SCREENING ACTINOMYCETES ASSOCIATED WITH LEMON GRASS (*CYMBOPOGON CITRATUS*) RHIZOSPHERE FOR ACTIVITY AGAINST MULTI DRUG RESISTANT BACTERIA

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ABSTRACT

Globally, there is increasing demand for novel bioactive molecules to combat the upsurge of multiple antibiotics resistant pathogenic bacteria. Consequently, researches are now directed towards different environments to identify microorganisms with capability of producing new, potent and safe compounds. In this study, sixty Actinomycetes isolates obtained from the rhizosphere of lemon grass were screened for antibacterial substance production using thirty-four multiple antibiotic resistant bacteria isolates isolated from soils obtained from dumpsites in Abraka metropolis, Delta State. Out of the 60 Actinomycete isolates, 6 (10%) belonging to the genus Streptomyces demonstrated antibacterial activity against at least one of the 34 test bacterial isolates. Although, 5(83.3%) out of the 6 *Streptomyces* spp exhibited antibacterial activity against only the Gram positive test bacterium, 1(2.04%) coded as Str1, demonstrated broad spectrum activity. This resulted in the extraction of its secondary metabolites using ethyl acetate. There were no significant differences at $p < 0.05$ between zones of inhibition (ranging from 19 to 24 mm) produced against various test isolate by the crude extract and that produced by the antibiotics used as control (ceftriaxone) indicating susceptibility of the strains to the crude extract. Analysis of the extract using GC-MS, led to the identification of 25 compounds. However, the main constituent with suspected antibacterial activity were 1,2 benzodiol,3,5-bis(1,1-dimethylethyl)-, 2-methyl-7-phenyl indole, 2,4,6, cycloheptatrien-1-one3,5, -bis-tri-methylsilyl and benzo[h]quinolone,2,4-dimethyl. Hence, the study concludes that the *Streptomyces* sp Str1 produced compounds with antibacterial properties against both Gram positive and negative multiple antibiotics resistant bacteria and thus is a potential candidate for the development of promising antibacterial agents.

KEYWORDS: Actinomycetes, Bacteria, Lemon grass, Multidrug resistant, Rhizosphere, Screening.

INTRODUCTION

Over the last few decades, there has been increasing reports of bacterial resistance to various antibiotics globally (Danquah *et al*., 2022; Al-Ansari *et al*., 2019; CDC, 2019; Manimaran *et al.,* 2017*;* Akponah, 2014). The major drivers of such antibiotic resistance have been attributed to both overuse and misuse of antibiotics (Suganya *et al*., 2022; WHO, 2021a). The quantum of antibiotics indiscriminately used for prophylactic and therapeutic measures in both man and farm animals have resulted in the selection of resistance to multiple antibiotics in several pathogenic bacteria (Al-Ansari *et al*., 2019; Nikaido, 2009). Pertinently, horizontal transmission of antibiotic resistant genes has also been reported among environmental bacterial isolates and in agricultural animal products (Suganya *et* al., 2022; Vinayamohan *et al*., 2022).

Multiple antibiotics resistance (MDR) is a global public health threat that need to be tackled in order to achieve the sustainable Developmental goals of the United Nations (Suganya *et al.*, 2022; WHO, 2021b). In furtherance to this, resistance to antibiotics and other antimicrobials, play a key role in the economy of any nation. It births the need for more expensive drugs hence draining the finances of those impacted as well as the government, especially, in countries where government attention is paid to subsidies in the health sector (WHO, 2021b). Antimicrobial resistance has also resulted in severe/or difficult to threat infections, prolonged stays in hospitals, disabilities and even death of teeming population irrespective of age (Rammali *et al*., 2022). This is very significant because, the productivity of patients including their attendants are greatly impeded. Also, many medical procedures like surgeries, organ transplantation, cancer chemotherapy have become very risky as a result of the resistance of bacteria to various antibiotics (Hummell and Kirienko, 2020; Rammali *et al*., 2022). In fact, the cost of multiple antibiotics resistance cannot be over-emphasized. Therefore, there is an urgent need for the development of new antibiotics to forestall the problem exerted by multi drug resistance in our modern day society (Danqua *et al*., 2022; Mapipa *et al.,* 2021; WHO, 2021b; Hummel and Kirienko, 2020; CDC, 2019).

