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Antimicrobial, and Antibiofilm Forming Activities of Red Pigment of *Talaromyces marneffei* Strain SA2a on Multidrug-Resistant *Burkholderia* sp.

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Abstract *Burkholderia* are Gram-negative bacteria found in moist environments, soil, and water, where they can survive for months. These bacteria are resistant to several antibiotics of choice in treating *Burkholderia*-associated infections and diseases. This study aims to evaluate the activities of red pigment of *Talaromyces marneffei* on multidrug-resistant *Burkholderia* sp. Pigment producing fungus was obtained from microbial culture collections and it was properly identified by sequencing of the 18S rRNA partial sequence. Red pigment from the fungus was extracted with ethyl acetate and characterized with a UV-visible spectrophotometer, FTIR, and GCMS. Antibacterial activities of the pigment were determined on multidrug-resistant *Burkholderia pseudomallei* and *Burkholderia cepacia* complex isolates with agar well diffusion method. Antibiofilm formation was evaluated in 96-well microtiter plates and antioxidant properties were also determined. The molecular characterization identified the fungus as *Talaromyces marneffei* strain SA2a. The ethyl acetate efficiently extracted the pigment, and UV-visible identified the red pigment as purpuride at a wavelength of 512nm. The FTIR revealed the functional group as alkanes, esters, and carbonyl groups, and the GCMS showed the prominent compounds in the pigment as Ethanol, 2-nitro- (m/z 91.07), Bis(n-propylthio)methane (m/z 164.3), Acetic acid (m/z 60.05), and Purpuride (m/z391.5). The pigment demonstrated good inhibitory potential on 18(90%) of the *B. pseudomallei* and 11(85%) of the *B. cepacia* complex isolates at 200mg/mL-12.5mg/mL with zones of inhibition around the wells. The pigment prevented biofilm formation with 25 - 96% biofilm inhibition on *B. pseudomallei* and 19 - 94% on *B. cepacia* complex isolates. The crude pigment showed 48.22% DDPH scavenging activities at the lowest concentration of 100mg/mL, 11.37±0.04 FRAP content at 100mg/mL and 11.58±0.01 ABTS at 100µg/mL. The result of this study suggests that the pigment of *Talaromyces marneffei* could be a promising alternative therapy in treating *Burkholderia* infections. Further research is required to show these potential applications in pharmaceutical industries for drug production.

Keywords: : Antibiofilm, Antimicrobial, *Burkholderia* sp, Multidrug resistance, *Talaromyces marneffei*

I. Introduction

Burkholderia pseudomallei complex is a Gram-negative environmental bacterium widely distributed in water and soil, and the group includes *B. mallei, B. pseudomallei, B. humptydoensis*, and *B. thailandensis* [1, 2]. They are distinguished by their genetic and morphological resemblance to *B. pseudomallei* [3]. *Burkholderia pseudomallei* is the melioidosis-causing agent, and the tropical illness can appear as a localized infection or community-acquired pneumonia or abscess formation, particularly in the spleen, liver, and prostate [4, 5]. This infection can be transmitted through ingestion or inhalation of this organism via exposure to soil or water. The infection is common in endemic locations, among people in

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rural areas and among agricultural workers [6]. The illness has been documented to be the cause of death in northern Australia, Southeast Asia, South and Central America and the Indian subcontinent [4,7].

Burkholderia cepacian complex (Bcc) is a group of non-fermenting, Gram-negative bacteria colonizing human or natural niches [8]. Bcc is majorly isolated in moist environments, soil, and water, where it can survive for months. Bcc can be isolated from the sputum of patients with cystic fibrosis and is linked with accelerated pulmonary function increase and decline mortality due to treatment challenges and antimicrobial resistance [2, 9]. Bcc can cause severe disease in patients suffering from chronic granulomatous illness, whose phagocytes are incapable of generating reactive oxygen species. Bcc can be transmitted from one person to another directly or indirectly by contact and/or droplets, particularly in individuals with cystic fibrosis. This can lead to lethal necrotizing pneumonia, often called "cepacia syndrome" [10, 11].

Most *B. cepacia* and *B. pseudomallei* clinical isolates resist β-lactam antibiotics due to class A β-lactamase, encoded by *penA* gene on chromosome 2 [3]. Three resistance nodulation cell division (RND) pumps in *B. pseudomallei* have been characterized. In most strains of *B. pseudomallei*, BpeAB-OprB confers host adaptability, quorum sensing in some isolates, and low-level resistance to macrolides, tetracyclines, chloramphenicol, and fluoroquinolones in regulatory mutants. AmrAB-OprA expression is responsible for intrinsic resistance to macrolides, aminoglycosides, and some resistance to tetracyclines. Regulatory mutants express BpeEF-OprC, which extrudes trimethoprim, tetracyclines, sulfamethoxazole, fluoroquinolones, and chloramphenicol [3, 12]. Invitro biofilm formation is a common trait of *Burkholderia* sp., and it has been associated with the persistence of *Burkholderia* infections. *Burkholderia* sp. has developed several strategies for survival by extrusion of antimicrobials from the cell through efflux pumps or the inactivation by catabolic enzymes [2, 13].

The treatment for melioidosis is usually quite extensive and involves 10 to 14 days of intravenous meropenem, ceftazidime, or imipenem, followed by 3 to 6 months of oral trimethoprim-sulfamethoxazole. *B. pseudomallei* has intrinsic resistance to several antibiotics, including macrolides, penicillin, rifamycins, cephalosporins first- and second-generation, aminoglycosides and polymyxins [4]. However, *Burkholderia* sp. now showed resistance to antibiotics of choice that are used in the treatment of infections and diseases caused by *Burkholderia* sp.

Studies have shown that secondary metabolites from *Talaromyces* have novel structures with good biological activity, which provides a basis for the development and application of endophytes. Compounds isolated from the secondary metabolites of *Talaromyces* include polyketones, esters, steroids, terpenoids, anthraquinones, alkaloids and others. Most of them have a wide range of biological activities, such as antibacterial, anti-inflammatory, and antitumor activities. In addition, species of this fungi have great potential in environmental protection, agriculture, cosmetics, food, and medicine [14, 15].

In order to provide an alternative therapy for the treatment of *Burkholderia* infections and to reduce the spread and burden of antimicrobial resistance of *Burkholderia* sp., this study aims to

evaluate antimicrobial and antibiofilm forming activities of red pigment of *Talaromyces marneffei* strain SA2a on multidrug-resistant *Burkholderia* complex isolates.

II. Materials and Methods

A. Collection and Molecular Identification of Fungal Isolate

The fungal isolate used in this study was obtained from microbial culture collections of African Centre of Excellence for Microbial Secondary Metabolites, Department of Microbiology, Osun State University, Nigeria. Fungal DNA was extracted with Zymo-research fungi DNA extraction kits following the manufacturer's protocol. Internal transcribe sequence region, ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'and - ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3'primers were used in the identification of 18S rRNA partial sequence. PCR amplification of the region was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR condition of initial denaturation at 94°C for 5 min, 35cycles, final denaturation at 94°C for 30 secs, annealing at 55°C, for 30 secs, extension at 72°C for 60 secs, and a final extension for 10 min at 72°C. The PCR product was run on 1.5% agarose gel and visualized with an ultraviolet transilluminator to check for successful amplification. The amplified fragments were purified and sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems with the manufacturer's manual. The obtained DNA sequences were compared to available sequences online in a GenBank database (http://www.ncbi.nlm.nih.gov). Homology search was performed using Bioinformatics tools available online, BLASTn www.ncbi.nlm.nih.gov/BLA. Homology sequences obtained were aligned and Molecular Evolutionary Analysis tools (MEGA11) were used to plot the neighbor-joining. The determined sequences were submitted to GenBank for GenBank association [16].

B. Production and Extraction of Fungal Pigment

The fungal isolate was inoculated on sterile Sabouraud dextrose agar (SDA) and incubated for 7 days at 25±2°C. The fungal mycelium (5mm) plug was transferred into sterile Sabouraud dextrose broth (SDB) (pH 5.0) and incubated at 25±2°C for 14 days in static condition. Fungal mycelium was sieved with cheese cloth, and the broth containing pigment was then centrifuged for 10 minutes at 7000 rpm and filtered with Whatman No. 1 to obtain debris-free pigment. The pigment was extracted with an equal volume of ethyl acetate with a 1000mL capacity separating funnel and stored in the dark at 4 °C to avoid photo-degradation of the pigments [17, 18].

C. Characterization of Fungal Pigment

i. UV-vis spectrophotometer

The pigment was characterized using the Pharo 300 15320070 2.20-Merck-2.20 UV-visible spectrophotometer. The pigments were analyzed with a wavelength range of 200-700 nm, to determine the highest absorption peak of the pigment, and ethyl acetate was used as blank [17, 19, 20, 21, 22, 23].

ii. Fourier- transform infrared spectroscopy (FT-IR) of pigment

The SHIMADZU FTIR-8400S was the FTIR spectrophotometer used to characterize pigments. The infrared spectrum of the FTIR

helps in structural analysis. The pigment was analyzed in the range of 4000 to 400cm^{-1} to identify the functional group present and their position **(**OH, N-H, C=C, C-H, C-N, C-H, and C-O) in the pigment [23, 24].

iii. Gas chromatography-mass spectrometry (GC-MS) of extracted pigment

Following the method of **[24], t**he Perkin—Elmer Clarus 680 system (Perkin Elmer Inc. USA) was utilized for the GCMS analysis of the extracted pigment with a fused silica column, packed with the elite **-** 5MS) capillary column (30 m in length*250 nm in diameter *0.25 nm in thickness). The carrier gas was unalloyed helium (99.99%) at a 1 mL/min constant flow rate. An electron ionization energy method was used with 70 eV (electron Volts) high ionization energy with 0.2 s scan time—fragments ranging from 40 to 600 m/z to detect the GCMS spectral. The injector temperature was maintained at 250 °C (constant). The column oven temperature was set at 50 °C for 3 min, raised at 10 degrees/min up to 280 °C, and the final temperature was increased to 300 °C for 10 min. The compounds present in the pigments were identified by comparing their retention time (min), peak area, peak height, and mass spectral patterns with the spectral database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library (NIST 2008).

