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Antimicrobial Properties of Actinomycetes Isolated from Menengai Crater in Kenya

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Abstract

A study was carried out to isolate and screen actinomycetes for antimicrobials from Menengai Crater in Kenya. The actinomycetes were isolated using starch casein agar, Luria Bertani agar and starch nitrate agar. Primary screening for antagonism was carried out using perpendicular method while secondary screening was done using agar disk technique. Extraction of the antimicrobials was carried out using ethyl acetate. Sensitivity testing of the crude extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Xanthomonas campestris*, *Erwinia carotovora*, *Candida albicans*, *Alternaria alternate and Fusarium oxysporum* was carried out using agar well technique. Biochemical tests and carbon source requirements were used in characterization of the selected antimicrobial producers. M1 was the best agar medium for isolation of actinomycetes. The number of actinomycetes from regions A, B, C, and D in the crater varied significantly ($F = 27.50$ $P = 0.000$). Out of the 156 actinomycetes isolates, 20 isolates were positive for both primary and secondary screening for antimicrobials. There was no significant difference in the zones of inhibition in primary screening of the actinomycetes for antagonistic properties against the test pathogens ($F = 1.6957$ $P = 0.0838$). The zones of inhibition after secondary screening varied significantly ($F = 2.4473$ $P = 0.0089$). Likewise, there was a significant difference ($F = 6.6046$ $P = 0.001338$) in the zones of inhibition after exposing the pathogens to ethyl extracts of the selected antagonistic actinomycetes. There is need to purify and characterize the antimicrobials obtained from the present study.

Keywords

Actinomycetes, Antimicrobials, Crater, Menengai, Metabolites, Sensitivity

1. Introduction

Naturally occurring antimicrobials are needed in the world today to cub the rising antimicrobial resistance among pathogenic micro-organisms [1]. Soils offer a favourable habitat for the growth of actinomycetes which are good producers of antimicrobials. The need for identifying undiscovered actinomycetes, which increases the likelihood of discovering new drugs is on the increase [2]. New antibiotics produced by such actinomycetes will help in solving challenges of drug resistance and in treating life-threatening diseases such as cancer [3].

Production of secondary metabolites by actinomycetes may offer solutions to these problems [4]. Currently, over 5000 antibiotics have been screened from Gram positive, Gram negative bacteria as well as fungi. However, only 100 of these antibiotics have been developed to the level of treating human, animal and plant diseases [5]. This has been attributed to toxicity of majority of the antibiotics [6].

Actinomycetes are said to be morphologically, physiologically and ecologically diverse organisms [7]. They are of great significance to the pharmaceutical world due to their ability to produce secondary metabolites of medical importance [8]. Most of their metabolites have been shown to have antibacterial (streptomycin, tetracycline, chloramphenicol), antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin) properties [9]. Indeed, most of the antimicrobials used today in remedying diseases caused by pathogens have been developed from actinomycetes.

Among the genera of actinomycetes, *Streptomyces* occupy the biggest ecological niche [10] and they produce the vast majority of antimicrobials known today. Nevertheless, the search for rare actinomycetes which can produce new antibiotics is still a fertile approach to the problem [11]. Besides increased chances of discovering antibiotics by searching for rare actinomycetes, exploring hostile environments such hot deserts, deep seas, saline environments and volcanic areas is another option [12]. This study was therefore carried out to isolate and screen actinomycetes for antimicrobials from the environmentally harsh Menengai Crater in Kenya.

2. Materials and Methods

2.1. Description of the Study Area

Soils were sampled from Menengai crater located in Rongai and Nakuru North sub-counties in Nakuru County, Kenya. The crater is located at an elevation of 2278 m above sea level. Currently, the crater is considered dormant but there are underground geothermal activities which raise the temperatures of the region to a maximum of 82˚C. After volcanic eruptions estimated to have occurred 2000 years ago, walls that were formed on its sides collapsed leading to formation of a large hole at the centre referred to as a caldera [13].

2.2. Soil Sampling

Based on land terrain and soil characteristics, the study area was divided into regions A, B, C, D and eight sampling points were randomly identified from each of the regions. Geothermal vents occurred in regions A and D and were referred to as vents A and vents D, respectively. Soil samples were separately collected from the top 5cm using a sterile trowel and placed in zip lock bags. The samples were transported to the Department of Biological Sciences laboratory, Egerton University. The soil samples were air dried on the laboratory benches for one week to kill some of the vegetative microorganisms. Heat treatment of the samples was carried out by separately placing the samples in 250 ml Erlenmeyer flask and holding them in a water bath at 50˚C for 1 h to further reduce the number of vegetative types of other bacterial flora.

