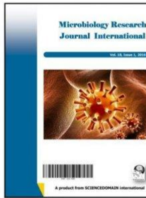

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Antibacterial Activity of Actinomycetes Isolated from Waste Dump Soil from Western Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAA designed the study, wrote the protocol, conducted the *laboratory experiments and wrote the first draft of the manuscript. Authors EE, MNJ and SHA managed the data entry and statistical analysis and authors MAO and JO managed the literature searches and reviewed the final drafted manuscript. All authors read and approved the final manuscript.*

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ABSTRACT

Aims: To determine the antibacterial activity of actinomycetes isolated from waste dump soil in Western Uganda.
Study Design: The study was an experimental laboratory design.
Place and Duration of Study: Waste dump soil samples were collected from Bushenyi, Kabale, Kasese, and Mbarara districts in Western Uganda, from May, 2016 to February, 2017.
Methodology: Actinomycetes were isolated from 22 waste dump soil samples collected using

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standard spread plate technique, all isolates were screened primarily using cross streak method against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Staphylococcus aureus* ATCC25923, resistant clinical bacteria: *Escherichia coli* 2966, *Pseudomonas aeruginosa* 2929, and *Staphylococcus aureus* 2876 (MRSA). Secondary screening was carried out by first growing all fifty six isolates (56) in broth media and their supernatants were tested for activity using agar well diffusion method. The remaining broths were extracted using ethanol. The ethanol extract was also tested for antibacterial activity using agar well diffusion method. The MIC was determined using tubes dilution technique and MBC was determined by culture method.

Results: Fifty six (56) actinomycetes isolates were isolated from 22 waste dump soil samples. Four (7.14%) isolates showed activity to at least one test bacteria during primary screening. Eight (14.29%) actinomycetes isolates fermented broth showed activity to at least one test bacteria during secondary screening, with mean zone of inhibition of 7.67±1.45 to 33.67±2.03 mm. Isolate KBMWDSb6 showed activity to all test bacteria with exception of resistant *Staphylococcus aureus* 2876 (MRSA) while isolates BRWDSc (SP) and KBRWDSa3 (RF) showed activity to all sensitive standard isolates and resistant *Staphylococcus aureus* 2876 (MRSA). Eleven (19.64%) actinomycets isolates ethanol extracts showed activity to at least one test bacteria with mean zone of inhibition 7.33±1.20 to 31.67±1.45 mm. The MIC and MBC of the extracts were found to be 0.07 to 0.62mg/mL and 0.15 to 1.25mg/mL respectively.

Conclusion: The findings of this study indicated that actinomycetes spp isolated from waste dump soil collected from Western Uganda have ability to produce bioactive compounds with activity against bacteria including clinically drugs resistant bacterial isolates. This could be a good source of novel antibiotics.

Keywords: Antibacterial; waste dump soil; actinomycetes; Western Uganda.

1. INTRODUCTION

There has been increasing reports of resistance to existing antimicrobial agents over three decades and surprisingly, there is also increasing infections of opportunistic pathogens due to immune compromising disease such as HIV, organ transplant, cancer and other conditions [1,2]. In early era of antibiotics resistance, the problems was considered only threat to hospital acquired infections and immunosuppressed patients as opposed to these days where whole global populations are seem to be at risk [2,3]. Of the Ugandan population of about 40 m, 1.14% had HIV and an estimated 9.2% (101,000) had a CD4 count <200 cells/µL [4]. Bacterial infections accounted for about 20% among hospitalized patients in Uganda [2]. Various studies have shown that there were increasing cases of resistant bacterial isolates such as *Staphylococcus aureus*, *Streptococcus pneumonia*, *Neisseria gonorrhoeae*, Enterococci, *Salmonella* Typhi and non-typhoidal salmonellae, Shigella, *Escherichia coli* and *Mycobacterium tuberculosis* to the most available antibiotics [2,5]. These have put more demands on search for novel antibiotics compounds to face this global challenge.

Actinomycetes are saprophytic, thermophilic, filamentous sometimes rod to coccoid in shape, gram positive and spores bearing bacteria belonging to the phylum Actinobacteria (order Actinomycetales) [6]. Most of actinomycetes are free living organisms that are widely distributed in natural habitats such as aquatic and terrestrial habitats. In terrestrial habitats, actinomycetes were reported to be the most populated microbes but their existence and composition differed with soil type [7,8]. Actinomycetes were considered one of the golden microbes of the 21st century due to their ability to produce different kinds of bioactive compounds including antibiotics. Many researchers from different parts of the world reported the ability of actinomycetes to produce bioactive compounds including antibiotics. For instances, George et al. [7]; Radhakrishnan et al. [9] from India, Ensieh and Maryam [10] from Iran and James et al. [11] from Kenya reported novel bioactive compounds from actinomycetes obtained from different ecosystems. Actinomycologists recommended for the search of novel bioactive compounds from less study habitat, area or geographical location as reviewed by Chand and Sambasiva [12].