D. Antimicrobial Activities of Extracted Pigment

The activity of the extracted pigment on multidrug-resistant *Burkholderia* isolates previously reported by [2] (Table 1) was carried out with agar well diffusion methods. 0.5 McFarland standards were prepared from 24hr old *Burkholderia* isolates culture and swabbed on Mueller Hinton Agar plates with a sterile swab stick. Wells of 6 mm were bored with sterile cork borer on the plates. Different concentrations of the dried crude pigment ranging from 200, 100, 50, 25, and 12.5 mg/mL were dissolved in 2% DMSO respectively. In each well, 100µL of each mixture concentration was dispensed into the wells accordingly, and 2% DMSO was employed as a negative control. The experiment was conducted in triplicate, and the bacteria plates were incubated at 37°C for 24 hrs. Zones of inhibition were observed and recorded on each plate and compared with European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical Laboratory Standards Institute (CLSI) breakpoints [19, 23, 25, 26].

E. Antibiofilm Formation Activities

With slight modification to the methods described by [27], [28], [29], [30], 200 µL/well of sterilized Tryptic soy broth consisting of Casein peptone 17 g/L, Dipotassium phosphate 2.5 g/L, Sodium chloride 5 g/L, Dextrose 2.5 g/L, and Soy peptone 3 g/L) medium supplemented with 1% glucose was dispensed to sterile flat bottom 96-well polystyrene plates. An overnight culture (50 µL) of each *B. pseudomallei* complex and *B. cepacia* complex isolate with optical density OD_{600} = 0.1 (10^6 colony forming units (CFU/mL)) was added into each well. Test wells were treated with 50 µL/well of 200 mg/mL of the crude pigment, and untreated wells contained no crude pigment. Negative control wells were filled with 200µL of TSB + 1% glucose only, and the experiment was conducted in triplicate. The plates were incubated for 24hrs at 37°C in a static condition. After 24 hrs of incubation, the cell growth in treated and untreated wells was measured with a microplate reader at a wavelength of 630nm and the

Table 1a: Multidrug-Resistant *burkholderia pseudomallei* complex Isolates and Resistant Antibiotics [2].

***Burkholderia pseudomallei* complex Isolate**	**Multidrug-Resistance Pattern**
S3L3a	AMC, MEM, CTX, CAZ, and K
S3L3b	AMC, CT, MEM, CTX, CAZ, and K
S3L3c	AMC, CT, IMP, MEM, CTX, CAZ, and K
S3L3d	AMC, MEM, CTX, CAZ, and K
S3L3e	AMC, IMP, MEM, CTX, CAZ, and K
S3L3f	AMC, CT, IMP, MEM, CTX, CAZ, and K
S3L3g	AMC, CT, IMP, MEM, CTX, CAZ, and K
S3L3h	AMC, CT, IMP, MEM, CTX, CAZ, and K
S3L1a	AMC, CT, IMP, MEM, CTX, CAZ, and K
S3L1b	AMC, CT, CTX, CAZ, and K
S3L1c	AMC, CT, CTX, CAZ, and K
S3L1d	AMC, CT, MEM, CTX, CAZ, and K
S3L1e	AMC, CT, CTX, CAZ, and K
S3L1f	AMC, CT, CTX, CAZ, and K
S3L1g	AMC, CT, CTX, CAZ, and K
S3L1h	AMC, CT, CTX, CAZ, and K
S3L1i	CTX, CAZ, and K
S3L1j	AMC, CT, MEM, CTX, CAZ, and K
S1L1a	AMC, CT, IMP, CTX, CAZ, and K
S1L1b	AMC, CT, IMP, CTX, CAZ, and K
S1L1c	AMC, CT, IMP, MEM, CTX, CAZ, and K
S1L1d	AMC, CT, IMP, MEM, CTX, CAZ, and K
S2L3a	AMC, CT, IMP, MEM, CTX, CAZ, and K
S2L3b	AMC, CT, IMP, MEM, CTX, CAZ, and K
S2L3c	AMC, IMP, MEM, CTX, CAZ, and K
S2L1a	AMC, CT, IMP, MEM, CTX, CAZ, and K
S1L3a	AMC, CT, IMP, MEM, CTX, CAZ, and K

Key: CT- Colistin (10µg), IMP- Imipenem (10µg), AMC- Amoxicillin/clavulanic acid (20/10µg), MEM- Meropenem (10µg), CTX- Cefotaxime (30µg), CAZ- Ceftazidime (30µg), and K- Kanamycin (30µg)

Table 1b: Multidrug-Resistant *Burkholderia cepacia* complex Isolates and Resistant Antibiotics [2]

Burkholderia cepacia complex Isolate	Multidrug-Resistance Pattern
S3L2a	Ct, AMC, CAZ, MEM, P and CTX
S3L2b	Ct, AMC, CAZ, MEM, P and CTX
S3L2c	Ct, AMC, CAZ, MEM, P and CTX
S3L2d	Ct, CAZ, MEM, P and CTX
S3L2e	Ct, AMC, CAZ, MEM, P and CTX
S3L2f	Ct, AMC, CAZ, P and CTX
S1L2a	Ct, AMC, CAZ, MEM, P and CTX
S1L2b	Ct, AMC, CAZ, P and CTX
S1L2c	Ct, AMC, CAZ, MEM, P and CTX
S1L3a	Ct, AMC, CAZ, MEM, P and CTX
S1L3b	CAZ, P and CTX
S1L3c	Ct, AMC, CAZ, P and CTX
S1L3d	Ct, AMC, CAZ, MEM, P and CTX
S1L3e	Ct, AMC, CAZ, P and CTX
S2L1a	Ct, AMC, CAZ, MEM, P and CTX
S2L1b	Ct, AMC, CAZ, MEM, P and CTX
S2L1c	Ct, AMC, CAZ, MEM, P and CTX
S2L1d	Ct, AMC, CAZ, MEM, P and CTX
S3L1a	Ct, AMC, CAZ, MEM, P and CTX
S3L1b	Ct, AMC, CAZ, P and CTX
S3L1c	Ct, AMC, CAZ, MEM, P and CTX
S3L3a	Ct, AMC, CAZ, MEM, P and CTX
S3L3b	Ct, AMC, CAZ, MEM, P and CTX
S3L3c	Ct, AMC, CAZ, P and CTX
S3L3d	Ct, AMC, CAZ, P and CTX
S2L2a	Ct, AMC, CAZ, MEM, P and CTX
S2L2b	Ct, AMC, CAZ, MEM, P and CTX

Key: CT- Colistin (10μg), MEM- Meropenem (10μg), AMC- Amoxicillin/clavulanic acid (20/10μg), CTX- Cefotaxime (30μg), CAZ- Ceftazidime (30μg), and P- Penicillin G (1Unit)

absorbance of the negative control wells was subtracted from the treated and untreated wells to determine the percentage of cell growth inhibition.

Biofilm quantitative assay was performed using the crystal violet (CV) method. The contents of the wells were washed thrice with 200µL/well of sterile phosphate buffer saline (PBS) at pH 7.4 to remove planktonic bacteria. Methanol 200µL/well was dispensed for 15 minutes to fix the biofilms. The methanol was decanted, and wells were allowed to completely evaporate. Then, 200µL/well of 1% crystal violet was added for 5 min, and the excess dye was washed with distilled water. 200µL/well of 33% acetic acid was used to dissolve the crystal violet, and the absorbance was measured at 570 nm. The absorbance of control wells was subtracted from the absorbance of treated and untreated wells, and the percentage biofilm inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Untreated OD}_{570\text{ mm}} - \text{Treated OD}_{570\text{ mm}}}{\text{Untreated OD}_{570\text{ mm}}} \times 100$$

F. Antioxidant Activity of Red Pigment of *Talaromyces Marneffei*

i. DPPH radical scavenging activity

With slight modification to the methods described by [31], [32], 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by taking various concentrations of the pigment (100-300 mg/g) and exposing it to 1.5 mL freshly prepared solution of DPPH in methanol for 20 min, and then measuring the absorbance at 517 nm. The inhibition percentage of DPPH in the medium was calculated by comparing it with the control (untreated DPPH). Percentage inhibition was calculated as below:

$$\% \text{ Inhibition} = \frac{\left(ODDPPH - OD_{sample}\right)}{oddpph} \times 100$$

ii. ABTS radical scavenging activity.