2.3. Preparation of the Culture Media

Actinomycetes were cultured on starch casein agar (SCA) (starch 10 g, K_2HPO_4 2 g, KNO_3 2 g, casein 0.3 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, $CaCO_3$ 0.02 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, agar 15 g, filtered sea water 1000 ml and pH 7.0 ± 0.1); Luria Bertani (M1) medium (starch 10 g, Peptone 2.0 g, Yeast Extract 4.0 g, Agar 18.0 g, distilled water 1000 ml, pH; 7.0 ± 0.1) and starch nitrate agar (soluble starch 20.0 g, K_2HPO_4 1.0 g, KNO_3 2.0 g, $MgSO_4$ 0.5G, $CaCO_3$ 3.0 g, NaCl 100 g, $FeSO_4$ 0.1 g, $MnCl_2$ 0.1 g, $ZnSO_4$ 0.1 g, Distilled water 100 ml, pH 7.0 ± 0.1). The media were dissolved in distilled water as guided by the manufacturers prior to autoclaving at 121˚C for 15 min. The media were supplemented with 25 $\mu g \cdot ml^{-1}$ nystatin do suppress growth of fungi and 10 $\mu g \cdot ml^{-1}$ nalidixic acid to minimize growth of Gram negative and some Gram-positive bacteria.

2.4. Isolation of Actinomycetes

Separately 1 g of soil sample was added to 9 ml of distilled water in a test tube. The test tubes were shaken in an orbital shaker rotating at 200 rpm for 10 min to release actinomycetes that were strongly attached to the soil particles. Aseptically, serial dilution was carried out up to 10^{-6}. Following this, 0.1ml of each sample was separately plated in the three-isolation media using spread plate technique. The plates were incubated at 30˚C for up to one month. The growing colonies were identified as actinomycetes using cultural characteristics such as colonies that were tough, leathery, and partially submerged into the agar. Colonies having these characteristics were sub-cultured onto yeast extract malt extract agar medium and incubated at 30˚C for up to one month. Sub-culturing was carried out until pure cultures were obtained. The pure cultures were preserved in slants and glycerol after coding using letters PAN followed by a number.

3. Biochemical Characterization of Actinomycetes

3.1. Gram's Staining

Crystal violet, gram's iodine, 95% ethyl alcohol and safranin were used in Gram

staining. Briefly, the isolated actinomycetes were separately placed on glass slides using a wire loop. Aseptically, crystal violet was added on the slides and allowed to stand for 1 min. The excess stain was drained off using tap water. Following this, Gram's iodine was added and the preparation allowed to stand for another 1 min. The excess Gram's iodine was removed using running water. Ethyl alcohol was added dropwise followed by washing with running water. A counter stain safranin was added and allowed to stand for 45s before observation of the culture under the microscope [14].

3.2. Use of API Strips

Biochemical characterization of the isolates was carried out through inoculating large volumes of the actinomycetes into 0.85% Nacl. McFarland units were used in standardizing the inocula. The inocula were applied into the wells of API strips. The strips were incubated at 30˚C for up to 7 d [15].

3.3. Carbon Source Utilization

The isolated actinomycetes were tested for their ability to utilize D-Glucose, D-Xylose, L-Arabinose, D-Fructose, D-Galactose, Raffinose, D-Mannitol, sucrose, maltose, lactose and cellulose. The isolates were mixed (1% w/v) with the basal medium followed by incubation at 30˚C for 7 d.

3.4. Primary Screening of Actinomycetes for Antagonism to Selected Pathogenic Microorganisms

The antibacterial activity of pure isolates of actinomycetes was determined using streak plate method [16]. Mueller-Hinton agar for bacterial pathogens and sabouraud dextrose agar (SDA) for fungal and yeast pathogens were prepared. The Mueller-Hinton plates were seeded with bacteria while sabouraud dextrose agar were seeded with fungi test organisms by a single streak at a 90˚ angle to actinomycete strains. The following pathogens: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (ATCC 49617), *Proteus vulgaris* (ATCC 49990), *Aspergillus niger* (ATCC 1015), *Fusarium oxysporum* (ATCC 16608) and *Ustilago maydis* (ATCC 14826) were used as test organisms. The test pathogens were retrieved from the culture collection center of the School of Biological Sciences, University of Nairobi. As positive control, vancomycine (30 μg) was used for bacteria and crotimazole (1% topical solution) for fungi in addition to use of plain plates as negative control. Antagonism was measured by determination of the size of the inhibition zone in millimeters following incubation of bacteria at 37˚C for 24 h and fungal pathogens at 28˚C for 5 - 25 days [17].

3.5. Secondary Screening of Actinomycetes for Antagonism to Selected Pathogenic Microorganisms

M1 agar was prepared and inoculated with isolated actinomycete cultures by

spread plate technique and incubated at 27˚C for 5 - 10 days. From well grown colonies, 6 mm agar disks of actinomycetes cultures were cut out using sterile cork borers. Disks were aseptically transferred to Mueller-Hinton agar plates having *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pneumoniae* (ATCC 49617) and *Proteus vulgaris* (ATCC 49990). Other disks were similarly transferred to sabouraud dextrose agar seeded with *Aspergillus niger* (ATCC 1015), *Fusarium oxysporum* (ATCC 16608) and *Ustilago maydis* (ATCC 14826). Vancomycine (30 µg) for bacteria and crotimazole (1% topical solution) for fungi were used as positive control while plates without the inocula were used as negative control [18].

