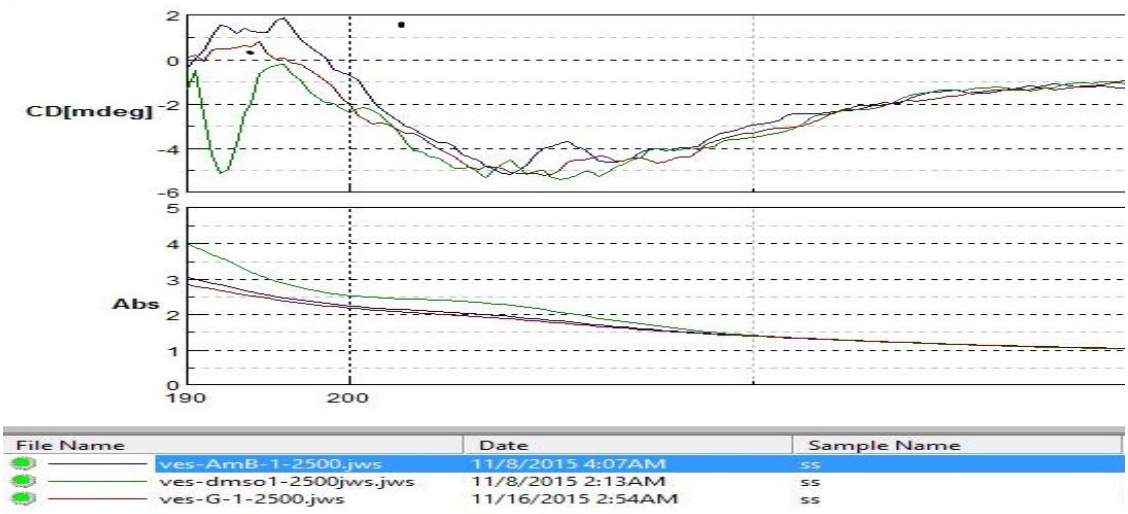


Fig. 2. Circular dichroism and absorption measurements to confirm membrane activity of fraction 0811 TSB (G-1-2500)

HPLC analysis was profiled based on the use of a sensitive and rapid method to estimate the production of secondary metabolites present in ethyl acetate fraction of *Sterptomyces* sp 0811. It was done by the HPLC technique using DAD detectors at 254 nm, and the results are shown in Fig. 3.

Extensive LC-MS fragmentation experiments on the extracted fraction resulted in the identification of the metabolites existing in the bioactive fraction and structurally elucidation of the corresponding structures shown in Fig. 4.

According to TLC results, the best mobile phase for purification of the bioactive strain fraction was ethyl acetate/hexane 50:50. In this solvent system TLC showed many metabolite spots that were produced by strain 0811, as indicated by their Rf values (0.22, 0.35, and 0.42). This mobile phase system was also used for PTLC to purify the compounds. After purification by PTLC, the individual metabolites were again spotted on the TLC plate for confirmation of their purity. Each fraction was analysed by HPLC. Pure fractions from strain 0811 (TSB) were named as PAA and PAB. The chromatogram isshown in Fig. 5. A sharp single peak wasobtained at the retention time of 13.60 min withpurity of about 95% for PAB and retention time of 13.72 min for PAA.