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical-scavenging activity of extract was determined according to [26, 31, 33] with slight modification, ABTS radicals were pregenerated by adding 5mL of a 4.9mM potassium persulfate solution to 5mL of a 14mM ABTS solution and kept for 16 h in the dark. Different concentrations of extract (100-300 µg/mL) were added to the above activated pregenerated ABTS solution. This solution was suitably diluted with distilled water to yield an absorbance of 0.70 at 734 nm and then used for antioxidant assay. Ascorbic acid (50 μg/mL) was used as a reference compound. 50 μL was added to 950μL of ABTS solution and vortexed for 10 seconds after 6 min, and then a reduction in absorbance was recorded at 734 nm, using distilled water as a blank. The same volume of test solutions of each extract was also taken in a similar manner. The result was compared with control (only ABTS solution).

iii. Ferric reducing antioxidant power (FRAP) assay

The total antioxidant potential of a sample was determined using the ferric-reducing ability of plasma FRAP assay by [34] as a measure of antioxidant power. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM

HCl, and 20 mM $FeCl_3$ at 10:1:1 (*v*/*v*/*v*). The reagent (3.400 µL) and sample solutions (100 µL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 30 min. All solutions were used on the day of preparation. Analyses were performed in triplicate on each extract.

G. Statistical Analyses

All assays were conducted in triplicates and expressed as mean ± standard deviation (SD). The software Microsoft Excel, version 2019, was used to analyze the data. Using one-way Analysis of Variance (ANOVA), a statistically significant difference was identified and $p \leq 0.05$ was used as a limit to indicate statistical significance.

III. Results and Discussion

A. Results

i. Identification of pigment-producing fungal isolate

The result of the molecular identification revealed the identity of the pigment-producing fungus obtained from the microbial culture collection of the African Centre of Excellence for Microbial Secondary metabolites as *Talaromyces marneffei* strain SA2a (OR387723.1) with 99 % similarity when the fungal DNA sequence obtained was subjected to multiple alignment algorithms against the closest published sequences using BLASTn. The phylogenetic tree generated using MEGA 11 software showed the most adjoining strain to the fungal isolate to be *Talaromyces marneffei* isolate FPE 3C-1 (OL780147.1) Figure 1.

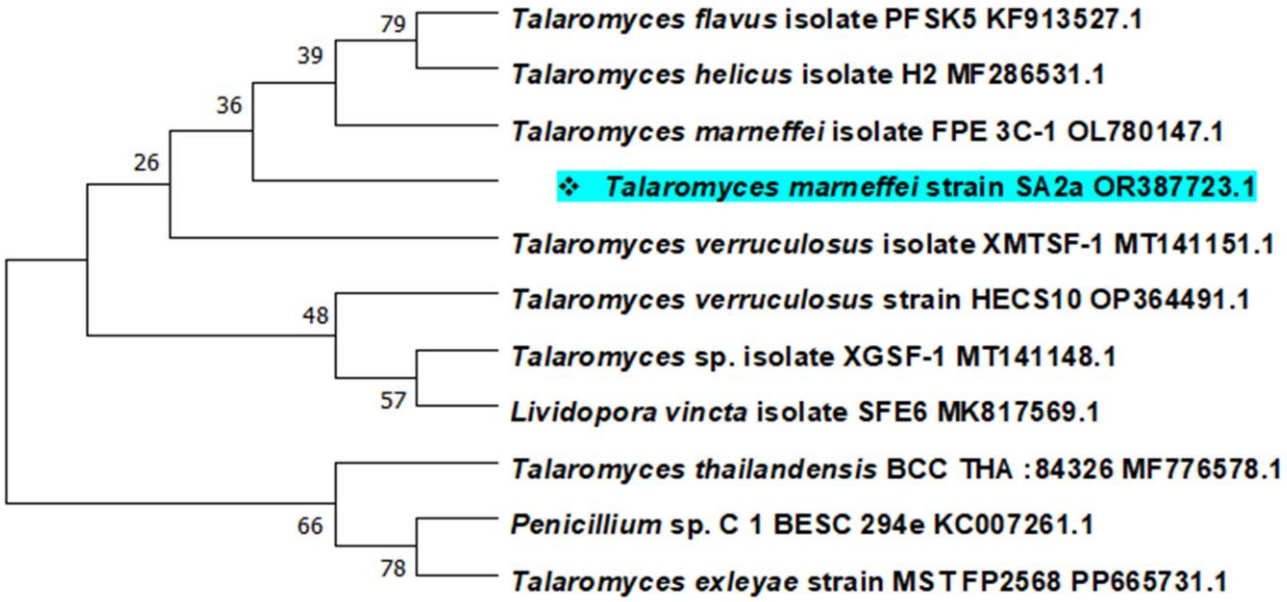

Figure 1: Neighbor-joining tree based on **18S rRNA (partial sequence)-ITS1-5.8S rRNA-ITS4-28S rRNA (partial sequence)** showing the phylogenetic position of the isolates **among** closely related taxa at a percentage of 1000 bootstrap replicates. **Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Evolutionary analyses were conducted in MEGA11**.

ii. Extraction and uv-vis spectrophotometer characterization of pigment

The *Talaromyces marneffei* strain SA2a was able to produce intense extracellular pigment in the SDB after 14 days of incubation. The pigment was observed to be red-coloured, and ethyl acetate was seen to efficiently extract the pigment. The UV-visible study of the extracted pigment showed diverse peaks at the UV end of the spectra, indicating the presence of several compounds in the pigment. The highest absorption was seen at a λmass of 310nm with an absorbance of 3.726 abs. Another sharp peak was observed at the λmass of 432nm with 2.533 abs and λmass of 512nm with 2.134 abs. In addition, the spectrophotometry analysis revealed that as the absorption wavelength increased, there was a decrease in the absorbance of the pigment, as shown in Figure 2.

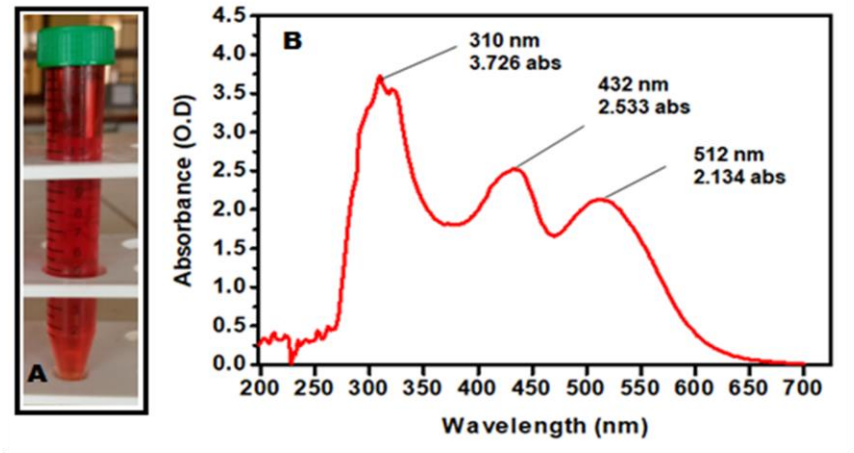

Figure 2: (A) Extracted extracellular red pigment (B) Uv-vis Spectrophotometer characterization of pigment produced by from *Talaromyces marneffei* strain SA2a (OR3877 23.1)
Key: nm- nanometer, abs- absorbance

iii. Fourier-transform infrared spectroscopy (FTIR) of extracted pigment from fungi isolates

The FTIR spectrum of extracted pigment from the *Talaromyces marneffei* strain is shown in Figure 3 & Table 3. The broad and long peak at 3410cm^{-1} is due to Dimeric O-H stretch. The peak at 2943cm^{-1} is due to N-H stretching of amine salt. The peak at 1996 cm^{-1} is due to CΞC stretching of alkyne. The peak at 1639 cm^{-1} is due to C=C stretching of alkene. The peak at 1508 cm^{-1} is due to N-O stretching of nitro compound. The peak at 1458cm^{-1} is due to C-H bending of alkane. The peak at 1242 cm^{-1} is due to C-N stretching of amine. The peak at 1153 cm^{-1}, 1078 cm^{-1}, and 1053 cm^{-1} is due to C-O stretching of tertiary alcohol. The peak at 1078 cm^{-1} is due to C-O stretching of primary alcohol. The peak at 528 cm^{-1} and 567 cm^{-1}, 416 cm^{-1} is due to C-I stretching of halo compound.

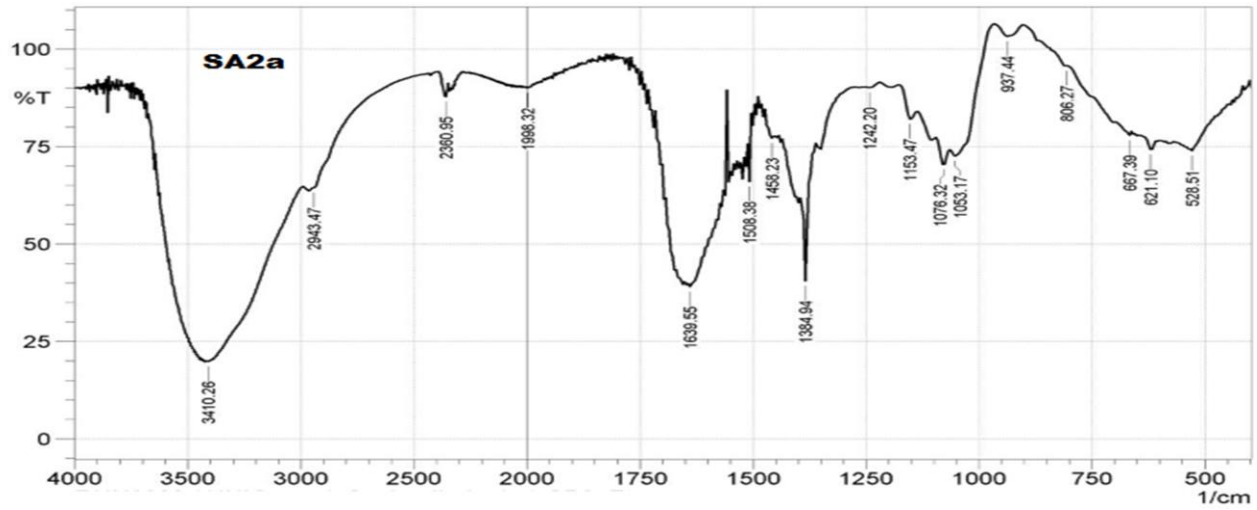

Figure 3: FTIR spectra of extracted pigment from *Talaromyces marneffei* strain SA2a
Key: SA2a (*Talaromyces marneffei*)