The inhibition zones (mm) were measured after incubating the bacteria for 24 - 48 h for 37˚C and the fungal pathogens for 5 - 10 days at 28˚C. Among the actinomycetes isolates, four potent strains that indicated greater activity against Gram-positive, Gram-negative bacteria and fungi and recorded the largest diameter of clear zone were selected for antimicrobial production studies.

3.6. Extraction of Crude Antimicrobial Compounds

The selected antagonistic antimicrobial strains were separately inoculated into 3L of M1 broth, and incubated at 28˚C in a shaker (Gallenkamp, Model 10X 400) (200 rpm) for seven days. After incubation, the broths were filtered through Millipore filters (Millipore Millex-HV Hydrophilic PVDF 0.45 Zm). The filtrates were separately transferred aseptically into a conical flask and stored at 4˚C for further assay. To the culture filtrate, equal volume of ethylacetate was added separately and centrifuged at 5000 rpm for 10 min to extract the antimicrobial compound. The compound obtained from each solvent and antimicrobial strain were tested for activity against the test pathogens by agar well diffusion method [19].

3.7. Separation of Antimicrobial Metabolites

Ethyl acetate was used in separately dissolving the selected antimicrobials. The solution was concentrated using a vacuum evaporator (Heidolph Laorota, 4001, Buchi Vacuum Conroller V-805) at 40˚C (50 rpm) to obtain the crude extracts. The crude antimicrobial extracts were collected and dried overnight in a vacuum oven Scwabach, DIN 40050-IP20, V+240, Hz 50/60 at 40˚C.

3.8. Data Analysis

The data obtained was analyzed using Statistical package for social sciences (SSPS) version 17.0 software. Means of actinomycetes from geothermal vents of region A and D were compared using t-test while comparison of the number of actinomycetes isolated using the three types of media were compared using ANOVA at $P = 0.05$.

4. Results

4.1. Isolation of Actinomycetes

A total of 156 actinomycetes stains were isolated from the soils of Menengai Crater. In region A, the actinomycetes isolates varied from $2 \pm 3 \times 10^8$ to $9 \pm 2 \times 10^3$ in starch casein agar (SC), Luria Bertani agar (M1) ($5 \pm 2 \times 10^8$ - $14 \pm 3 \times 10^3$) and starch nitrate (SN) ($4 \pm 3 \times 10^8$ - $14 \pm 2 \times 10^3$) (Table 1). On the other hand, variation of the actinomycetes isolates in region B were SC ($15 \pm 2 \times 10^8$ - $25 \pm 3 \times 10^3$), M1 ($20 \pm 3 \times 10^8$ - $30 \pm 2 \times 10^3$) and SN ($14 \pm 2 \times 10^8$ - $27 \pm 3 \times 10^3$). In addition, actinomycetes isolated from region C ranged from ($16 \pm 2 \times 10^8$ - $24 \pm 2 \times 10^3$) in SC, M1 ($20 \pm 2 \times 10^8$ - $31 \pm 3 \times 10^3$) and SN ($17 \pm 3 \times 10^8$ - $28 \pm 2 \times 10^3$). Besides, actinomycetes ranges in region D were SC ($5 \pm 2 \times 10^8$ - $9 \pm 2 \times 10^3$), M1 ($7 \pm 2 \times 10^8$ - $15 \pm 3 \times 10^3$) and SN ($4 \pm 3 \times 10^8$ - $13 \pm 3 \times 10^3$). There was a significant difference in the number of actinomycetes isolated using the three types of media ($F = 3.315$ $P = 0.04218$). Likewise, the number of actinomycetes isolated from region A, B C, and D varied significantly ($F = 27.50$ $P = 0.000$).

4.2. Primary Screening of Antimicrobials from Actinomycetes

A total of 20 actinomycetes showed antagonism against the test pathogenic microorganisms. The mean zone of inhibition for PAN 4 was 8.91 ± 2 mm, PAN 9 (12.91 ± 3), PAN 18 (11.73 ± 3), PAN 30 (21.36 ± 2), PAN 37 (21.27 ± 2), PAN 41 (23.27 ± 3), PAN 50 (8.91 ± 2), PAN 62 (5.18 ± 1), PAN 71 (8.73 ± 2), PAN 83 (6.09 ± 1), PAN 90 (6.82 ± 1), PAN 101 (11.00 ± 3), PAN 110 (11.18 ± 3), PAN 117 (10.91 ± 3), PAN 126 (5.82 ± 1), PAN 130 (5.82 ± 1), PAN 132 (11.64 ± 2), PAN 137 (6.73 ± 2), PAN 150 (11.18 ± 3) and PAN 154 (20.55 ± 3 mm) (Table 2). However, there was no significant difference in the zones of inhibition among the isolates ($F = 1.6957$ $P = 0.0838$) (Figure 1).

Table 1. Number of actinomycetes isolated from soils of Menengai crater using different types of media.