In Uganda, there has been little literature to ascertain the exploration of soil actinomycetes producing novel antibiotics especially in waste dump soil found in different region with different composition [13]. Therefore, there is an urgent need to explore antibacterial activity of actinomycetes isolated from waste dump soil from selected districts in Western Uganda.

Hence, this study was designed to determine the antibacterial activity of actinomycetes isolated from waste dump soil from selected districts in Western Uganda.

2. MATERIALS AND METHODS

2.1 Sampling Sites and Samples Collection

A total of 22 waste dump soil samples were collected from two different temperate areas of Western Uganda: cold areas Bushenyi district 00°32'30'S, 30°11'16E (6 samples); Kabale district 01°15'S30°0E/1.250°S, 30.000°E (6 samples) and warm areas (Kasese district 00°11'N30°05'E/0.183°N, 30.083°E (4 samples); Mbarara district 00°36'S 30°36'E/0.600°S, 30.600° E (6 samples). The samples were collected from market waste, residential waste and manure plantation farm. Two plots (120x120 cm) were mapped out from each sampling site and three samples (3-15 cm depth) were collected randomly from each of the plots using a sterile stainless spoon with hand core and sterile gloves to avoid contamination. The collected soils were mixed to have one representative sample per plot [14]. The samples were placed in sterile polythene bags [15] and then transported to the Microbiology laboratory at Department of Microbiology and Immunology Kampala International University Western Campus for further study. Temperature of each sampling site was recorded during soil collection while pH and moisture content of each sample was determined immediately using digital pH metre and oven dried method respectively as shown in Table 1 in the results section.

2.2 Isolation of Actinomycetes

Isolation of actinomycetes was carried out according to the method described by George et al. [7]; Ismail et al. [15]; Arifuzzaman et al. [16]. Two (2) grams of each soil samples was air dried for 10 days at room temperature, and approximately 1 g of air dried soil sample were suspend in 9 mL of sterile distilled water supplemented with 0.9% of NaCl and incubated in a Gas bath thermostats oscillator (THZ-82B) for 1 h at 200 rpm and 55°C. The suspension was serially diluted (10^{-1} - 10^{-7}). One hundred microliters (100 µL) from dilution 10^{-2} were spread on starch casein nitrate agar (Composition in media g/L: Starch 10, Casein 0.3, KNO_3, NaCl 2, K_2HPO_4 2, $MgSO_4.7H_2O$ 0.5, $CaCO_3$ 0.02, $FeSO_4.7H_2O$ Agar 18 pH adjusted 7± 2) [17]. Glycerol casein agar (Kuster agar) (Composition in media g/L: Glycerol 10, casein 0.3, KNO_3 2, K_2HPO_4 2, $MgSO_4$ 0.05, $CaCO_3$ 0.02, Fe_2 $(SO_4)3.6H_2O$ 0.01, Agar 15 and pH was adjusted to 7 ± 2) [18] and yeast extract starch casein agar (YSCA) (Composition in media g/L: yeast extract 3, peptone 3, casein 3, starch 8, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.5, NaCl 2, agar 15 and pH 7.0 -7.6) [19]. The inoculated plates were incubated at 28°C for 7 – 14 days. Colonies with limiting growth, appeared dry powdery or velvety, tough leathery or chalky texture; dry or folded and branching filamentous with or without aerial mycelia and clear zone of inhibition were sub-cultured on clean agar plates to obtained pure cultures [19,20]. The pure cultures were maintained at 4°C for short storage and -80°C for long storage for further studies.

2.3 Production of Antibacterial Compounds

2.3.1 Test organisms

The following test organisms were obtained from Department of Medical Microbiology Makerere University, Kampala: Standard drug sensitive bacteria (*Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *and Staphylococcus aureus* ATCC25923), drug (s) resistant clinical bacterial isolates (*Escherichia coli* 2966, *Pseudomonas aeruginosa* 2929, *and Staphylococcus aureus* 2876). Drug resistance profile of the resistant clinical bacterial isolates is presented in Table 2 as shown in result section.

2.3.2 Primary screening

Primary screening was performed using cross streak method as described by Gulve and Deshmukh [21]. Seven day old actinomycetes isolates was inoculated as strict line or a circle on yeast extract starch casein agar (YSCA). The composition of medium supporting the growth of both actinomycetes and test organisms (Composition in media g/L included: yeast extract 3, peptone 3, casein 3, starch 8, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.5, NaCl 2, agar 15 and pH 7.0 - 7.6) and incubated at 28°C for 5-7 days after which the test organisms were inoculated perpendicularly and incubated at 37°C for 24h after which the plates were observed for decrease or absent of growth at site of test organisms and the results were recorded.