Table 3: Spectrum range of FTIR Result for Extracted Pigments from *Talaromyces marneffei* Strain SA2a

Absorption (cm^{-1})	Group	Compound Class
600-500	C-I stretching	halo compound
690-515	C-Br stretching	halo compound
840-790	C=C bending	Alkene
895-885	C=C bending	Alkene
1070-1030	S=O stretching	Sulfoxide
1085-1050	C-O stretching	primary alcohol
1205-1124	C-O stretching	tertiary alcohol
1250-1020	C-N stretching	Amine
1342-1266	C-N stretching	Aromatic amine
1390-1310	O-H bending	Phenol
1600-1300	C-H bending	Alkane
1550-1500	N-O stretching	nitro compound
1648-1638	C=C stretching	Alkene
2000-1650	C-H bending	Aromatic compound
2140-1990	CΞC stretching	Alkyne
2400-2000	O=C=O stretching	Carbon dioxide
3000-2840	N-H stretching	Amine salt
3550-3200	O-H stretching	Alcohol

iv. GCMS analysis of extracted pigment

The gas chromatography-mass spectroscopy analysis of the compounds in the extracted pigment revealed the presence of 91 different compounds from a retention time of 3.2 min to 31.5 min Figure 4. Among the compounds identified, the four most prominent compounds were identified as Ethanol, 2-nitro-(m/z 91.07) at a retention time of 4.82 min accounting for 43.20% peak area, Bis(n-propylthio)methane (m/z 164.3) at 3.60 min with 15.28% peak area, Acetic acid (m/z 60.05) at 4.10 min, with 11.46% peak area and Purpuride (m/z391.5) at 22.27 min, with 3.70% peak area. Other noticeable compounds with bioactivities were also accounted for in Table 4, and the structures are presented in Figure 5.

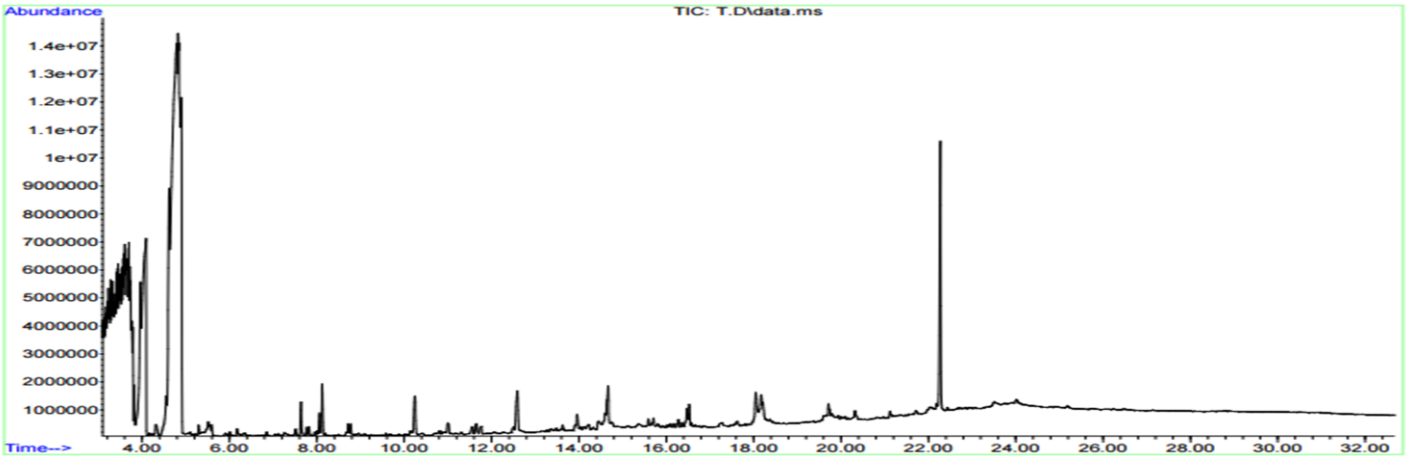

Figure 4: Spectra Scan of the Compounds Identified in the GCMS Analysis of the Pigment

Table 4: Compounds Present in the Pigment Extracted from *Talaromyces marneffei* Strain SA2a.

S/N	RT (min)	Area (%)	Library/ID	Molecular Formula	M/Z
1	3.28	2.23	4-Hydroxy-2-butanone	$C_4H_8O_2$	88.11
2	3.60	15.28	**Bis(n-propylthio)methane**	$C_7H_{16}S_2$	164.3
3	4.10	11.46	**Acetic acid**	$C_2H_4O_2$	60.05
4	4.82	43.20	**Ethanol, 2-nitro-**	$C_2H_5NO_3$	91.07
5	6.85	0.06	alpha-Pinene	$C_{10}H_{16}$	136.23
6	14.67	1.19	Hexadecane	$C_{16}H_{34}$	226.44
7	17.63	0.52	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	233
8	18.17	1.01	2- Chloropropionic acid, octadecyl ester	$C_{21}H_{41}ClO_2$	361
9	19.71	1.37	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	224
10	22.27	3.70	**Purpuride**	$C_{22}H_{33}NO_5$	391.5
11	23.51	1.35	Pyrido[2,3-d]pyrimidine, 4-phenyl-	$C_{13}H_9N_3$	270.23
12	24.02	1.76	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{48}O_7Si_8$	563

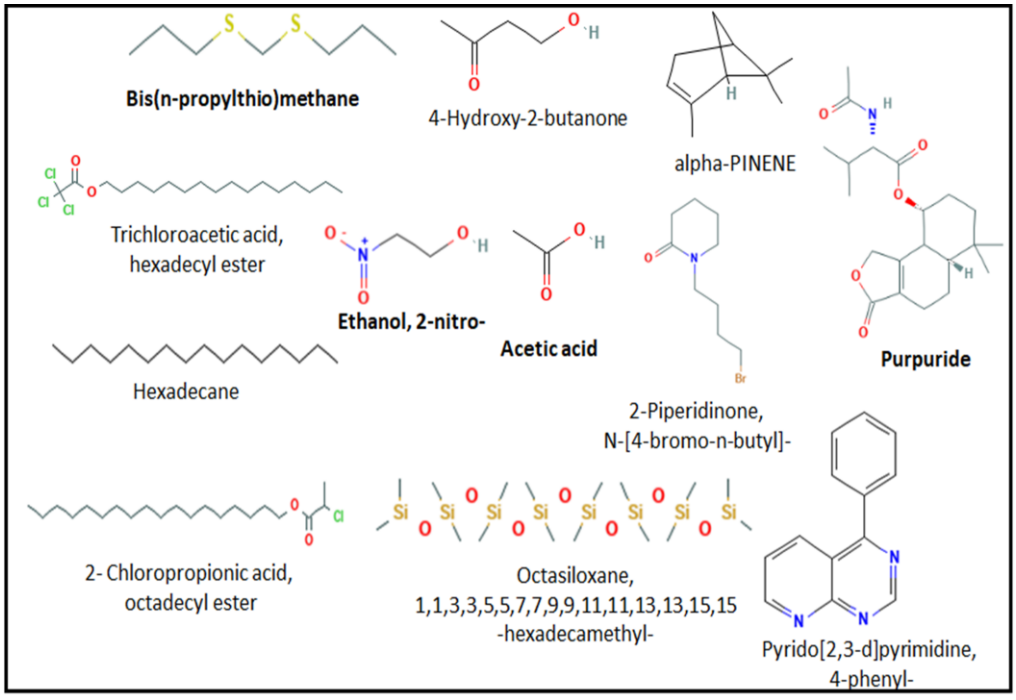

Figure 5: (A) Structure of Some Compounds in the Pigment with Bioactivities (B) Spectra of Most Abundant Compound

v. Inhibitory activities of pigment on *Burkholderia* isolates

The result of the inhibitory activities of the pigment on multidrug-resistant *Burkholderia* isolates revealed the presence of clear zones on the tested isolates. Out of the 27 *Burkholderia pseudomallei* complex and 27 *Burkholderia cepacia* complex isolates, the crude pigment at a concentration of 200mg/mL, inhibited the growth of the isolates. Zones of inhibition were recorded in 20 *Burkholderia pseudomallei* complex and 13 *Burkholderia cepacia* complex isolates Figure 6. 15 of the *B. pseudomallei* isolates showed resistance to the lowest concentration of 12.5 mg/mL tested with no zones of inhibition, whereas inhibitory zones between 10-15mm around the wells were observed in 5 of the isolates. In addition, 11 of the *Burkholderia cepacia* isolates showed resistance to the lowest concentration, 12.5 mg/mL of the pigment, with no zones of inhibition observed, and 8±0.5 and 8±0.6 mm zones were recorded in 2 of the isolates Table 5. When the zones of inhibition were compared to EUCAST and CLSI antibiotics breakpoint, it was observed that 18(90%) of the *Burkholderia pseudomallei* isolates were susceptible to the 200mg/mL of the pigment with similar zones of inhibition to imipenem and meropenem (10µg). Similarly, 11(85%) of the *Burkholderia cepacia* complex isolates were susceptible to the 200mg/mL of the pigment with zones of inhibition to ceftazidime (30µg), meropenem (10µg), minocycline (30µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg).