DF	Number of actinomycetes											
	Region A			Region B			Region C			Region D		
	SC	M1	SN	SC	M1	SN	SC	M1	SN	SC	M1	SN
10^{-3}	9 ± 2	14 ± 3	14 ± 2	25 ± 3	30 ± 2	27 ± 3	24 ± 2	31 ± 3	28 ± 2	9 ± 2	15 ± 3	13 ± 3
10^{-4}	6 ± 2	12 ± 2	10 ± 2	21 ± 3	30 ± 2	24 ± 3	22 ± 2	31 ± 2	25 ± 2	7 ± 3	11 ± 3	10 ± 3
10^{-5}	4 ± 3	10 ± 2	7 ± 2	19 ± 2	28 ± 3	21 ± 3	22 ± 2	30 ± 3	22 ± 3	5 ± 2	10 ± 3	11 ± 2
10^{-6}	3 ± 2	8 ± 3	8 ± 3	18 ± 2	26 ± 2	18 ± 3	17 ± 3	24 ± 2	21 ± 3	6 ± 2	10 ± 2	5 ± 2
10^{-7}	3 ± 3	7 ± 2	5 ± 2	15 ± 3	27 ± 3	15 ± 2	18 ± 3	22 ± 2	19 ± 2	5 ± 2	9 ± 2	3 ± 2
10^{-8}	2 ± 3	5 ± 3	4 ± 3	15 ± 2	20 ± 3	14 ± 2	16 ± 2	20 ± 2	17 ± 3	5 ± 2	7 ± 2	4 ± 3
Mean	**4.5** ± 3	**9.3** ± 2	**8.0** ± 2	**18.8** ± 2	**27.3** ± 3	**19.8** ± **3**	**19.8** ± **3**	**27.0** ± 2	**22.0** ± 3	**6.2** ± **2**	**10.3** ± 3	**7.67** ± 3

Each value represents the means (±SD) of five independent experiments, DF; Dilution factor, M1; Luria Burtani, SN; Starch nitrate.

Table 2. Zones of inhibition (mm) of selected pathogenic micro-organisms in primary screening of the actinomycetes isolates.

S. No.	Isolate	Zone of inhibition (mm)										
		S. aur	*B. sub*	*E. fae*	*E. col*	*K. pne*	*S. typ*	*X. cam*	*E. car*	*C. alb*	*A. Alt*	*F. oxy*	Mean
1	PAN 4	11 ± 2	15 ± 3	19 ± 2	15 ± 2	0 ± 0	14 ± 3	13 ± 3	10 ± 2	0 ± 0	0 ± 0	0 ± 0	8.91 ± 2
2	PAN 9	15 ± 3	14 ± 3	17 ± 2	17 ± 3	10 ± 2	15 ± 3	11 ± 2	10 ± 2	12 ± 3	10 ± 3	11 ± 3	12.91 ± 3
3	PAN 18	11 ± 3	17 ± 2	10 ± 3	12 ± 3	9 ± 2	12 ± 2	15 ± 3	11 ± 3	11 ± 2	10 ± 3	11 ± 2	11.73 ± 3
4	PAN 30	20 ± 2	23 ± 3	23 ± 2	25 ± 2	19 ± 3	21 ± 2	21 ± 2	21 ± 2	21 ± 3	20 ± 2	21 ± 2	21.36 ± 2
5	PAN 37	21 ± 2	20 ± 2	21 ± 2	24 ± 2	21 ± 2	22 ± 2	20 ± 3	22 ± 3	22 ± 2	21 ± 3	20 ± 3	21.27 ± 2
6	PAN 41	21 ± 3	25 ± 3	24 ± 3	25 ± 2	23 ± 3	24 ± 3	22 ± 2	24 ± 2	23 ± 3	22 ± 2	23 ± 2	23.27 ± 3
7	PAN 50	18 ± 2	0 ± 0	14 ± 3	15 ± 3	15 ± 3	0 ± 0	0 ± 0	0 ± 0	15 ± 2	10 ± 3	11 ± 3	8.91 ± 2
8	PAN 62	0 ± 0	0 ± 0	0 ± 0	14 ± 2	0 ± 0	12 ± 2	0 ± 0	0 ± 0	11 ± 2	9 ± 2	11 ± 2	5.18 ± 1
9	PAN 71	13 ± 2	13 ± 3	13 ± 2	15 ± 2	0 ± 0	13 ± 2	0 ± 0	0 ± 0	10 ± 3	9 ± 2	10 ± 3	8.73 ± 2
10	PAN 83	10 ± 3	15 ± 2	12 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 3	10 ± 2	10 ± 2	6.09 ± 1
11	PAN 90	15 ± 2	16 ± 3	11 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 2	10 ± 3	12 ± 3	6.82 ± 1
12	PAN 101	13 ± 3	12 ± 2	10 ± 2	10 ± 3	12 ± 2	11 ± 2	9 ± 3	12 ± 3	11 ± 3	11 ± 3	10 ± 3	11.00 ± 3
13	PAN 110	13 ± 2	14 ± 3	11 ± 2	11 ± 3	10 ± 3	12 ± 3	10 ± 2	10 ± 3	10 ± 2	10 ± 3	12 ± 2	11.18 ± 3
14	PAN 117	12 ± 3	11 ± 2	10 ± 3	12 ± 3	13 ± 3	10 ± 2	11 ± 2	11 ± 2	9 ± 3	11 ± 2	10 ± 2	10.91 ± 3
15	PAN 126	11 ± 2	11 ± 2	9 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 2	11 ± 2	12 ± 3	5.82 ± 1
16	PAN 130	9 ± 2	9 ± 3	10 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 2	12 ± 3	14 ± 2	5.82 ± 1
17	PAN 132	12 ± 3	11 ± 2	11 ± 2	10 ± 2	10 ± 2	13 ± 2	14 ± 2	14 ± 2	11 ± 2	11 ± 2	11 ± 2	11.64 ± 2
18	PAN 137	14 ± 3	11 ± 3	12 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 3	12 ± 3	14 ± 3	6.73 ± 2
19	PAN 150	12 ± 2	10 ± 3	14 ± 3	11 ± 3	12 ± 3	10 ± 2	11 ± 3	10 ± 3	10 ± 3	10 ± 2	13 ± 3	11.18 ± 3
20	PAN 154	19 ± 3	20 ± 2	22 ± 3	20 ± 2	21 ± 3	22 ± 3	20 ± 2	19 ± 2	20 ± 2	20 ± 3	23 ± 2	20.55 ± 3