2.3.3 Secondary screening

2.3.3.1 Fermentation

All isolates were subjected to fermentation; this was to confirm their ability to produce bioactive compounds in the solid media and in liquid media [22]. Fermentation was carried out by the submerged culture in Erlenmeyer flask (500mL). Seven days old culture of actinomycetes was inoculated in yeast starch broth (g/L: yeast extract 3, Peptone 3, Casein 3, Starch 8, Glycerol 3, $CaCO_3$ 0.75, K_2HPO_2 0.5, $MgSO_4.7H_2O$ 0.5, NaCl 12 and pH 7.4) and incubated in gas bath thermostats oscillator (THZ-82B) at 28°C and 200 ± 5 rpm for 7 to 14 days after which the broth was centrifuged at 3000rpm for 20 minutes and filtered using filter paper (Whatman No. 1) [18,23].

2.3.3.2 Antibacterial activity of fermented broth

Agar well diffusion method was employed to assess the antibacterial activity of the filtered broth. Cell concentration of test organisms was adjusted to 0.5 McFarland turbidity standards and inoculated on Mueller Hinton agar (HIMEDIA M173-500G) plates by using sterile cotton swabs and wells were bored by sterile 1000 µL micro pipette tip [23]. The wells were filled with 200µL of supernatant of centrifuged broth and the plates were incubated at 37°C for 24 h. Two point five percent (2.5%) of dimethyl sulphoxide (DMSO) (Thomas Baker) was used as negative control. All experiments were performed in triplicates.

2.3.4 Extraction of bioactive compounds

The fermented broth was centrifuged and filtered using Whatman No. 1 filter paper. The filtered broth was extracted using a solvent by adding equal volume (1:1) of ethanol (95%). The solution was shaken vigorously on a rotatory shaker for 24 h. The solvent phase was collected and evaporated in hot air oven at 40°C. The extracts were dried and stored at 4°C for further studies [23,24].

2.3.5 Antibacterial activity of the solvent extract

The dried extracts were re-dissolved in 2.5% dimethyl sulphoxide (DMSO) (THOMAS BAKER) [23] to concentration of 2.5 mg/mL and antibacterial activity was determined by agar well diffusion method [24,25]. Cell concentration of test bacteria were adjusted to 0.5 McFarland turbidity standards and inoculated on Mueller Hinton agar plates using sterile cotton swabs. Wells were bored using sterile 1000 µL micro tip [24]. The wells were filled with 200 µL of 2.5 mg/mL ethanol extract and the plates were incubated at 37°C for 24 h. Ciprofloxacin at 0.2 µg/mL and 2.5% DMSO were used as positive and negative control respectively. Plates were incubated at 37°C for 24 h after which the zone of inhibition was measured. All experiments were performed in triplicates.

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

Minimum inhibitory concentration of the ethanol extract was determined according to the method described by Iqbal and Farrukh [26]. Brain heart infusion broth medium was prepared and various concentrations (0.019 – 1.25 mg/mL) were made using serial dilution from the initial concentration of extract (2.5 mg/mL). One milliliter (1 mL) of 0.5 McFarland turbidity standards cell of test bacterium was added to each test tube and incubated at 37°C for 24hr. On the other hand, the minimum bactericidal concentration of the extracts was determined by inoculating 0.1mL from each test tube with no growth on freshly prepared nutrient agar and incubated at 37°C for 24h. The plate without growth was considered as MBC for the extract.

2.5 Identification of Actinomycetes

The active actinomycetes isolates that showed activity during primary and secondary screening were identified using macroscopic, microscopic and biochemical methods [10,24].

2.5.1 Macroscopic and microscopic study

The macroscopic features of the active isolates observed are colony color, aerial mycelium, substrate mycelium, pigment production and colony surface [10,24]. Surface appearances of the selected actinomyecets isolate were studied using dissecting microscope. While spores arrangements were study using slide culture method. Blocks of Starch casein nitrate agar were cut and placed on sterile glass slides. The active actinomycetes isolate was inoculated on the block by streaking over the agar block surface, a cover slip was placed over the block, and the entire set up was incubated at 28°C for 7 days. The cover slip was removed and stained using Gram's staining techniques. Cover slip was covered with crystal violet for 60 seconds and

washed off with water, followed by Gram's iodine for 60 s, decolorized with alcohol for 05 seconds, and washed with water. Finally cover slip was stained with safranin counter stain for 1 min. After washing and drying, the test was microscopically observed under high power using phase-contrast microscope (X100) [24,27].

2.5.2 Biochemical characterization

The biochemical characterisation of the active actinomycetes isolates was carried out according to the methods describe by [10,24]. and these includes : Gelatin hydrolysis Starch hydrolysis, Esculin degradation, Methyl Red – Voges – Proskauer test (MR-VP) Catalase test, Indole test, Urease test, Nitrate test, Triple iron sugar test, Citrate Utilization.

2.6 Data Analysis

Data was analyzed using past software (Version 3.14). Pearson's correlation coefficient was used to compare between environmental factors (temperature, percentage moisture content and pH) with percentage colonies distribution, value at (*P* =.05) were considered to be significant. One way analysis of variance (ANOVA) was used to compare between antibacterial activity of fermented broth and ethanol extract, control and ethanol extract and (*P* = .05) was considered to be significant.