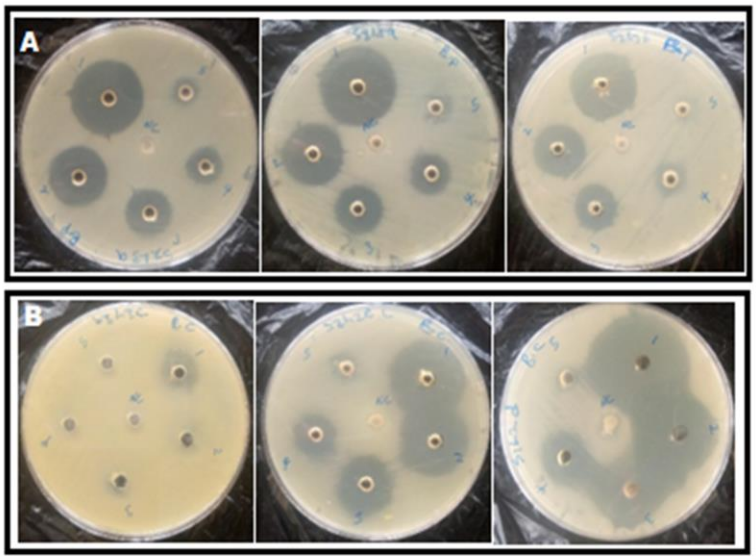

Figure 6: Inhibitory Appearance of Different Concentrations of Crude Pigment on (A) *Burkholderia Pseudomallei* Complex Isolates (B) *Burkholderia cepacia* complex in Agar Well Diffusion Methods

Table 5a: Zones of Inhibition of Different Concentrations of Pigment on Biofilm-Forming and Multidrug-Resistant *Burkholderia pseudomallei* Isolates

	Concentrations / Zones of Inhibition				
Isolates	200mg/mL (mm)	100mg/mL (mm)	50mg/mL (mm)	25mg/mL (mm)	12.5mg/mL (mm)
S2L3b	24±1.2	22±0.5	17±0.6	0±00	0±00
S3L3c	28±0.3	21±0.6	16±0.00	12±0.3	0±00
S3L3e	22±0.3	10±0.3	0±00	0±00	0±00
S3L3f	30±00	25±0.3	23±0.6	20±0.6	10±0.6
S3L3g	40±0.5	36±0.3	27±0.3	20±0.0	15±0.6
S3L3h	33±0.6	28±0.3	23±0.6	17±0.5	10±0.3
S1L1b	38±00	30±1.0	25±0.3	20±00	10±0.3
S1L1c	36±0.3	30±0.3	28±0.3	22±00	12±0.3
S2L3a	26±00	21±0.3	17±0.3	13±0.6	0±00
S3L1a	27±0.3	22±0.3	17±00	14±0.3	0±00
S3L1b	28±0.3	22±0.3	16±0.5	13±0.6	0±00
S3L1c	27±0.5	22±0.00	16±0.5	12±0.3	0±00
S3L1e	27±0.6	22±0.5	17±0.3	0±00	0±00
S3L1g	27±0.3	21±0.6	16±0.3	12±00	0±00
S3L1h	20±0.5	26±00	17±0.6	12±0.3	0±00
S3L1f	26±0.3	22±0.3	19±0.5	13±00	0±00
S3L1i	30±0.3	27±00	0±00	0±00	0±00
S3L1j	27±00	18±0.3	17±0.6	11±00	0±00
S3L3a	21±0.6	15±0.6	0±00	0±00	0±00
S3L3b	25±0.3	19±0.3	15±0.6	10±0.3	0±00

Table 5b: Zones of Inhibition of Different Concentrations of Pigment on Biofilm-Forming and Multidrug-Resistant *Burkholderia cepacia* Isolates

	Concentration / Zones of Inhibition				
Isolate	200mg/mL (mm)	100mg/mL (mm)	50mg/mL (mm)	25mg/mL (mm)	12.5mg/mL (mm)
S1L2b	22±0.3	14±0.3	0±00	0±00	0±00
S1L2d	38±0.3	27±00	23±0.3	18±00	0±00
S3L3a	19±0.5	15±00	0±00	0±00	0±00
S3L3c	14±0.3	10±0.5	8±0.3	0±00	0±00
S3L1a	27±0.3	24±0.3	13±00	10±0.3	0±00
S3L1c	33±00	23±0.3	0±00	0±00	0±00
S1L3a	11±00	6±0.5	0±00	0±00	0±00
S2L1a	32±0.6	28±0.3	23±0.5	17±00	8±0.5
S2L1b	19±0.3	11±0.3	0±00	0±00	0±00
S2L1d	17±0.3	10±0.3	0±00	0±00	0±00
S1L3e	16±0.5	13±00	0±00	0±00	0±00
S3L2c	18±00	11±0.5	0±00	0±00	0±00
S3L2e	26±0.3	23±0.3	21±00	14±00	8±0.6

vi. Antibiofilm forming activities

The result of the biofilm inhibitory activities of the pigment on the multidrug resistance *Burkholderia pseudomallei* complex and *Burkholderia cepacia* complex isolates in 96- wells microtiter plates revealed the inhibitory potentials of the pigment. It was observed that after 24 hrs of incubation, growth of *Burkholderia* isolates was seen in untreated wells compared to wells that were treated with 200mg/mL of the pigment. In *Burkholderia pseudomallei* isolates, a minimum of 94% and a maximum of 98% cell growth inhibition was recorded, whereas, in *Burkholderia cepacia* complex isolates, a minimum of 91% and a maximum of 97% cell growth inhibition was recorded in Figure 7. Furthermore, biofilm forming inhibitory activities of the pigment showed the potential of the pigment to prevent biofilm formation. It was observed that a minimum of 25% and a maximum of 96% biofilm-forming inhibition were recorded in the tested *Burkholderia pseudomallei* complex isolates. In addition, a minimum of 19% and a maximum of 94% biofilm formation inhibition were recorded in the tested *Burkholderia cepacia* complex isolates Figure 8**.**

vii. Antioxidant activities of red pigment

The result of the antioxidant activities of the red pigment showed that the pigment possessed 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid scavenging activities, 2,2-Diphenyl-1-picrylhydrazyl2, and ferric ion-reducing ability. It was observed that at 100 mg/g of the crude pigment, 48.22% DDPH scavenging activities of the crude pigment were recorded. In addition, 100 mg/mL of the pigment showed 11.37±0.04 mg/mL FRAP concentration was also recorded. Furthermore, 11.58±0.01 µg/mL of the ABTS were accounted for in 100µg/mL Table 6.

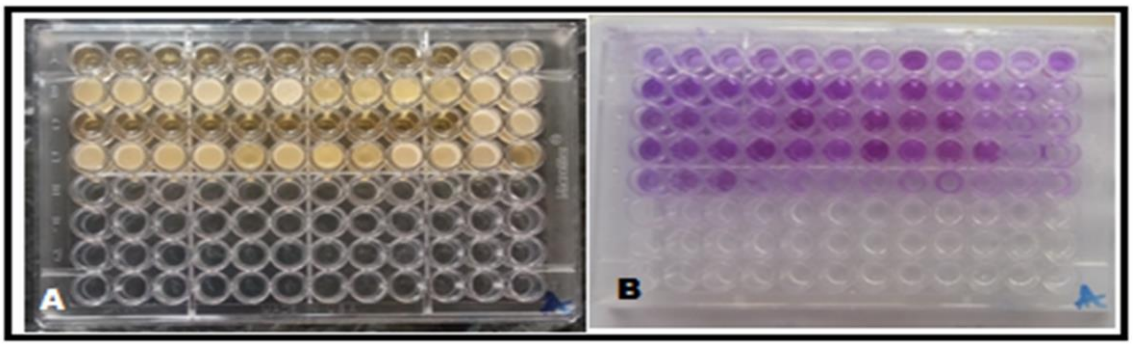

Figure 7a: (A) Cell Growth Inhibition and (B) Biofilm Formation Inhibition of Pigment on Multidrug-Resistant *Burkholderia I*solates in 96-well Microtiter Plates