Each value represents the means (±SD) of five independent experiments, *S. aur*; *Staphylococcus aureus*, *B. sub*; *Bacillus subtilis*, *E. fae*; *Escherichia faecalis*, *E. col*; *Escherichia coli*, *K. pne*; *Klebsiella pneuminiae*, *S. typ*; *Salmonella typhi*, *X. cam*; *Xanthomonas campestris*, *E. car*; *Erwinia carotovora*, *C. alb*; *Candida albicans*, *A. alt*; *Alternaria alternata*, *F. oxy*; *Fusarium oxysporum*.

4.3. Secondary Screening for Antimicrobials from Actinomycetes

There was significant difference in the zones of inhibition among the isolates (F = 2.4473 P = 0.0089) (Table 3). The mean zone of inhibition for PAN 4 was 9.91 ± 2 mm, PAN 9 (14.18 ± 3), PAN 18 (13.18 ± 3), PAN 30 (22.27 ± 2), PAN 37 (21.00 ± 2), PAN 41 (24.36 ± 3), PAN 50 (9.82 ± 2), PAN 62 (5.91 ± 1), PAN 71 (10.00 ± 2), PAN 83 (6.91 ± 2), PAN 90 (7.82 ± 1), PAN 101 (12.09 ± 3), PAN 110 (12.73 ± 3), PAN 117 (11.82 ± 3), PAN 126 (6.36 ± 1), PAN 130 (6.45 ± 1), PAN 132 (12.73 ± 2), PAN 137 (6.82 ± 2), PAN 150 (12.72 ± 2) and PAN 154 (20.91 ± 3 mm).

4.4. Screening for Antibiotics of Ethyl Acetate Extracts

There was a significant difference in the zones of inhibition between the extracts (F = 6.6046 P = 0.001338). The mean zone of inhibition for PAN 41 was 37.64 ± 2 mm which was bigger than that of PAN 30 (35.36 ± 2), PAN 37 (34.09 ± 2) and