3. RESULTS AND DISCUSSION

Fifty six (56) actinomycetes isolates were isolated from 22 waste dump soil samples collected from four districts (Bushenyi, Kabale, Kasese, and Mbarara districts) of Western Uganda. The samples were collected from Market waste dump soil, Residential waste dump soil and Manure plantation farm. Three media (Starch casein nitrate agar, Glyceral casein agar and Yeast extract starch casein agar) were used for the isolation of actinomycetes. Glycerol casein agar was found to support high growth of actinomycetes followed by yeast extract starch casein agar (Result not shown).

Although starch casein agar nitrate showed less growth it was chosen for the storage of pure isolates due to its ability to support the growth of all actinomycetes isolated from different media. Although the studied areas has high percentage moisture content and lower temperature, significant number of actinomycetes isolates (56) were isolated.

Table 1 showed the results of pH, temperature, percentage moisture, colonies and percentage colonies distribution from the studied areas. The pH value ranged from 7.32 to 8.63 which is slightly alkaline, temperature was 18 to 57°C, percentage moisture content was 33% to 93% and colonies and percentage colonies distribution ranged from 1 to 6 and 1.79% to 10.71% respectively. The colonies and percentage colonies distribution were found to have a positive weak correlation with pH (which is slightly alkaline) of the soil samples but it was not statistically significant (r = 0.206, *P*=.05). This was in line with findings of [28,29] who reported that actinomycetes distribution is affected by pH and its desired neutral to alkaline pH condition than acidic environment. Basilio et al. [30] also added that actinomycete loads drop at pH less than 5 which is acidic. There was positive weak correlation between percentage colonies distribution with percentage moisture and negative weak correlation with temperature but it was not statistically significant (r = 0.146, *P* =.05 and r = - 0.053, *P* =.05 respectively). This was in accordance to the findings of [6,28] who reported that environmental factors like temperature, moisture can affects the distribution of actinomyctes and tends to be abundant in wasteland than the moist soil. However, this was slightly different to the findings of [30] who reported negative correlation between actinomycetes loads and moisture content and positive correlation between actinomycetes loads and temperature.

All isolates actinomycetes isolates (56) were subjected to primary screening using cross streak method. Four actinomycets isolates (7.14%) showed antibacterial activity against one or more test organism(s) during primary screening (Table 3). Isolate KBMWDSb6 (M6) showed activity to all test organisms with exception of clinical resistant *S. aureus* 2876 (MRSA) while isolates BRWDSc (SP) and KBRWDSa3 (RF) showed activity to all standard test bacteria and clinical resistant *S. aureus* 2876 (MRSA) isolate. Our findings revealed that majority 52(92.86%) of the actinomycetes spp did not produced antibacterial compounds in the solid medium which could be as results of lack of nutrients requirement or due to their cell structure as some actinomycetes not have been found to produce bioactive compound on the solid media or the bioactive compounds produced cannot diffuse in the solid medium due to the presence of polar or non-polar functional group which requires the polar solvent to dissolve. This was in

line with findings of [31] from India where he isolated 11 actinomycetes isolates from hospital waste dump soil and only 1 (9.09) produced antimicrobial activity against test organisms. Ensieh and Maryam [10] from Iran also isolates 34 actinomycetes isolates and 9 (26.47%) produced activity to at least one test organism. However, Rotich et al. [32] from Kenya also isolates 107 and 34 (36.4%) shows activity to at least one test bacterium during primary screening. This was contrary to the finding of [9] who reported that most actinomycetes produced bioactive compound in solid media and loose ability to produce analogous in liquid media.

All actinomycetes isolates (56) were subjected to secondary screening involving two parts: Testing for antibacterial activity of shake flask filtered broths and Ethanol extract. The results from the shake flask filtered broth showed that, some isolate that have not activity during primary screening had activity after subjecting them into liquid broth culture and vice versa. Eight (14.29%) isolates showed activity on at least one test bacterium (Table 4 and Fig. 1). The actinomycetes isolate KBMWDSb (M6) maintained broad spectrum activity to all test bacteria with exception of clinical isolates *S. aureus* 2876 (MRSA) which was also resistant during primary screening. Isolates BRWDSc (SP) and KBRWDSa3 (FR) shake flasks filtered broth showed activity to all sensitive standard bacteria and resistant clinical isolate *S. aureus* 2876 (MRSA). However, it was also observed that shake flask filtered broth of isolates KBMWDSb6 (M6) produced activity on all Gram negative and positive sensitive standard test bacteria and two Gram negative resistant clinical isolates *E. coli* 2966 and *P. aeruginosa* 2929 while isolate BRWDSc (SP) and KBRWDSa3 (RF) also produced activity on Gram negative and positive sensitive standard test bacteria and only Gram positive resistant clinical *S. aureus* 2876 (MRSA). The mean standard error zones of inhibition of antibacterial activity of shake flask filtered broth ranges from 7.67±1.45 to 33.67±2.03 mm including the diameter of the 1000 micropipette tip. The zone of inhibition was more on Gram positive resistant clinical bacterium *S. aureus* 2876 (MRSA) followed by Gram negative resistant clinical isolates bacteria *E. coli* 2966 when compared to their counterpart Gram negative resistant clinical isolates *P. aeruginosa* 2929.