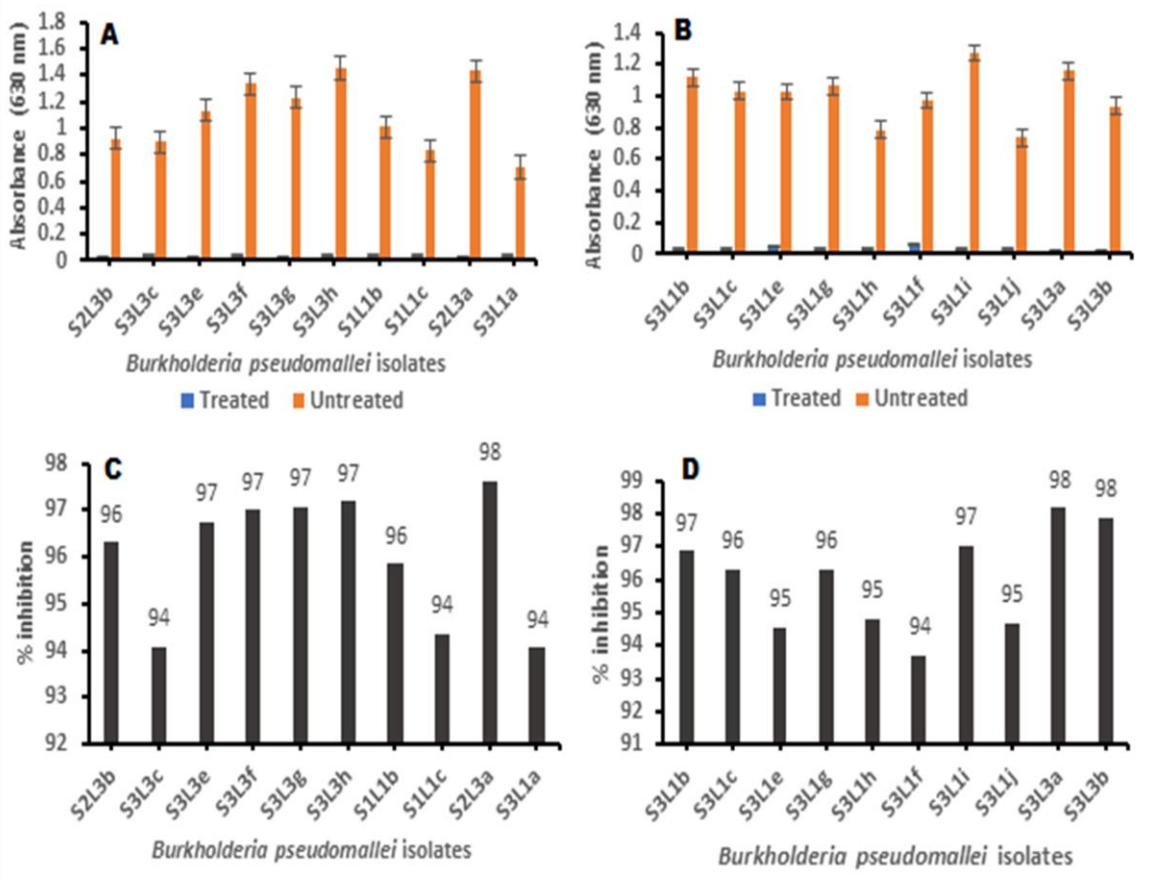

Figure 7b (A&B) Cell Growth of Treated and Untreated *Burkholderia pseudomallei* Isolates in 96 Well Microtiter Plates (C&D) Percentage Inhibition of *Burkholderia pseudomallei* Isolates in 96 Well Microtiter Plates

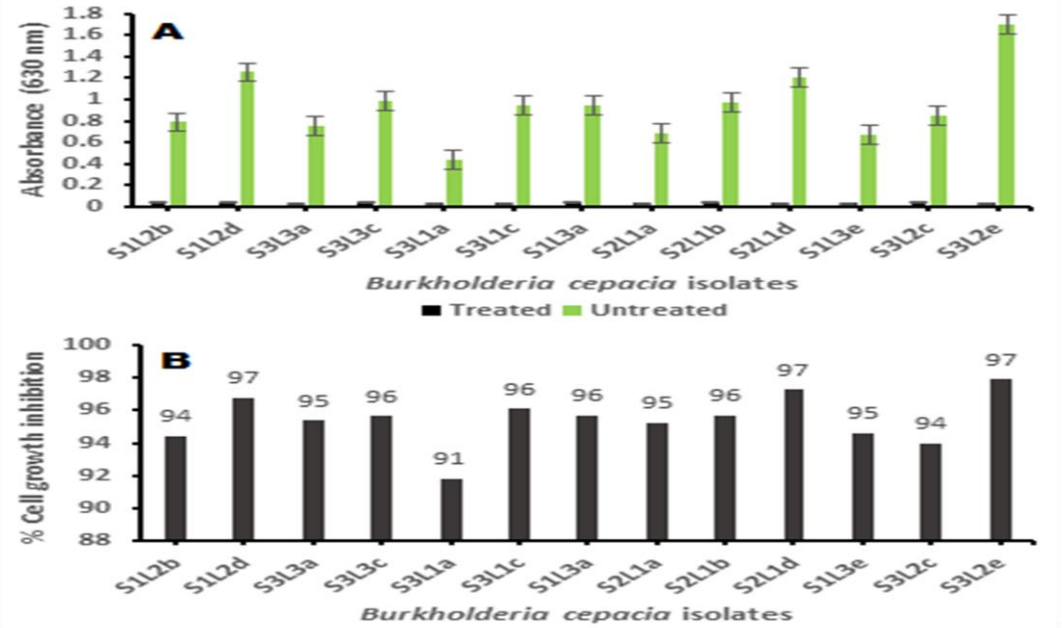

Figure 7c: (A) Cell Growth of Treated and Untreated *Burkholderia cepacia* Isolates in 96 Well Microtiter Plates (B) Percentage Inhibition of *Burkholderia cepacia* Isolates in 96 Well Microtiter Plates

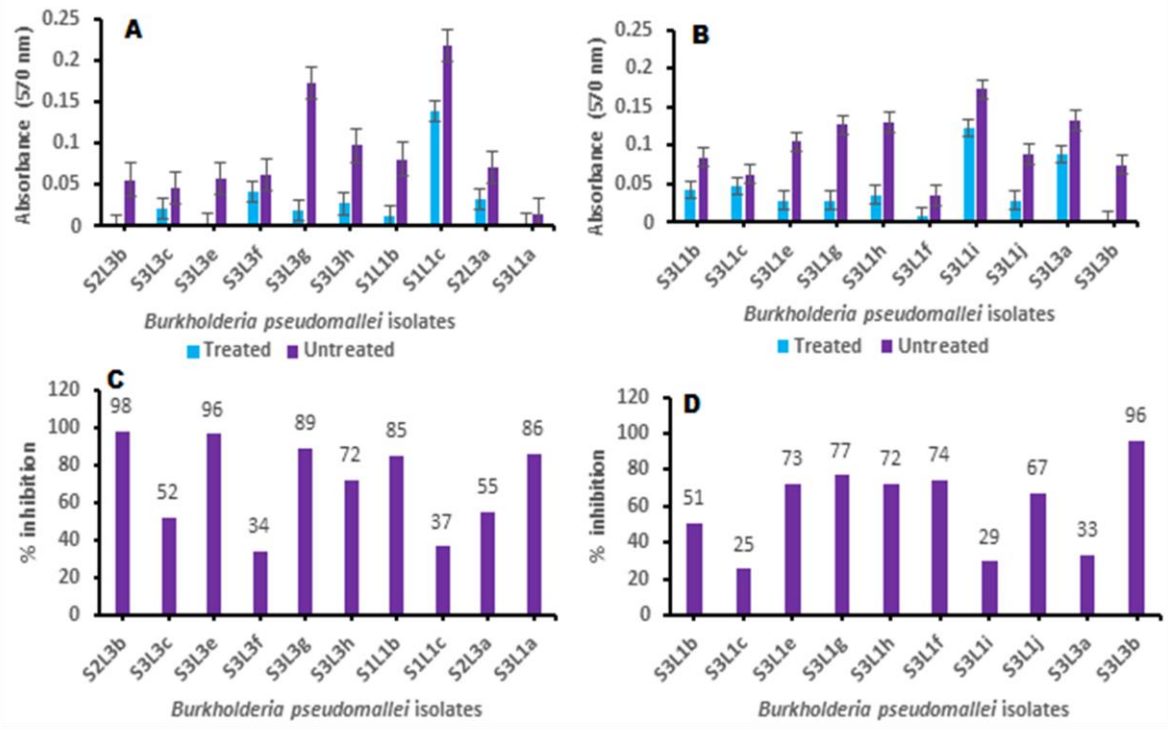

Figure 8a: (A&B) Biofilm Formation of Treated and Untreated *Burkholderia pseudomallei* Isolates in 96 Well Microtiter Plates (C&D) Percentage Biofilm Inhibition of Crude Pigment on *Burkholderia pseudomallei* in 96 Well

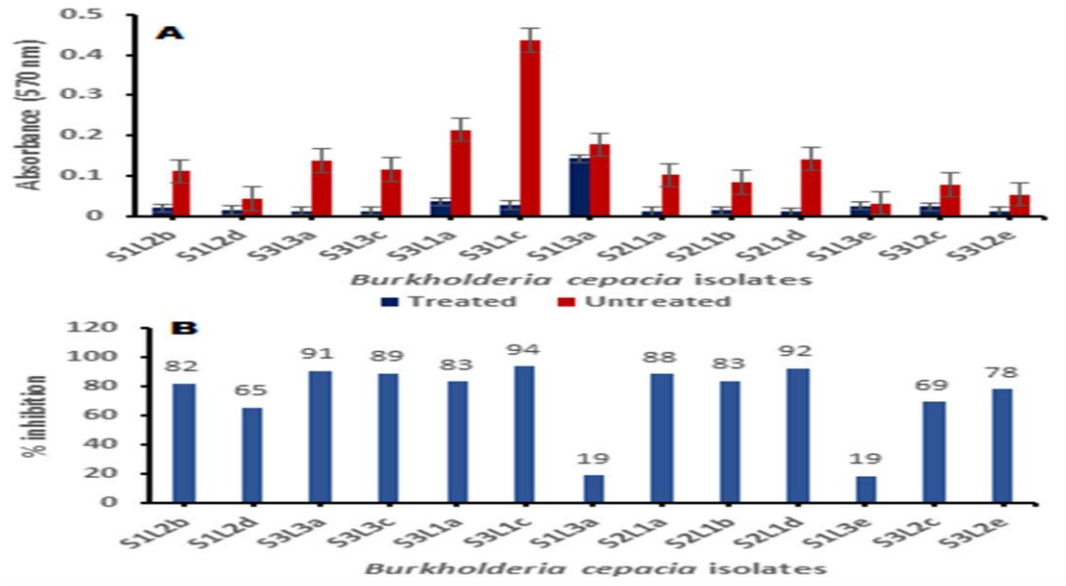

Figure 8b: (A) Biofilm Formation of Treated and Untreated *Burkholderia cepacia* Isolates in 96 Well Microtiter Plates (B) Percentage Biofilm Inhibition of Crude Pigment on *Burkholderia cepacia* in 96 Well