In 2021, WHO reported a prevalence of 12.11%, 64%, 92.9%, 79.4% in methicillin resistant *Staphylococcus aureus*, fluoroquinolone resistant *Escherichia coli* and carbapenem resistant *Klebsiella pneumonia* in countries that report to the Global Antimicrobial Resistance and Use Surveillance System (GLASS). Recently, the US Center for Disease and Prevention issued a health advisory to warn the public of an increase in drug resistance in bacteria since there are limited

recommendations for treatment for infections caused by such bacteria. According to the CDC (2019) report, percentage of infections caused by drug resistant strains of Shigella increased from 0 in 2015 to 5% in 2019. Furthermore, the report states that there are nearly 3 million antimicrobial resistant infections each year and more than 35000 people die as a result in the US and about 5 million deaths recorded worldwide. These records are expected to increase to 10 million by 2050 if efforts are not made to stop the spread of antimicrobial resistance (Santos-Beneit *et al.,* 2022).

The rapid onset of resistance to several drugs, requires a constant supply of new drugs for the effective treatment of infections (Arya *et al*., 2019; Al-Ansari *et al*., 2019; Manimaran *et al*., 2017; Gill *et al*., 2011). Thus, screening and discovery of novel drugs have become a necessity (Danquah *et al*., 2022; Manimaran *et al*., 2017; Parsaeimehr *et al*., 2013). Natural products play a pivotal role in antibiotic drug discovery (Rammali *et al*., 2022) and microorganisms are considered as reservoir of such natural products and hence untapped antimicrobials, (Danquah *et al*., 2022; Shukla, 2015). They offer a plethora of new, cheap, alternative bioactive compounds that can be used excellently to combat the upsurge of pathogenic antibiotic resistant strains (Danquah *et al*., 2022 Rakholiya *et al.,* 2013).

Microbial metabolites produced especially by Actinomycetes have been the basis for the synthesis of many antibiotics (Promnuan *et al*., 2020) hence making this group of organisms highly important in the medical and pharmaceutical sectors. The metabolites of Actinomycetes have not only exhibited antimicrobial activity but have also shown anti-tumor, anti-viral, anti-cancer anti-inflammatory as well as immunosuppressive activities (Rammali *et al*., 2022; Gomes *et al.*,2018; Shukla, 2015). Actinomycetes are a group of Gram positive, acid fast, facultatively anaerobic, filamentous bacteria possessing the tendency to break or fragment into cocci or bacillary forms (Gottelt *et al*., 2010). The morphological complexity and diversity of this group put them in limelight as they are widely distributed in both aquatic and terrestrial environments (Selim *et al*., 2021) and are well known sources of an array of bioactive secondary metabolites (Promnuan *et al*., 2020; Al-Ansari *et al*., 2019; Ramanzani *et al*., 2013; Gottelt *et al*., 2010). More than 50 % of the explored bioactive molecules have been produced by Actinomycetes (Gomes *et al*., 2018; Shukla, 2015) hence, the group stay indispensable in the race to ameliorate the impact of drug resistant pathogens (Mast and Stegmann, 2019).

As crucial as the foregoing, there has been a decrease in the discovery of new bioactive compounds from actinomycetes in the last two decades (Santos-Berneit *et al*., 2022; Mojica *et al*., 2021; WHO, 2021b). Therefore, researches are now, re-directed towards unexploited environments (Santos-Berneit *et al*., 2022; Manimaran *et al*., 2017) to obtain Actinomycetes with potentials for vast and new compounds that would be able to effectively and safely target resistant pathogens (Rammali *et* al., 2022) To this end, this study was designed to screen lemon grass rhizosphere (a culinary and medicinal grass common in the Southern part of Nigeria) for common soil-derived/indigenous Actinomycetes capable of producing bioactive compound(s) with activity against multi-drug resistant bacteria.

MATERIALS AND METHOD

Source of Samples/Actinomycetes isolates

Rhizosphere samples were obtained from 30 lemon grasses growing at three different sites (designated Station A, B and C) in Abraka, Delta State following the method described by Ubogu *et al*., 2019 and Kumar *et al.,* 2012. Loose soils around the root of each uprooted grass, was shaken off while soil firmly attached to the root was carefully but vigorously shaken into sterile black polyethylene bag. After which, the rhizosphere soil samples were transported to the laboratory within 30 minutes of collection for isolation of Actinomycetes.

The Actinomycete isolates were obtained by culture method. Each rhizosphere sample was weighed in 1 g amount and diluted using the ten-fold serial dilution method. Thereafter, 0.1 mL of various dilution factors were inoculated separately into freshly prepared starch casein agar using the pour plate method. Incubation at ambient temperature for 48-72 h, followed immediately. At the end of incubation period, plates of an appropriate dilution factor that produced distinct colonies ranging from 30 -300 were selected for isolation and further studies. The composition of the starch casein agar in g/L is as follows: $FeSO_4.7H_2O$; 0.01, $MgSO_4.7H_2O$; 0.05, $CaCO_3$; 0.02, NaCl; 2.00, KNO_3; 2.0, K_2HPO_4; 2.0, casein; 0.3, starch 10, and agar 15.