Table 1. The pH, temperature, moisture, colonies and percentage colonies distribution from the studied areas

S/No.	Sampling site	pH	Temperature °C	Moisture (%)	No. of isolates (%)
1	BRWDSa	7.81	20	36.98	3 (5.36)
2	BRWDSb	7.48	22	89.39	2 (3.57)
3	BRWDSc	8.24	25	65.56	1 (1.79)
4	BMWDSa	7.71	25	92.31	4 (7.14)
5	BMWDSb	8.53	29	47.06	2 (3.57)
6	BMWDSc	7.36	32	74.83	1 (1.79)
7	KBRWDSa	8.33	20	52.85	3 (5.36)
8	KBRWDSb	7.42	22	52.91	2 (3.57)
9	KBRWDSc	7.65	24	93.82	2 (3.57)
10	KBMWDSa	8.43	18	82.80	1 (1.79)
11	KBMWDSb	8.02	21	66.67	6 (10.71)
12	KBMWDSc	7.87	19	50.15	2 (3.57)
13	KSWDSa	7.81	49	60.77	5 (8.92)
14	KSWDSb	7.42	53	57.73	3 (5.36)
15	KSWDSc	8.01	56	48.81	1 (1.79)
16	KSWDSd	8.63	57	33.69	4 (7.14)
17	MBWDS a	7.78	29	38.12	2 (3.57)
18	MBWDS b	7.52	34	55.84	2(3.57)
19	MBWDS c	7.57	36	48.37	1 (1.79)
20	MBWDS d	8.36	39	37.36	5 (8.92)
21	MBWDS e	7.94	35	31.93	1 (1.79)
22	MBWDS f	8.01	33	56.21	3 (5.36)
	Total	**-**	**-**	**-**	**56 (100)**

Key: BRWDS: Bushenyi residential waste dump soil, BMWDS: Bushenyi market waste dump soil, KBRWDS: Kabale residential waste dump soil, KBMWDS: Kabale market waste dump soil, KSWD: Kasese waste dump soil, MBWDS: Mbarara waste dump soil

Table 2. Antibiotics resistant profile of the clinical isolates test bacteria

Test organisms	Laboratory number	Resistant profile
Escherichia coli	2966	Chloramphenicol Erythromycin Ampicillin Cloxacillin Augmentin Ceftazidime Cefuroxime
Pseudomonas aeruginosa	2929	Erythromycin Ampicillin Cloxacillin Ceftriaxone Augmentin Ceftazidime Gentamicin Nitrofurantion Cefuroxime
Staphylococcus aureus (MRSA)	2876	Erythromycin Gentamicin Oxacillin Penicillin Tetracycline Ceftazidime Ceftriaxone Cloxacillin Ofloxacin Augmentin Cefuroxime

The antibacterial activity of ethanol extract result showed that some shake flask filtered broth had activity after using ethanol for extraction of bioactive compound. Eleven (19.64%) actinomycetes isolate showed activity to at least one test bacterium. The mean standard error zone of inhibition of ethanol extracts ranges from 7.33±1.20 to 31.67±1.45 mm including the diameter of the 1000 micropipette tip (Table 4 and Fig. 2). The actinomycetes isolate KBMWDSb (M6) ethanol extract maintained broad spectrum activity to all test bacteria including clinical isolates *S. aureus* 2876 (MRSA) which was resistant during primary screening and secondary screening (shake flask filtered broth). BRWDSc (SP) and KBRWDSa3 (RF) ethanol extracts maintained the same activity as recorded from fermented broth. There was no activity observed from the negative control (DMSO). The ability of some actinomycetes to produce antibacterial activity in the liquid medium and when solvent (ethanol) was used for extraction could be as result of presence of polar and week polar functional group of bioactive compounds which can easily dissolve in polar solvents (water and ethanol) which was used during fermentation and extraction. Ethanol was used to extracts bioactive compound due to polarity of the solvent which makes it to extract both polar and week polar compounds [10]. The inability of the majority of the actinomycetes isolates to produce bioactive compounds in fermented broth (86.71%) and ethanol extract (80.36%) could be due to presence of nonpolar compounds which cannot dissolve in polar solvents. This was in lined with findings of [33] who reported that some bioactive compounds could be missing during fermentation and extraction process due to the lack of appropriate fermentation conditions or suitable solvents for extractions. Actinomycetes isolate KBMWDSb6 (M6) produced activity on all Gram negative and positive sensitive standard test bacteria and two Gram negative resistant clinical isolates *E. coli* 2966 and *P. aeruginosa* 2929 while isolate BRWDSc (SP) and KBRWDSa3 (RF) also produced activity on Gram negative and positive sensitive standard test bacteria and only Gram positive resistant clinical *S. aureus* 2876 (MRSA). The diameter mean zone of inhibition of both shake flask and ethanol extract seem to be higher on Gram positive bacteria than the Gram negative bacteria. This could be due to the nature of the outer membrane of Gram negative strains (presence of lipopolysaccharide membrane) which does not permit antimicrobial agent penetration [10].