Table 6: Antioxidant Activities of Red Pigment from *Talaromyces marneffei* Strain SA2a

Antioxidants	Conc(mg/g)	Abs 517nm	%Scavenging Inhibition
DDPH	100	0.428±0.00	48.22
	200	0.407±0.00	50.77
	300	0.394±0.00	52.26
	Conc (mg/mL)	**Abs 593nm**	**FRAP (mg/mL)**
FRAP	100	1.328±0.00	11.37±0.04
	200	1.525±0.00	13.05±0.03
	300	1.529±0.00	13.09±0.02
	Conc (µg/mL)	**Abs 734nm**	**ABTS (µg/mL)**
ABTS	100	2.144±0.00	11.58±0.01
	200	2.195±0.00	11.85±0.02
	300	2.204±0.00	11.90±0.01

Key: ABTS- 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, FRAP- Ferric reducing antioxidant power, DDPH- 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

B. Discussion

The pigment-producing *Talaromyces marneffei* strain SA2a obtained from the culture collections of African Center of Excellence for Microbial Secondary Metabolites showed to be the most adjoining strain to *Talaromyces marneffei* isolate FPE 3C-1 (OL780147.1) at a percentage of 1000 bootstrap replicates and produced a red colour pigment in SDB at 25±2°Cfor 14 days in a static condition. The production of these extracellular red pigments from the fungus could be that the fungus attained stationary stage of growth and produced the pigment as secondary metabolites, which are not required for the growth. The result of this study is similar to the report of [35], who obtained orange to red colour pigments from *Talaromyces albobiverticillius* strains (A, B, C) in potato dextrose broth incubated for 10 days at 24°C. [17] optimized pigment production from *Talaromyces verruculosus* in potato dextrose liquid medium incubated for 14 days at a temperature of 28°C to obtain extracellular red pigment. This result also corresponds to the findings of [36] on the production of red pigment from *Talaromyces atroroseus* in yeast malt peptone medium at 30°C for 7 days in a static condition. likewise, [26] reported the production of red pigment in potato dextrose broth from *Talaromyces assiutensisin* at 28°C for 10 days in a static and dark condition. The production of these red pigments from These *Talaromyces* species could be that they have ability to synthesize red pigment as secondary metabolites during their growth.

The ethyl acetate used as an extraction solvent in our study efficiently extracted the red pigment produced by the *Talaromyces marneffei* strain SA2a by separating the red pigment from the broth. The efficient extraction of this red pigment could be a result of the nature of the pigment and other compounds present, which may be regarded as non-polar compounds. This corresponds to the report of [22], who utilized ethyl acetate, n-butanol, and chloroform to extract different coloured pigments from *Penicillium* species. In a similar study, [37] extracted the red pigment of *Talaromyces purpurogenus* LC128689 with vigorous shaking in ethyl acetate and concentrated the pigment to dryness using a rotary evaporator at 40°C. However, contrary to the present study, [38] reported the extraction of diffused water-soluble pigment from *Talaromyces purpureogenus* strain F in Czapek yeast autolysate agar with 70% ethanol under continuous shaking conditions, which were then centrifuged and filtered. Likewise, [17], obtained the highest concentration of red pigment produced by *Talaromyces verruculosus* by boiling the mycelia of *T. verruculosus* together with the media containing the diffused pigment. In a similar study, [36] extracted red pigment produced by *Talaromyces atroroseus* TRP-NRC in Yeast malt peptone medium, filtered with Whatman No. 1 filter paper and centrifuged for 15 min at 10,000 rpm. *Talaromyces assiutensis* red pigment was extracted in Potato dextrose broth, filtered through a Whatman filter paper using a Büchner funnel, and further lyophilized for further estimation of extrolites [26].

The absorption spectra of the *Talaromyces marneffei* strain SA2a pigment were measured at 200-700 nm to determine the maximum absorbance wavelength of the pigment components in the UV and visible areas. The red pigment extracted in this study was absorbed at three different peaks with a wavelength of 310, 432, and 512nm. The absorption at these three UV-visible regions could be as a result of the presence of more than one compound in the pigment. The sharp peak at 512 nm in this study indicated the presence of purpuride pigment, a

red pigment produced by *Talaromyces* species which absorbed between 500 - 520 nm. The result of this study corresponds to the red pigment of *Talaromyces purpureogenus* strain F, which is absorbed in a wavelength of 510 nm and identified as a purpuride [38]. Similarly, [17] identified a red pigment from *Talaromyces verruculosus* with a UV-visible spectrophotometer at a wavelength of 520nm. Additionally, [36] identified the red pigment of *Talaromyces atroroseus* TRP-NRC at a maximum absorbance of 500 nm. Likewise, [37] determined a red pigment from *Talaromyces purpurogenus* LC128689 at a λmass of 500nm. Following the same trends, [35] characterized a red pigment from *Talaromyces albobiverticillius* at a λmass of 500nm. The result of this study, with other previous reports, showed the trends of purpuride red pigment produced by *Talaromyces* species which absorbed between the wavelength of 500- 520 nm

The FTIR showed the presence of aliphatic amines, alkyl halides, alcohols, alkanes, esters, carbonyl groups, and nitro. This finding validates the report of [39], who reported the presence of NH/OH, aromatic CH, aliphatic CH groups and ether groups in a red pigment from *Penicillium marneffei*. Similarly, [24] reported the presence of alkane, alkene, alkynes, alcohols, esters, and sulfate in a propyprodiosin pigment produced by *Brevundimonas olei* strain RUN-D1.

The GCMS identified 91 compounds from a retention time of 3.2 min to 31.5 min in the red pigment. The prominent compounds were identified as Ethanol, 2-nitro-(m/z 91.07) at a retention time of 4.82 min accounting for 43.20% peak area, Bis(n-propylthio)methane (m/z 164.3) at 3.60 min with 15.28% peak area, Acetic acid (m/z 60.05) at 4.10 min, with 11.46% peak area and Purpuride (m/z 391.5) at 22.27 min, with 3.70% peak area. The GCMS characterization of the red pigment produced by the *Talaromyces marneffei* strain SA2a in this study indicated the identity of the red pigment as Purpuride with a m/z 391.5. Interestingly, it also identified other Ethanol, 2-nitro-, Bis(n-propylthio)methane and Acetic acid were present in abundance in the metabolite, which has not been previously reported to be produced by *Talaromyces* species. The purpuride identified in this study is in agreement with the report of [36], who characterized a red pigment from *Talaromyces atroroseus* TRP-NRC using UPLC/HRESI-MS and identified purpuride with a m/z 392.2 in the pigment. Likewise, [38] identified purpuride in the extracts of *Talaromyces purpurogenus* with HPLC-PDA ESi/MS. Many *Talaromyces* species produce striking diffusing red pigments, especially *T. purpurogenus, T. albobiverticillius, T. atroroseus, and T. marneffei, T. minioluteus*. These red pigments are typically composed of the purpuride and azaphilone pigments monascorubrin, rubropunctatin, threonine derivative of rubropunctatin, monascorubramine, PP-R (= 7–(2–hydroxyethyl)-monascorubramine), rubropunctamine, N-glutarylrubropunctamine, and PP–V [40]. Several studies reported the production of a herqueinone-like pigment from *Talaromyces marneffei*, Monascus-like azaphilone pigments (N-glutarylmonascorubramine and N-glutarylrubropunctamine) from *Talaromyces purpureogenus* industrially important red pigments (mitorubrin, monascorubrin, PP-R, glauconic acid, purpuride, and ZG-1494α) from *Talaromyces atroroseus*, trihydroxyanthraquinones (emodin, erythroglaucin, and catenarin) from *Talaromyces stipitatus*, and a xanthone dimer (talaroxanthone) from *Talaromyces* sp [41]. To the best of our knowledge, the *Talaromyces marneffei* strain SA2a in this study produced purpuride, which has not

been previously identified in *Talaromyces marneffei* red pigment.