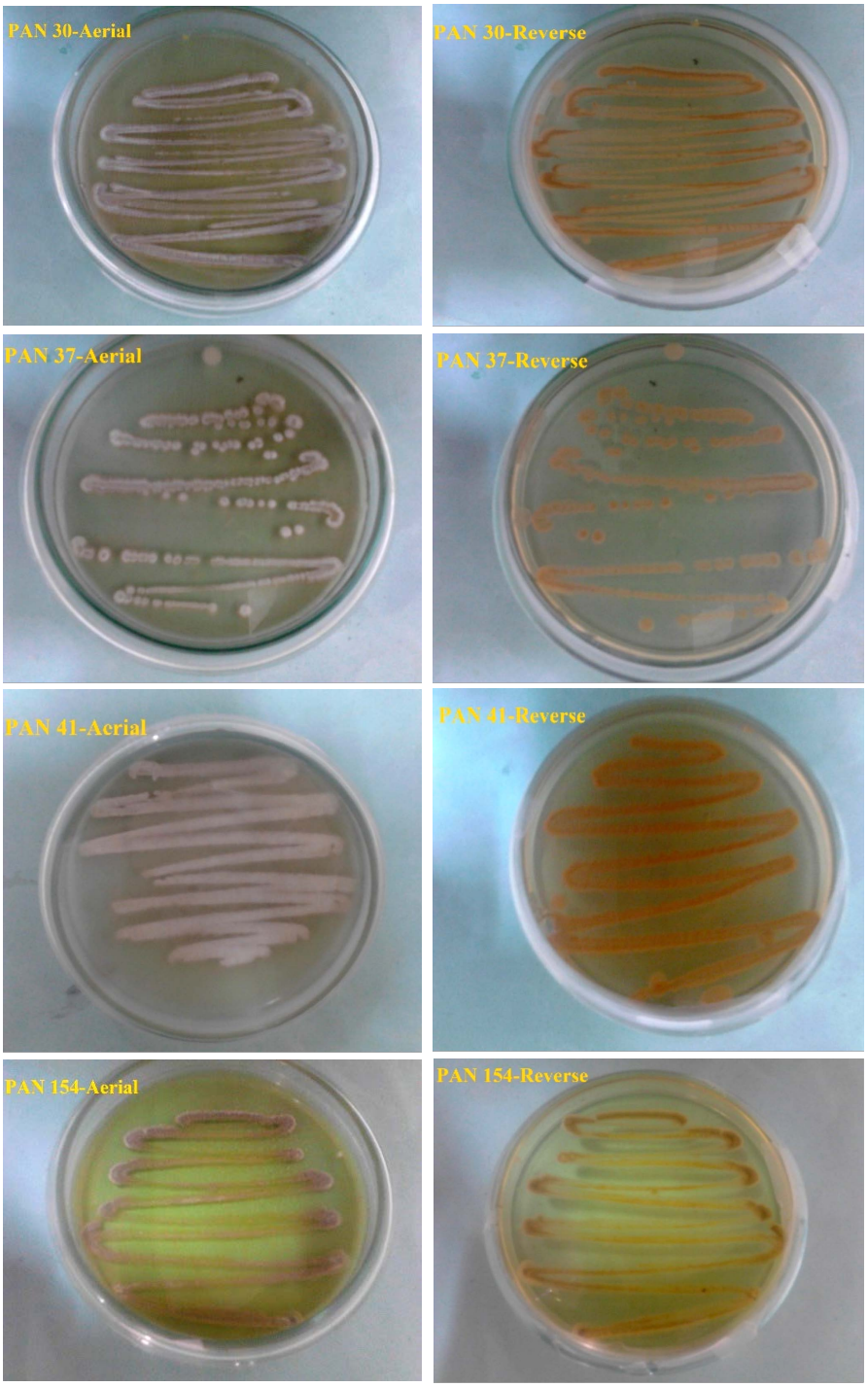

Figure 1. The selected actinomycetes on Luria Bertani agar (M1).

154 (26.27 ± 2 mm) (**Table 4**). However, the biggest zone of inhibition was shown by PAN 41 (49 ± 3 mm) against *Escherichia coli* while the least was 20 ± 3 mm exhibited by PAN 154 against *Bacillus subtilis*.

4.5. Biochemical Test of the Selected Actinomycetes

All the actinomycetes isolates tested positive for Gram stain, catalase, oxidase,

Table 3. Zones of inhibition (mm) of the selected pathogenic microorganisms in secondary screening of the actinomycetes isolates.