Comprising between shake flasks filtered broth and ethanol extract mean zone of inhibition using one way ANOVA showed no significant difference at (P =.05) between the two extracts though the concentrations were not the same.

Table 5 shows the comparison between of ethanol extract (2.5 mg/mL) of active actinomycetes isolates and positive control (Ciproflaxacin 0.2 µg/mL). The findings showed that clinical resistant isolates *E. coli* and *P. aeruginosa* were sensitive to both ethanol extract and positive control while *S. aureus* (MRSA) were sensitive to ethanol extract of the three isolates but resistant to positive control at (0.2 µg/mL) concentration but the results was not statistically significant at (P =.05). The comparison between positive control (Ciproflaxacin 0.2 µg/mL) and ethanol extract (ethanol extract 2.5 µg/mL) showed that the mean zone diameter of the positive control was higher than that of ethanol extract against two clinical resistant Gram negative isolates (*E. coli*

2966 and *P. aeruginosa* 2929) but negative on *S. aureus* 2876 clinical resistant isolate.

The result of minimum inhibitory concentration and minimum bactericidal concentration of the three active isolates were determined (Table 6). The isolates were chosen due to their ability to produced activity to both sensitive and drug resistant clinical test bacteria. The minimum inhibitory concentration ranges from 0.07 to 0.62 mg/mL while the minimum bactericidal concentration range from 0.15 to 1.25 mg/mL.

Table 3. Primary screening of actinomycetes isolates against test bacteria

S/No	Active Isolates code	Test organisms					
		***E. coli* ATCC 25922**	***E. coli* 2966**	***P. aeruginosa* ATCC 27853**	***P. aeruginosa* 2929**	***S. aureus* 2876 (MRSA)**	***S. aureus* ATCC 25923**
1	BRWDSc (SP)	+	-	+	-	+	+
2	KBMWDSb6 (M6)	+	+	+	+	-	+
3	KBRWDS (N1)	+	-	+	-	-	+
4	KBRWDSa3 (RF)	+	-	+	-	+	+

Key: +: positive and –: Negative

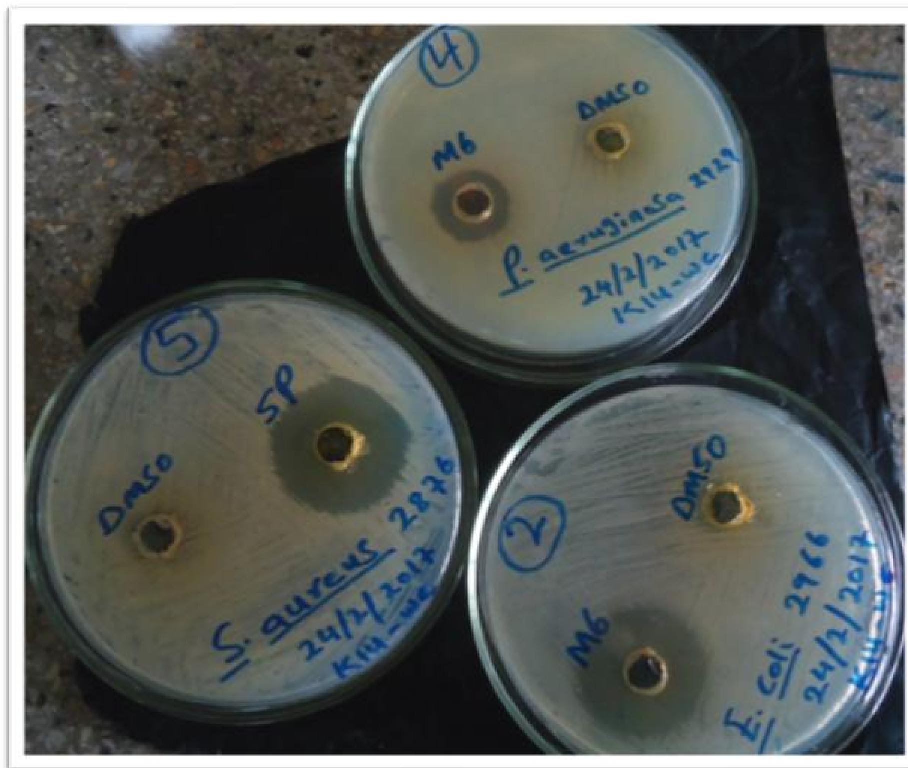

Fig. 1. Secondary screening. Antibacterial activities of shake flask broths of KBMWDSb (M6) and BRWDSc (SP) using well diffusion method against three resistant clinical bacterial isolates