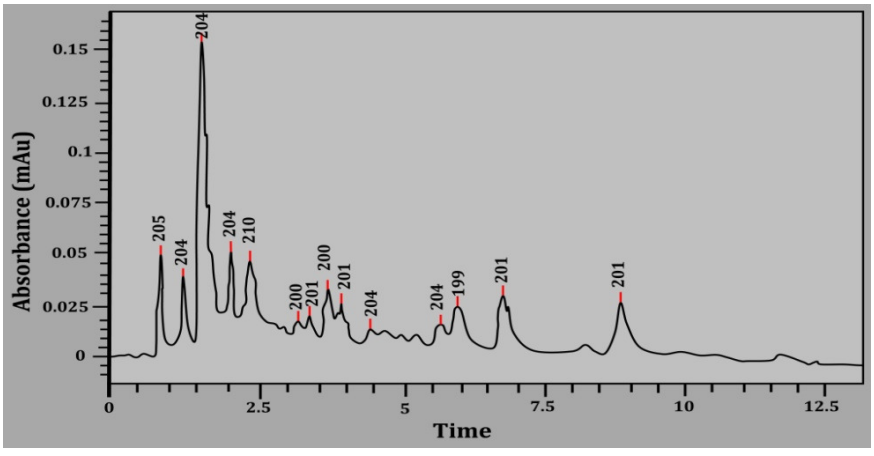


Fig. 3. HPLC-DAD analyses detected at 254 nm of the crude ethyl acetate fraction from *Streptomyces* strain code 0811 cultured in TSB

Hence, the isolated pure bioactivecompound was characterized using various spectroscopic studies. The mass spectrum of the purified compounds is shown in Fig. 6. The results of the present study for the purified PAA and PAB compounds in the positive ion mode and the ESI spectrum showed major (M+H) peak at 146.9 (m/z) and 224.9 (m/z). The molecular mass of the purified compounds was found to be 145.9 for (PAA) and 223 for (PAB).

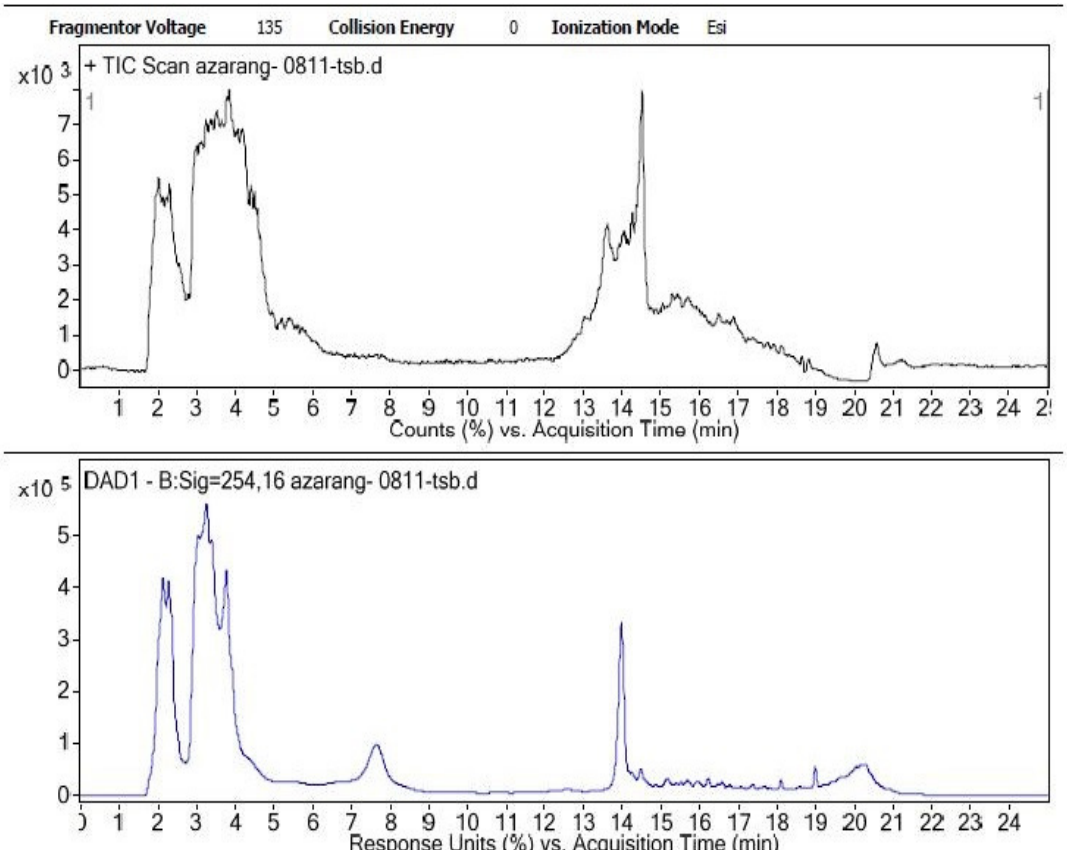
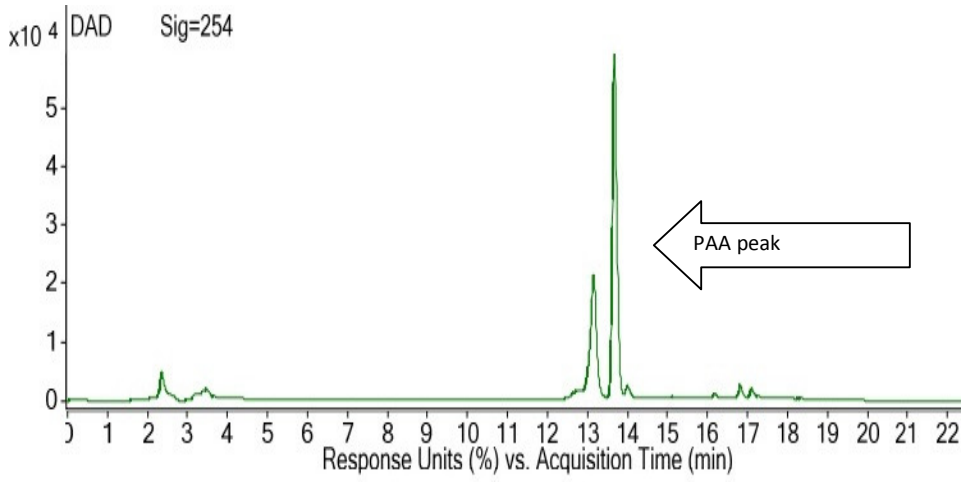