The crude red pigment from the *Talaromyces marneffei* strain SA2a demonstrated good antimicrobial activities on the tested multidrug-resistant *Burkholderia* complex isolates. The 200mg/mL of pigment had zones of inhibition on 20 *Burkholderia pseudomallei* complex and 13 *Burkholderia cepacia* complex. The zones of inhibition on 18(90%) of the *Burkholderia pseudomallei* isolates are similar to the zones of inhibition of imipenem (10μg) and meropenem (10μg) likewise, the zones of inhibition on 11(85%) of the *Burkholderia cepacia* isolates are similar to the zones of inhibition of ceftazidime (30μg), meropenem (10μg), minocycline (30μg), and trimethoprim-sulfamethoxazole (1.25/23.75 μg) reported in EUCAST and CLSI breakpoint for *Burkholderia* sp. Additionally, MIC value of 12.5 mg/mL had inhibitory zones on 5 *B. pseudomallei* isolates with 10-15mm zones around the wells. While MIC value of 12.5 mg/mL had inhibitory zones on 2 of the *Burkholderia cepacia* isolates. The inhibitory potential of this crude pigment on the multidrug-resistant *Burkholderia* complex isolates could be a result of the Ethanol, 2-nitro-, Bis(n-propylthio)methane, Acetic acid and Purpuride that were abundant in the pigment, which may prevent DNA synthesis or cell formation in *Burkholderia* sp. In a similar study, [26] evaluated the antimicrobial activities of different concentrations of pigment from *Talaromyces assiutensis* strain CPEF04 against *Salmonella typhimurium, Staphylococcus* aureus, *Vibrio cholerae* and methicillin-resistant *Staphylococcus* aureus. The pigment demonstrated maximum zones of inhibition on *S. aureus* (24.5±0.5), MRSA (22.05 ± 0.07), and *V. cholera* (24.78 ± 0.2) at 400μg/disc and minimum inhibitory concentration of 64.0, 128.0, and 256.0 μg /mL against the tested *S. aureus*, MRSA, and *V. cholera*, respectively. In addition, [42] evaluated the effect of different concentrations of Umonium[38] (0.5%, 1%, 1.5%, 2%, 2.5%, and 3% (v/v)) against *P. aeruginosa*, MRSA, *E. coli,* and *B. pseudomallei*. Interestingly, the Umonium[38] effectively inactivated the tested *P. aeruginosa*, MRSA, *E. coli,* and *B. pseudomallei* at a concentration of ≥ 0.5% with a contact time of at least 15 min, and the effect remained for 14 days. Furthermore, [43] conducted the activities of 0.5 mg/mL of snake venoms and phospholipase PLA_2 (Crotoxin B and Daboiatoxin) enzymes purified from different snake venoms on *Burkholderia pseudomallei*. The result of their study showed that crotoxin B (24.8 ± 0.089 and 27.6 ± 0.133) and daboiatoxin (24.8 ± 0.103 and 26.2 ± 0.121) display the strongest antibacterial activity on *B. pseudomallei* KHW and *B. pseudomallei* TES respectively. To the best of our knowledge, this study will be the first to report the antimicrobial activities of red pigment on multidrug-resistant *Burkholderia* species.

The crude pigment exhibited an inhibitory effect on the growth of tested multidrug-resistant *Burkholderia* isolates in the 96-well microtiter plate. The pigment demonstrated 98% cell growth inhibition on *Burkholderia pseudomallei* isolates, and 97% cell growth inhibition on *Burkholderia cepacia* isolates when the inhibition percentage was calculated. The inhibition of cell growth in this study could be a result of the active compounds in the pigment targeting the folate synthesis pathway or inactivating the enzymes required for cell formation and multiplication. Noticeably, when treated wells were compared with untreated wells, the pigment prevented *Burkholderia* isolates from forming biofilm in 96-well microtiter plates. This could be that the pigment inhibited quorum sensing, prevented cell aggregation and cell

multiplication in *Burkholderia* isolates. This result corresponds to the study of [27], who evaluated the effect of ethyl acetate extract of *Passiflora edulis* on *Chromobacterium violaceum*. Their study showed that the extract had significantly reduced biofilm formation in the presence of 2 mg/mL (90.7%), 1 mg/mL (69.4%), and 0.5 mg/mL (51.8%), respectively, on the *Chromobacterium violaceum*. Similarly, [29] investigated antibiofilm and staphyloxanthin inhibitory potential of terbinafine against *Staphylococcus aureus*. The terbinafine inhibited biosynthesis of staphyloxanthin and prevented a multilayer of aggregates and adherent cells in *S. aureus* ATCC 6538. Likewise, [44] tested different concentrations of promethazine hydrochloride (PTZ) on biofilm formation and lipase activity in *B. thailandensis* E264 and revealed a decrease in both biofilm biomass and loosed biofilm structure. However, further study needs to be carried out to understand the mechanism of action of crude red pigment from the *Talaromyces marneffei* strain SA2a on the multidrug-resistant *Burkholderia* isolates.

The antioxidant activities of the crude pigment showed that the pigment demonstrated good antioxidant properties. *Talaromyces* species can produce many other bioactive secondary metabolites, and these compounds have been found to have antibacterial, anti-inflammatory, antitumor, antioxidant, nematocidal, and other effects in medical research [15]. The pigment demonstrated significant DDPH scavenging activities at a concentration of 100, 200, and 300 mg/g with 48.22, 50.77, and 52.26% scavenging inhibition, respectively. Interestingly, the pigment also possessed high FRAP contents, accounting for 11.37±0.04, 13.05±0.03, and 13.09±0.02 mg/mL. In addition, high contents of ABTS were recorded with 11.58±0.01, 11.85±0.02, and 11.90±0.01 μg/mL. This result is in line with the study of [38], who evaluated the antioxidant activities of pigment from *Talaromyces purpureogenus* strain F. The extracellular pigment obtained in their study had high ABTS scavenging capacity and also increased the ability of ferrous ion chelating. High ferrous ions chelating abilities of pigments will inhibit the free radical-induced lipid peroxidation, thus would be beneficial to food, animal health, and cosmetic stability [38]. In a study by [32], carotenoid extract scavenged stable free radical DPPH. Our findings also concur with the significant antioxidant activity of pigment from *Talaromyces assiutensis* reported by [26].

Having thoroughly searched the literature, this study is the first to report the production of Purpuride pigment from *Talaromyces marneffei*. In previous studies, *Talaromyces purpureogenus* and *Talaromyces atroroseus* are known to be the producers of Purpuride pigment. In addition, this study is the first to report the antimicrobial activities and biofilm inhibition potential of pigments on multidrug-resistant *Burkholderia* sp. The graphical representation of the production, characterization, and antimicrobial activities of the red pigment on multidrug-resistant *Burkholderia* sp. is shown in Figure 9.

IV. Conclusion

In this study, we isolated red pigment from *Talaromyces marneffei* strain SA2a and successfully identified the pigment as Purpuride with UV-visible, FTIR and GCMS analysis. The pigment demonstrated good antimicrobial and antibiofilm formation activities on multidrug-resistant *Burkholderia* sp. The result of this study suggests that the pigment of *Talaromyces marneffei* could be a promising alternative therapy in treating *Burkholderia*-associated infections and

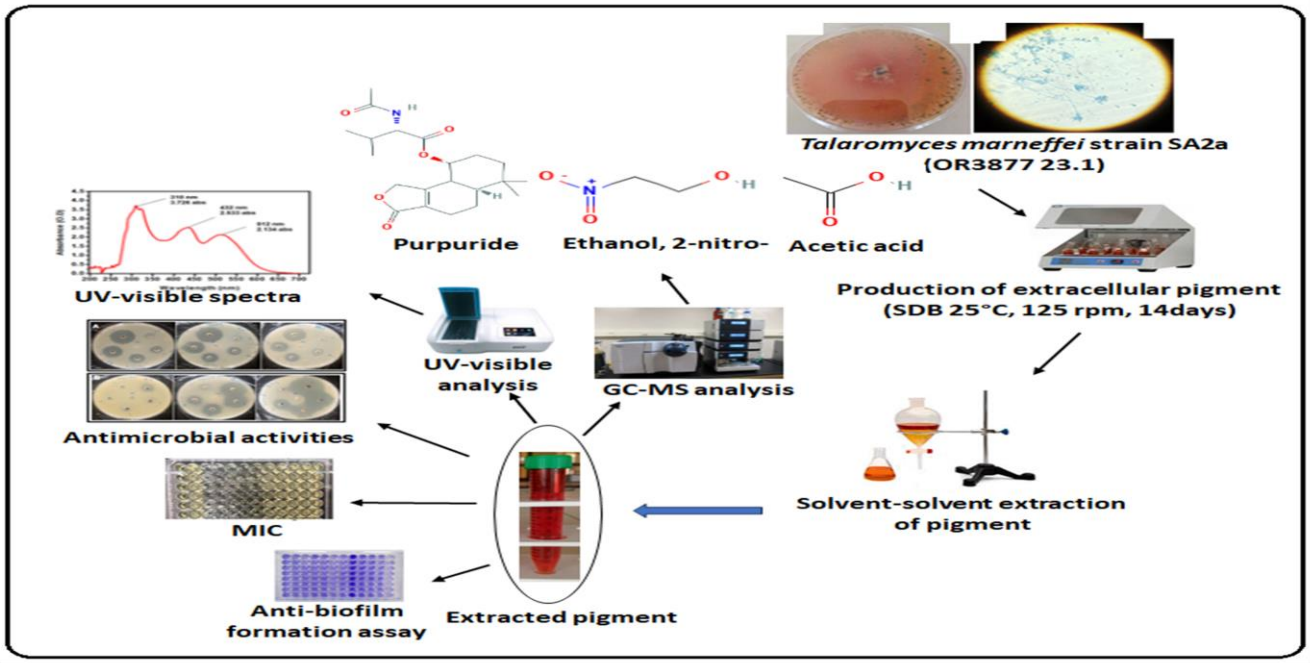

Figure 9: Graphical Representation of the Production and Activities of Red Pigment of *Talaromyces marneffei* Strain SA2a on Multidrug-Resistant *Burkholderia* sp.

diseases. If the pigment is purified it could be employed in pharmaceutical industries for drug production.

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