Table 4. Mean standard error zone of inhibition of antibacterial activity of fermented broth (FB) and ethanol extract (EE) of active actinomycetes isolates

S / No.	Isolates code	*E. coli* ATCC 25922		*E. coli* 2966		*P. aeruginosa* ATCC 27853		*P. aeruginosa* 2929		*S. aureus* 2876		*S. aureus* ATCC 25923	
		FB	**EE 2.5 mg/mL**	**FB**	**EE 2.5 mg/mL**	**FB**	**EE 2.5 mg/mL**	**FB**	**EE 2.5 mg/mL**	**FB**	**EE 2.5 mg/mL**	**FB**	**EE 2.5 mg/mL**
1	BRWDSc (SP)	21.00±1.53	21.00±1.73	0.00±0.00	0.00±0.00	17.00±1.15	22.33±1.20	0.00±0.00	0.00±0.00	33.67±2.03	31.67±1.45	21.67±0.88	24.33±0.33
2	KBMWDSb3 (M18)	15.33±1.45	17.67±2.60	0.00±0.00	0.00±0.00	0.00±0.00	21.67±2.02	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.67±1.45	13.00±2.08
3	KBMWDSb6 (M6)	22.67±1.45	20.67±1.20	29.00±0.58	22.33±1.45	22.00±1.52	17.33±1.33	16.33±0.88	16.00±1.73	0.00±0.00	22.00±1.15	13.00±0.58	22.00±1.15
4	KBMWDSc (D1)	0.00±0.00	15.33±1.45	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	23.00±1.53	20.67±2.33
5	KBRWDSa (RF)	16.33±1.45	20.67±0.88	0.00±0.00	0.00±0.00	22.33±1.12	20.33±1.45	0.00±0.00	0.00±0.00	22.33±1.20	20.33±1.45	26.00±1.15	19.00±1.15
6	KBRWDS (N1)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.33±1.67	14.67±2.03	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
7	KSWDSc (G)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.67±1.45	15.67±2.60	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	14.00±1.73
8	KSWDSd4	0.00±0.00	7.33±1.20	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
9	MBWDSe	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	11.00±1.73
10	MBWDSf1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.67±1.45
11	MBRWDSf2 (J)	10.33±1.45	15.67±1.45	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	8.33±1.20

*Key: **FB**: Fermented broth and **EE**: Ethanol extracts. There was no significant different between the two extracts (P=.05)*

Table 5. Comparative study of ethanol extract (2.5 µg/mL) with Standard antibiotic (Ciproflaxacin 0.2µg/mL)

Test organisms	Mean zone of inhibition (mm)											
	BRWDSc (SP)	KBMWDSb3 (M18)	KBMWDSb6 (M6)	KBMWDSc (D1)	KBRWDSa (RF)	KBRWDS (N1)	KSWDSc (G)	KSWDSd4	MBWDSe	MBWDSf1	MBRWDSf2 (J)	Control Ciproflaxacin 0.2µg/mL
E. coli ATCC 25922	21.00	17.67	20.67	15.33	20.67	0.00	0.00	0.00	0.00	0.00	15.67	32.00
E. coli 2966	0.00	0.00	22.33	0.00	0.00	0.00	0.00	7.33	0.00	0.00	0.00	27.33
P. aeruginosa ATCC 27853	22.33	21.67	17.33	0.00	20.33	14.67	15.67	0.00	0.00	0.00	0.00	27.64
P. aeruginosa 2929	0.00	0.00	16.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	25.33
S. aureus 2876	31.67	0.00	22.00	0.00	20.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. aureus ATCC 25923	24.33	13.00	22.00	20.67	19.00	0.00	0.00	0.00	11.00	7.67	8.33	28.00

Key: Comparative study of ethanol extract (2.5 µg/mL) with Standard antibiotic (Ciproflaxacin 0.2 µg/mL) There was no significant different between the positive control and ethanol extracts (*P* =.05)

Table 6. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of three active *Actinomycetes* spp

Test organisms	Minimum inhibitory concentration of Ethanol extract (mg/mL)					
	KBMWDSb6 (M6)		BRWDSc (SP)		KBRWDSa3 (RF)	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli ATCC 25922	0.15	0.31	0.31	0.62	0.16	0.31
E. coli 2966	0.31	0.31	-	-	-	-
P. aeruginosa ATCC 27853	0.07	0.15	0.31	0.62	0.62	1.25
P. aeruginosa 2929	0.62	1.25	-	-	-	-
S. aureus 2876	0.15	1.25	0.62	0.62	-	-
S. aureus ATCC 25923	0.07	0.15	0.07	0.31	0.31	0.62

Key: (-) no inhibition was observed.