Fig. 4. LC-MS chromatogram from ethyl acetate fraction of 0811/ TSB. Top is total ion count (TIC) and bottom is diode array detector (DAD)



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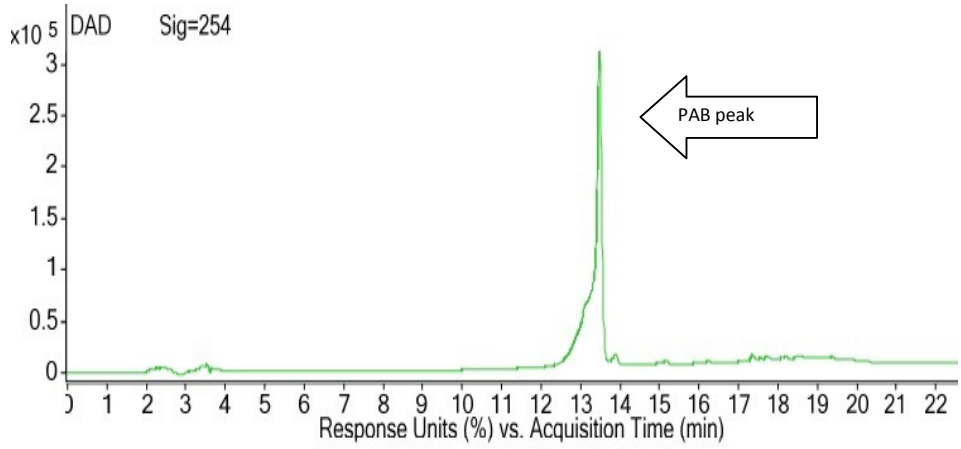