Identification of Actinomycete Isolates

Preliminary identification of isolates was based on observation of cellular and colonial morphologies. Isolates were further characterized biochemically by subjection to criteria as in Bergey's Manual of Determinative Bacteriology (Goodfellow *et al*., 2012).

Source of 'Test Bacteria' Isolates

Multiple antibiotics resistant bacteria (MDRB) isolates obtained from dumpsite soils (4) receiving several municipal wastes served as the test organisms. After careful removal of all solid waste, composite soil sample at a depth of 5cm (vertical profile) was collected from the dumpsites using sterile soil auger and transported in black polyethylene bags to the laboratory within 30 minutes of collection. Bacterial isolates were obtained by diluting 1g of each soil sample using the ten-fold serial dilution method and then inoculating 0.1mL aliquot of appropriate dilution factor into Muller Hinton agar. Plates were then incubated at room temperature for 24 to 72 h. The isolates obtained

were characterized biochemically according to criteria in *Bergey's manual of determinative bacteriology* (1994) and multidrug resistance determined by antibiotic susceptibility test as described below.

Screening test bacteria for antibiotic Susceptibility.

The Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines was adopted. Each test bacterium was standardized by sub-culturing 3 colonies of the respective isolate into sterile peptone water contained in a test tube and incubated at $37^{O}C$ until turbidity marched that of 0.5 $BaSO_4$ (McFarland standard). The standardized inoculum was then inoculated using a sterile swab stick onto the surface of freshly prepared Muller Hinton agar. Then, plates were allowed to stand for about 5 minutes and various antibiotic discs were aseptically placed on the surface of the already seeded agar plate. The antibiotics used to assess the sensitivity of the isolates were septrin (30µg), chloramphenicol (30 µg), sparfloxacin (10µg), ciprofloxacin (10µg), augmentin (30µg), amoxicillin(30µg), gentamycin (10 µg), streptomycin (30 µg), cefuroxime (30 µg) and ceftriaxone (30 µg). Incubation followed immediately at $37^{O}C$ for 18 -24 h. At the end of incubation, zones of inhibition were measured in mm and interpreted according to CLSI (2018) guideline. Isolates that were resistant to three or more classes of antibiotic were considered as MDR and were selected as the test bacteria. Also, percent occurrence (prevalence) of MDRB were determined.

Preliminary Screening of *Streptomyces* spp. for the production of Antibacterial Compounds

Preliminary screening for the production of bioactive substance (antibacterial) was done using the cross streaking method. Each of sixty Actinomycete isolates as obtained from lemon grass rhizosphere, was streaked across one-third portion of freshly prepared nutrient agar plates and incubation at room temperature for 7 days followed immediately. After which, the test bacterium (MDRB) was streaked perpendicularly, to the already grown Actinomycete isolate. Further incubation followed immediately at $37^{o}C$ for 24 h. At the end of incubation period, zone of inhibition was measured and regarded as indication for the production of bioactive substance with antibacterial activity.

Secondary Screening of *Streptomyces* spp. for the production of Antibacterial Compounds

The Actinomycete strain that elicited significant zone of inhibition against at least one strain of each MDR bacterium was selected for the secondary screening experiment. The experiment was done following a two-step procedure**.** In the first step, crude extract of the metabolites of the Actinomycete was obtained while the second step comprised evaluation of the antibacterial activity of the crude extract as described in the succeeding sections.

Production of crude extract of the metabolites from *Streptomyces* spp.

The choice strain of Actinomycete (*Streptomyces* sp.) was subjected to fermentation in starch casein broth. Cell concentration of 1.00 x 10^7 CFU/mL was inoculated into 100mL of the broth contained in a 250mL Erlenmeyer flask. Then, incubation at 30ºC for 7 days under shaken conditions of 120 rpm using shaker incubator (SEARCHTECH) was done immediately. Crude extracts of the metabolites produced was then, obtained from the supernatant using 1% v/v ethyl acetate following the method described by Dhananjeyan *et al*., 2010. Having harvested the cells, ethyl acetate was mixed with the supernatant in ratio 1:1. The mixture was allowed to stand overnight, after which the separated upper layer of ethyl acetate was evaporated to dryness at 40ºC. Concentrated bioactive compounds was reconstituted in sterile deionized water for further analysis.