Table 7. Macroscopic, microscopic and biochemical characteristics of active *Actinomycetes* spp

S / NO	Active Isolates code	Colony colour	Aerial mycelium	Substrate mycelium	Pigment production	Colony surface	Gram's reaction	Catalase test	Citrate test	Indole test	Urease test	Gelatin hydrolysis	Methyl Red test	Voges – Proskauer test	Nitrate test	Esculin degradation	Starch hydrolysis	Triple Sugar Iron test				
																		Glucose	sucrose	Lactose	Gas production	H_2S
1	BRWDSc (SP)	Light yellow	Light yellow chalky	Khaki2	Light yellow	Smooth	+	+	-	-	-	+	-	-	-	+	+	+	+	+	+	+
2	KBMWDSb3 (M18)	Light Pink	White	Light pink	Nil	Rough	+	-	+	-	+	-	+	-	+	-	-	+	-	-	-	-
3	KBMWDSb6 (M6)	Pink	Pink	Red	Red	Smooth	+	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-
4	KBRWDSc (D1)	White chalky	White chalky	Tan2	Nil	Rough	+	+	+	-	-	-	-	-	+	-	+	-	-	-	-	-
5	KBRWDSa3 (RF)	Grey 98	Grey 94 chalky	Light yellow	Yellow2	Smooth	+	-	-	+	-	-	+	-	-	+	+	+	+	+	+	+
6	KBRWDS (N1)	White chalky	White chalky	Khaki2	Nil	Smooth	+	+	+	-	-	+	-	-	-	+	+	+	+	+	+	-
7	KSWDSc (G)	Light yellow	Light yellow chalky	Black	Black	Smooth	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-
8	KSWDd4	Grey	Light ash	Khaki	Nil	Smooth	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
9	MBWDSe	White chalky	Whit	Tan	Nil	Smooth	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-
10	MBWDSf1	Ash	Ash	Khaki	Tan	Rough	+	-	+	-	+	-	+	-	-	+	-	+	-	-	-	+
11	MBWDS f2 (J)	Grey	Grey	Tan	Nil	Rough	+	-	-	-	-	+	-	-	+	-	+	+	-	-	-	-

*Key: **+**: positive, **-** : negative, H_2S: Hydrogen sulpha*

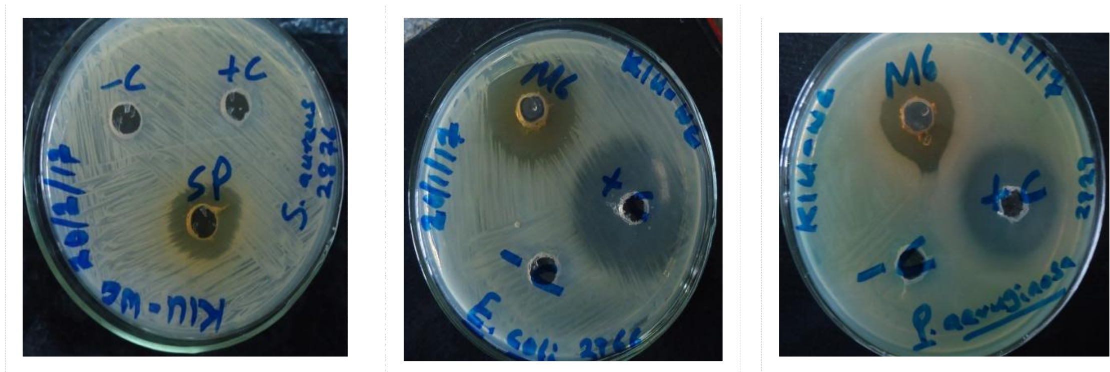

Fig. 2. Secondary screening. Antibacterial activity of ethanol extracts of BRWDSc (SP) and KBMWDSb6 (M6) using well diffusion method against resistant bacteria with both positive and negative controls

Macroscopic, microscopic and biochemical characteristics of the active actinomycetes isolates were presented in (Table 7). The macroscopic features of the isolates showed that majority were white chalky, grey and pink in color, smooth to rough surface colonies with aerial and substrate mycelium and pigment production. Microscopically, the isolates were all Gram positive and filamentous structures. The macroscopic, microscopic and biochemical features of the isolates showed similarity to the genera of actinomycetes found listed in Bergey's manual of determinative bacteriology 9th edition [34].

4. CONCLUSION

This finding showed that actinimycetes producing antibacterial compounds can be isolated from waste dump soil in Western Uganda. Among the isolates obtained, isolates KBMWDSb6 (M6), BRWDSc (SP) and KBRWDSa3 (RF) produced activity against both drug resistant clinical isolates and sensitive standard bacterial isolates. As this research is the first research conducted to this region to isolates actinomycetes producing antibacterial compound (s), these organisms will be subjected to further studies in order to discover their novelty as an antibacterial agent (s).

ETHICAL APPROVAL

Ethical approval of the study was obtained from Kampala International University (KIU) Institutional Research and Ethics Committee (IREC). All experiments were performed in accordance to the ethical standards of the microbiology laboratory operation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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