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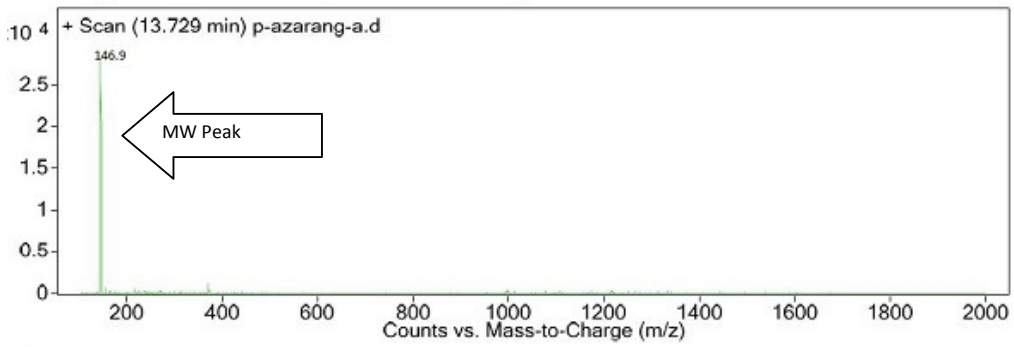
Fig. 5. HPLC profile of the purified PAA (1), PAB (2) compounds at 254 nm.



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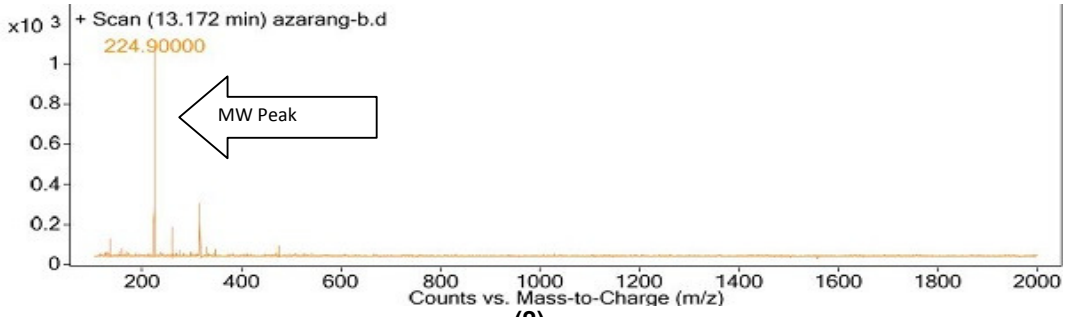

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Fig. 6. LC-MS spectrum of the purified compounds PAA (1), PAB (2)

^{1}HNMR of the purified compounds was obtained using a 300 MHz instrument. From the ^{1}HNMR spectra, the chemical shifts were observed and are calculated as shown below:

PAA: 9.87 (1H, s, N-H6), 8.22 (1H, d, H-1´), 8.09 (1H,d,H-3), 7.54 (1H, d, H-5), 6.32 (1H, t, H-2), 6.88 (1H, d, H-4), 2.14 (3H,t,H-8), 1.27 (1H,s, H-7).

PAB: 8.54 (1H,s , NH-2) , 7.89 (1H, s, NH-7) , 7.42 (2H, d, H-4,4') , 6.73 (2H, d, H-3,3′), 2.18 (2H, t, H-5), 1.88 (1H, s, H-OH), 1.27 (2H, s, H-5), 1.10 (2H,t, H-6), 0.89 (3H, d, H-9).^{1}HNMR (MeOD-d4, 300MHz).

2D-NMR by COSY experiments were performed for (MeOD-d4, 300 MHz). Inter-molecular cross peaks between H-1 and H-2, H-4 and H-5, H-2

and H-3 appear in the contour plots of the 2D spectra of **PAA.** In **PAB**, cross peaks between protons are H-8 and H-9, H-5 and H-6, H-3 and H-4, and H-3' and H-4'. Intra-molecular cross peaks appear in the contour plots of the 2D-NMR spectra, indicating that cross peaks between protons are separated from each other. These chemical assignments obtained from the UV, LC-ESI-MS, and ^{1}HNMR spectroscopy and COSY suggested the structure **PAA** and **PAB**, as shown in Fig. 7. The molecular formula of the purified compounds was determined to be $C_{11}H_{16}N_2O_3$ for PAB and $C_{10}H_{11}N$ for PAA.