S. No.	Isolate	Zone of inhibition (mm)										
		S. aur	*B. sub*	*E. fae*	*E. col*	*K. pne*	*S. typ*	*X. cam*	*E. car*	*C. alb*	*A. Alt*	*F. oxy*	Mean
1	PAN 4	12 ± 2	16 ± 3	22 ± 2	16 ± 2	0 ± 0	16 ± 3	15 ± 3	12 ± 2	0 ± 0	0 ± 0	0 ± 0	9.91 ± 2
2	PAN 9	17 ± 3	15 ± 3	19 ± 2	18 ± 3	11 ± 2	16 ± 3	12 ± 2	13 ± 2	14 ± 3	11 ± 3	10 ± 3	14.18 ± 3
3	PAN 18	14 ± 3	19 ± 2	11 ± 3	13 ± 3	10 ± 2	13 ± 2	16 ± 3	12 ± 3	13 ± 2	11 ± 3	13 ± 2	13.18 ± 3
4	PAN 30	22 ± 2	25 ± 3	24 ± 2	26 ± 2	18 ± 3	20 ± 2	22 ± 2	20 ± 2	25 ± 3	21 ± 2	22 ± 2	22.27 ± 2
5	PAN 37	21 ± 2	20 ± 2	22 ± 2	23 ± 2	20 ± 2	21 ± 2	18 ± 3	23 ± 3	22 ± 2	20 ± 3	21 ± 3	21.00 ± 2
6	PAN 41	24 ± 3	28 ± 3	25 ± 3	27 ± 2	24 ± 3	23 ± 3	20 ± 2	25 ± 2	25 ± 3	23 ± 2	24 ± 2	24.36 ± 3
7	PAN 50	19 ± 2	0 ± 0	15 ± 3	17 ± 3	16 ± 3	0 ± 0	0 ± 0	0 ± 0	18 ± 2	11 ± 3	12 ± 3	9.82 ± 2
8	PAN 62	0 ± 0	0 ± 0	0 ± 0	15 ± 2	0 ± 0	15 ± 2	0 ± 0	0 ± 0	13 ± 2	10 ± 2	12 ± 2	5.91 ± 1
9	PAN 71	15 ± 2	14 ± 3	15 ± 2	17 ± 2	0 ± 0	16 ± 2	0 ± 0	0 ± 0	12 ± 3	10 ± 2	11 ± 3	10.00 ± 2
10	PAN 83	12 ± 3	15 ± 2	16 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 3	12 ± 2	11 ± 2	6.91 ± 2
11	PAN 90	17 ± 2	18 ± 3	14 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	12 ± 2	11 ± 3	14 ± 3	7.82 ± 1
12	PAN 101	15 ± 3	13 ± 2	11 ± 2	10 ± 3	13 ± 2	11 ± 2	10 ± 3	13 ± 3	14 ± 3	11 ± 3	12 ± 3	12.09 ± 3
13	PAN 110	14 ± 2	16 ± 3	13 ± 2	12 ± 3	11 ± 3	13 ± 3	11 ± 2	12 ± 3	13 ± 2	11 ± 3	14 ± 2	12.73 ± 3
14	PAN 117	12 ± 3	11 ± 2	12 ± 3	14 ± 3	15 ± 3	12 ± 2	11 ± 2	10 ± 2	11 ± 3	10 ± 2	12 ± 2	11.82 ± 3
15	PAN 126	12 ± 2	13 ± 2	10 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	10 ± 2	12 ± 2	13 ± 3	6.36 ± 1
16	PAN 130	11 ± 2	10 ± 3	10 ± 3	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 2	14 ± 3	15 ± 2	6.45 ± 1
17	PAN 132	14 ± 3	13 ± 2	12 ± 2	10 ± 2	12 ± 3	14 ± 2	13 ± 2	15 ± 2	12 ± 2	13 ± 2	12 ± 2	12.73 ± 2
18	PAN 137	15 ± 3	12 ± 3	13 ± 2	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 3	11 ± 3	13 ± 3	6.82 ± 2
19	PAN 150	12 ± 2	12 ± 3	15 ± 3	11 ± 3	14 ± 3	11 ± 2	13 ± 3	11 ± 3	14 ± 3	12 ± 2	15 ± 3	12.72 ± 2
20	PAN 154	21 ± 3	20 ± 2	21 ± 3	19 ± 2	20 ± 3	21 ± 3	20 ± 2	19 ± 2	23 ± 2	22 ± 3	24 ± 2	20.91 ± 3

Each value represents the means (±SD) of five independent experiments, *S. aur*; *Staphylococcus aureus*, *B. sub*; *Bacillus subtilis*, *E. fae*; *Escherichia faecalis*, *E. col*; *Escherichia coli*, *K. pne*; *Klebsiella pneuminiae*, *S. typ*; *Salmonella typhi*, *X. cam*; *Xanthomonas campestris*, *E. car*; *Erwinia carotovora*, *C. alb*; *Candida albicans*, *A. alt*; *Alternaria alternata*, *F. oxy*; *Fusarium oxysporum*.

Table 4. Zones of inhibition (mm) of selected pathogenic microorganism to ethyl acetate extracts from the selected actinomycetes.

Extracts	Zone of inhibition (mm)											Mean
	S. aur	*B. sub*	*E. fae*	*E. col*	*K. pne*	*S. typ*	*X. cam*	*E. car*	*C. alb*	*A. Alt*	*F. oxy*	
PAN 30	35 ± 2	42 ± 1	41 ± 3	47 ± 3	28 ± 2	37 ± 3	28 ± 2	40 ± 1	30 ± 3	29 ± 1	32 ± 2	35.36 ± 2
PAN 37	32 ± 1	41 ± 2	38 ± 2	47 ± 1	25 ± 2	36 ± 3	32 ± 3	41 ± 2	30 ± 1	27 ± 2	26 ± 3	34.09 ± 2
PAN 41	39 ± 1	40 ± 1	45 ± 3	49 ± 3	35 ± 2	43 ± 3	38 ± 2	37 ± 3	25 ± 2	30 ± 3	33 ± 1	37.64 ± 2
PAN 154	26 ± 2	20 ± 3	25 ± 2	24 ± 2	25 ± 3	28 ± 2	25 ± 3	27 ± 2	28 ± 2	31 ± 2	30 ± 1	26.27 ± 2

Each value represents the means (±SD) of five independent experiments, *S. aur*; *Staphylococcus aureus*, *B. sub*; *Bacillus subtilis*, *E. fae*; *Escherichia faecalis*, *E. col*; *Escherichia coli*, *K. pne*; *Klebsiella pneuminiae*, *S. typ*; *Salmonella typhi*, *X. cam*; *Xanthomonas campestris*, *E. car*; *Erwinia carotovora*, *C. alb*; *Candida albicans*, *A. alt*; *Alternaria alternata*, *F. oxy*; *Fusarium oxysporum*.

urea hydrolysis and gelatin liquefication (Table 5). They were all negative for deaminase, Indole production, beta-galactosidase and lysine decarboxylase. Isolates PAN 30 and 154 were positive for ornithine decarboxylase and citrate utilization while PAN 37 and 41 were negative. In addition, PAN 37, PAN 41 and PAN 154 were positive for hydrogen sulphide production while PAN 30 was negative.