Determination of the anti-bacterial activity of crude extract of metabolites produced by *Streptomyces* sp.

The paper disc diffusion technique was used in the determination of the anti-bacterial activity of the crude extract obtained as described in the preceding section. The Kirby –Bauer disc diffusion method was adopted. Paper disc (3mm in diameter were cut from Whatman No 1 filter paper. After which they were allowed to cool and impregnated with 0.1mL of 0.01mg/L of reconstituted crude extract of bioactive compounds. This was then aseptically placed on the surface of an already seeded Muller Hinton agar medium. Seeding was done by respective streaking of standardized MDR bacterial isolate all over the surface of each medium. (The MDR bacteria used in this experiment included only the isolates that demonstrated susceptibility to anti-bacterial substance producing Streptomyces in preliminary screening) The antibiotics ceftriaxone was used as control.

All plates were incubated immediately at 37^oC for 24h. At the end of incubation period, zones of inhibition produced were measured.

Determination of the bioactive compounds present in crude extract.

Investigation to identify the bioactive compounds present in the ethyl-acetate extract obtained as described above, was done using gas chromatography coupled with mass spectrometer detector (Agilent Technologies). Compound identification was achieved based on reference to mass spectra database in NIST (National Institute of Standard and Technology) library.

Statistical Analysis

Data obtained were expressed as mean and percentage. Statistical significance was assessed by t-test and $p < 0.05$ was considered as statistical significance.

RESULTS

Two hundred and two bacteria isolates belonging to five genera including *Salmonella*, *Escherichia*, *Pseudomonas*, *Acinetobacter* and *Staphylococcus* were obtained from soil samples collected from dump sites. Upon screening for antibiotic susceptibility, varied degrees of sensitivity to the antibiotics used in the assay were noticed in the 210 isolates. it was observed that 34 (16.8%) of the 202 bacterial isolates demonstrated multiple antibiotic resistance as presented in Table 1. The prevalence of multiple antibiotic resistance observed among isolates of *Staphylococcus aureus* (16), *Acinetobacter* spp. (36), *Salmonella* spp. (51), *Escherichia coli* (69), and *Pseudomonas aeruginosa* (30) were 37.5, 13.9, 9.8, 26.7 and 14.5 (%) respectively (Table 1). On the basis of the multiple antibiotic resistance, these 34 bacterial isolates were selected as test organisms for preliminary screening of various Actinomycetes isolates for potential of anti-bacterial substance production.

Furthermore, a perusal of the data as presented in Table 2, revealed that at least 15% of the isolates of the various test bacteria (*Pseudomonas aeruginosa, Acinetobacter* spp., *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus*) were resistant to sceptrin, chloramphenicol, sparfloxacin, ciprofloxacin, gentamycin, streptomycin and amoxicillin. However, all isolates with the exception of *Pseudomonas aeruginosa*, were susceptible to ceftriaxone (hence its choice as control antibiotics in subsequent secondary screening experiment). Two strains (6.7%) of *Pseudomonas aeruginosa* demonstrated resistance to ceftriaxone. Also, most isolates were susceptible to augmentin though percentages of population observed as resistant to it ranged from 0 - 8.3.

A total of sixty Actinomycetes isolates were obtained from lemon grass rhizosphere soils, of which, 49 (81.67%) were *Streptomyces* spp. *Micromonospora* and *Nocardia* were 5 (8.33%) and 6 (10%) respectively as presented in Table 3. Out of these 60 Actinomycete isolates, 6 (10%) belonging only to the genus Streptomyces demonstrated antibacterial activity against at least one of the 34 MDR bacterial isolates as depicted in Table 4. The results of the preliminary screening revealed that, 5(83.3%) out of the 6 *Streptomyces* spp. that exhibited antibacterial activity, were more active against the Gram-positive test bacterium than the Gram-negative bacteria (Table 5). However, 1(2.04%) demonstrated activity against all the MDRB used in the assay as also, presented in Table 5.

In addition, the Streptomyces strain coded as Str1 was the only Streptomyces species capable of producing significant zones of inhibition against at least one isolate of each of the five genera of MDR test organisms irrespective of Gram response. Consequently, its metabolites were extracted following fermentation in starch casein broth. The results of the anti-bacterial activity of the extract from Str1, is as presented in Fig. 1. The crude extract produced zones of inhibitions ranging from 23-25(mm), 19-21(mm), 21-23(mm), 20-24(mm) and 22-24(mm) against *Staphylococcus aureus, Acinetobacter* spp., *Pseudomonas aeruginosa, Escherichia coli* and *Salmonella* spp. respectively. Again, broad spectrum activity was noticed and zones of inhibition were all greater than or equal to zones of inhibition produced by the control antibiotics. There were no significant differences at $p < 0.05$ between zones of inhibition produced against each isolate by the crude extract and that produced by the antibiotics used as control indicating susceptibility of the strains to the crude extract. The ethyl acetate extract demonstrated better inhibitory properties against the multidrug resistant bacteria than all the assayed antibiotics except ceftriaxone and augmentin.