4. DISCUSSION

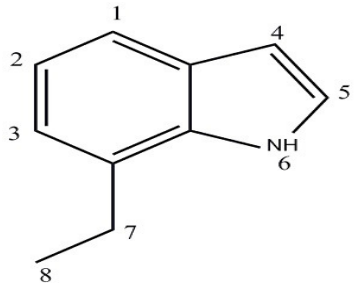
Extracts from natural products usually contain a high number of different new drugs. The present study was designed to investigate antimicrobial properties through membrane activity by an attempt to identify and pick up *Streptomyces* strains that display antimicrobial activity.

Results of the antimicrobial assay showed that the fractions tested in this study exhibited antimicrobial activities against bacteria and fungi. MIC values ranged from 46.8 to 1000 µg/ml. According to Kuete [35] the antimicrobial activity can be classified as potent for MIC values below 100 µg/ml, moderate when 100<MIC<625 µg/ml and weak if MIC>625 µg/ml. Therefore, the overall antibacterial activity exhibited in this study varied from moderate to significant. Among the two bacterial strains tested, the Gram-positive bacteria (*S. aureus*) were the most sensitive to the fraction 0811 TSB and the Gram negative bacteria strain (*E. coli*) were the most resistant. These results were consistent with the notion that Gram-positive bacteria are generally more sensitive than Gram-negative bacteria. The resistance of Gram-negative bacteria towards antibacterial substances is due to their outer membrane, which contributes to the intrinsic resistance by acting as an efficient permeability barrier [36].

The membrane activity was monitored by a Lipid/PDA test. As mentioned, the PDA polymer used in the present vesicles model had some important characteristics, such as transition from blue to red colour under external perturbation, which could be visible by the naked eye and also supported by interaction with the membrane components followed by CD spectropolarimetric experiments.

Various chromatographic techniques have been used for fractionation and purification of compounds from our biologically active sample. PTLC and HPLC are the most popular and widely used separation techniques to characterize organic materials in chemical analysis. In this research, successful application of PTLC and HPLCtechniques have been reported for the isolation and characterisation of biologically active secondary metabolites from the *Streptomyces* sample.

Monitoring of sample complexity and purification in this study has been performed by TLC and PTLC. Determination the structure was carried out by ^{1}HNMR, COSY, and LC-MS. The CD technique was also applied for monitoring membrane interaction and confirming the changes in the conformation of membrane as observed by colorimetric study of artificial vesicles. ^{1}HNMR and COSY was employed to determine the chemical structure of purified molecules and to confirm the neighbouring functional groups. The mass spectrometry helped in determining the molecular weight. Then, a search in



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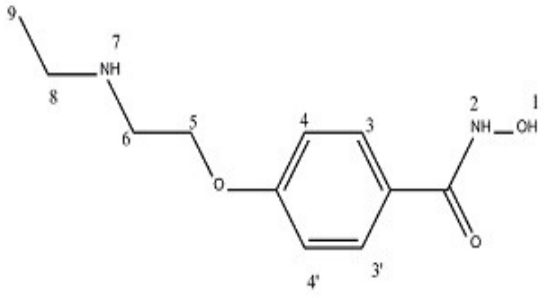

(2)

Fig. 7. The proposed structure of the two compounds isolated from ethyl acetate fraction of strain sp 0811. PAA (1), PAB (2)

important databases, such as ChEBI, confirmed that **PAB** is a new natural product from the hydroxybenzamide family, but compound **PAA**was previously reported by Takahashi as a novel compound from *Streptomyces* sp [37]. We found in other reports that new hydroxybenzamide derivatives have been synthesized [38]. All the synthesized compounds were screened against Gram-positive and Gram-negative bacteria and fungi. Some of them showed antibacterial and antifungal activities compared to the standard drugs [38]. In fact, the π electron delocalisation in PAB that enhances proton-donating capacity of the N-hydroxyl group, and the multi-nucleophilic nature of the compound easily helps the interface with the microbial components, thus stopping their growth.

5. CONCLUSION

Our results indicate that we could identify different new molecules with novel mechanism of action. Most microorganisms have developed resistance to existing antibiotics. Therefore, it is a motivation for continual research on the production of new drugs to overcome the resistant microorganism and to determine the structure of active compounds. In continuation of this research, the synthesis of derivatives and similar isolated compounds is underway.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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