4.6. Carbon Source Utilization of Actinomycetes Isolated from Menengai Crater

All the isolates utilized D-glucose and D-galactose. Isolates PAN 30 and Pan 154 utilized sucrose, lactose and maltose while PAN 37 and 41 did not. PAN 154 utilized meso-inositol while PAN30, 37 and 41 were unable to (Table 6). In addition, PAN 30, 37 and 41 utilized L-arabinose while PAN 154 did not. None of the isolates utilized cellulose, D-Mannitol, salicin and raffinose.

5. Discussion

Three isolation media—starch casein, Luria Bertani (M1) and starch nitrate agar were used in the isolation of actinomycetes from the soils of Menengai crater. Based on the number of actinomycetes isolates, M1 agar was the best medium for isolation of actinomycetes from this region. This is contrary to studies carried elsewhere [20] [21] [22] [23] which cite starch casein agar as the best isolation medium. This difference can be attributed to differences in the soil sampling region. Menengai crater is a hostile environment implying the possibility

Table 5. Biochemical characteristics of the selected actinomycetes.

Isolate	Biochemical test											
	GS	ONPG	CAT	GLU	LDC	ODC	CIT	H_2S	URE	TDA	IND	GL
PAN 30	+	−	+	+	−	+	+	−	+	−	−	+
PAN 37	+	−	+	+	−	−	−	+	+	−	−	+
PAN 41	+	−	+	+	−	−	−	+	+	−	−	+
PAN 154	+	−	+	+	−	+	+	+	+	−	−	+

GS: Gram stain, ONPG: beta-galactosidase, CAT: catalase test, GLU: oxidase, LDC: lysine decarboxylase, ODC: ornithine decarboxylase, CIT: citrate utilization, H_2S: Hydrogen sulphide production, URE: urea hydrolysis, TDA: deaminase, IND: Indole production, GL: Gelatin liquefication.

Table 6. Carbon source utilization of the selected actinomycetes.

Isolate	Carbon source											
	DG	DGal	Cellulose	Su	DM	Lactose	Mal	Salicin	DF	Raffinose	Meso	LA
PAN 30	+	+	−	+	−	+	+	−	+	−	−	+
PAN 37	+	+	−	−	−	−	−	−	+	−	−	+
PAN 41	+	+	−	−	−	−	−	−	+	−	−	+
PAN 154	+	+	−	+	−	+	+	−	+	−	+	−

DG; D-Glucose, DGal; D-Galactose, Su; sucrose, DM; D-Mannitol, Mal; Maltose, DF; D-Fructose, Meso; Meso-Inositol, LA; L-Arabinose.

of variation in the population composition of actinomycetes and therefore need for different nutrients from those isolated from other regions. [24] asserts that the soil nutrient composition leads to differences in nutrient requirements in actinomycetes.

Primary screening is an important procedure for preliminary identification of actinomycetes with antagonistic properties to pathogens. Although many actinomycetes may be isolated from a given soil sample, not all of them may show antagonism to pathogenic microorganism [25]. [26] isolated actinomycetes from the soils of Punjab and obtained a total of 15 isolates out of which 12 indicated antagonism against the tested organisms. This disagreed with the current study. Differences in antimicrobials produced could be a contributing factor to the differences.

In a similar study carried out by [27] in North Iran, the zones of inhibition obtained after carrying out secondary screening were *Bacillus subtilis* (8 mm), *Staphylococcus aureus* (12 mm), *E. coli* (9 mm) and *Klebsiella* spp. (0.00). These were lower than the ones obtained in the current study. This could be attributed to differences in the genetic codes that are responsible for production of antimicrobials. [28] explains that the genetic constitution of a particular actinomycete to a large extent dictates the antimicrobials produced.

This study obtained bigger zones of inhibition after testing for sensitivity of the pathogens to ethyl acetate extracts than previous studies carried out in other regions [29]. According to [30], different actinomycetes yield different types of antimicrobials Further, [31] asserts that the environment in which actinomycetes are growing in to a great extent, influences the metabolic activities of the organisms thus influencing the types of antimicrobials produced.

Results on biochemical tests obtained in this study slightly differ with those of previous studies carried out elsewhere [32] [33] [34]. [35] explains that different stains of actinomycetes present varying biochemical reactions. However, the results of carbon utilization obtained in this study agreed with a previous study carried out in a foot-hill of Western Ghats in India [36]. This may have been caused by similarity in carbon source requirement as explained by [37].

6. Conclusion

Actinomycetes were successfully isolated from Menengai crater. Although a total of 156 actinomycetes were isolated, 20 actinomycetes showed antagonism against the test pathogens. Four antimicrobial metabolites showed the highest antagonism against the test pathogens. The study indicated that Menengai crater has the potential of producing actinomycetes that can produce antimicrobials with high capability of treating diseases caused by tested pathogenic microorganisms.

Recommendation

There is need to purify and characterize the antimicrobials obtained from the

present study.

Conflict of Interest

The authors declare no conflict of interest

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