Analysis of the extract using GC-MS, led to the identification of 25 compounds (25 peaks). However, the main constituent with suspected antibacterial activity as shown in Table 6, were 1,2 benzodiol,3,5-bis(1,1-dimethylethyl)-, 2-methyl-7-phenyl indole, 2,4,6, cycloheptatrien-1-one3,5, -bis-tri-methylsilyl and benzo[h]quinolone,2,4-dimethyl. The resemblance of the mass spectrum of each of these compounds to the structure in the reference library were greater than 70% while their area ranged from 6.202 to 8.630 (%). All of these biomolecules are heterocyclic containing the benzenoid nucleus and their elution periods lasted between 12.984 and 16.687 minutes.

Table 1: Prevalence of multiple antibiotic resistant bacteria among isolates screened for antibiotic susceptibility

Organism	Number isolates from dump sites	Number of Isolates that were MDR	Prevalence of MDR Isolates (%)
S. aureus	16	6	37.5
Acinetobacter sp.	36	5	13.9
Salmonella sp.	51	5	9.8
P. aeruginosa	30	8	26.7
E. coli	69	10	14.5
Total	202	34	16.8

Table 2: Antibiogram of isolates obtained from dumpsite Soils

Antibiotic	Number of Resistant isolates (percentage)				
	P. aeruginosa	*Acinetobacter* spp.	*Salmonella* spp.	*E. coli*	*S. aureus*
SEP	15(50.0%)	11(30.6%)	15(29.4%)	16(23.2%)	8(50.0%)
CHL	11(36.7%)	9(25%)	8(15.7%)	13(18.8%)	3(18.8%)
SPA	10(33.3%)	12(33.3%)	10(29.4%)	14(20.3%)	6(37.5%)
CIP	11(36.7%)	12(33.3%)	13(19.6%)	14(20.3%)	6(37.5%)
AUG	2(6.7%)	3(8.3%)	0(0%)	4(5.8%)	1(6.3%)
AMX	9(30.0%)	14(38.9%)	18(35.3%)	6(8.7%)	7(43.8%)
GEN	7(23.3%)	6(16.7%)	14(27.5%)	13(18.8%)	2(12.5%)
STR	8(26.7%)	6(16.7%)	15(29.4%)	11(15.9%)	2(12.5%)
CEF	2(6.7%)	2(5.6%)	0(0%)	0(0%)	1(6.3%)
CER	2(6.7%)	0(0%)	0(0%)	0(0%)	0(0%)

SEP=Sceptrin, CHL=Chloramphenicol, SPA=Sparfloxaci, CIP= Ciprofloxacin, AUG= Augmentin, GEN= Gentamycin, STR= Streptomycin, CEF = Cefuroxime, CER= Ceftriaxone, AMX = Amoxicillin

Table 3: Frequency of occurrence of various Actinomycetes in the rhizosphere of Lemon grass from different stations

Actinomycetes	Number of isolates obtained from lemon grass rhizosphere.			Total
	Station A	Station B	Station C	
Streptomyces spp.	14	16	19	49(81.7%)
Micromonospora spp.	3	2	0	5(8.3%)
Nocardia spp.	3	2	1	6(10%)

Table 4: Percentage of Actinomycetes that demonstrated anti- bacterial activity.

Actinomycetes (n)	Percentage that Possessed antibacterial activity
Streptomyces spp. (49)	12.24
Nocardia spp. (6)	0
Micromonospora spp. (5)	0
Total (60)	10

Table 5: Response of various strains of MDRB to inhibitory action of antibacterial substance producing *Streptomyces* spp.

Antibacterial Producing Streptomyces Isolates	Proportion of Susceptible MDRB (%)				
	Staphylococcus aureus (n= 6)	*Acinetobacter* spp. (n = 5)	*Pseudomonas aeruginosa* (n = 8)	*Escherichia coli* (n = 10)	*Salmonella* spp. (n = 5)
Str1	50	100	50	60	60
Str2	16.7	0	12.5	10	0
Str3	16.7	0	12.5	0	0
Str4	50	0	0	0	0
Str5	33.3	0	0	10	0
Str6	33.3	0	12.5	10	0

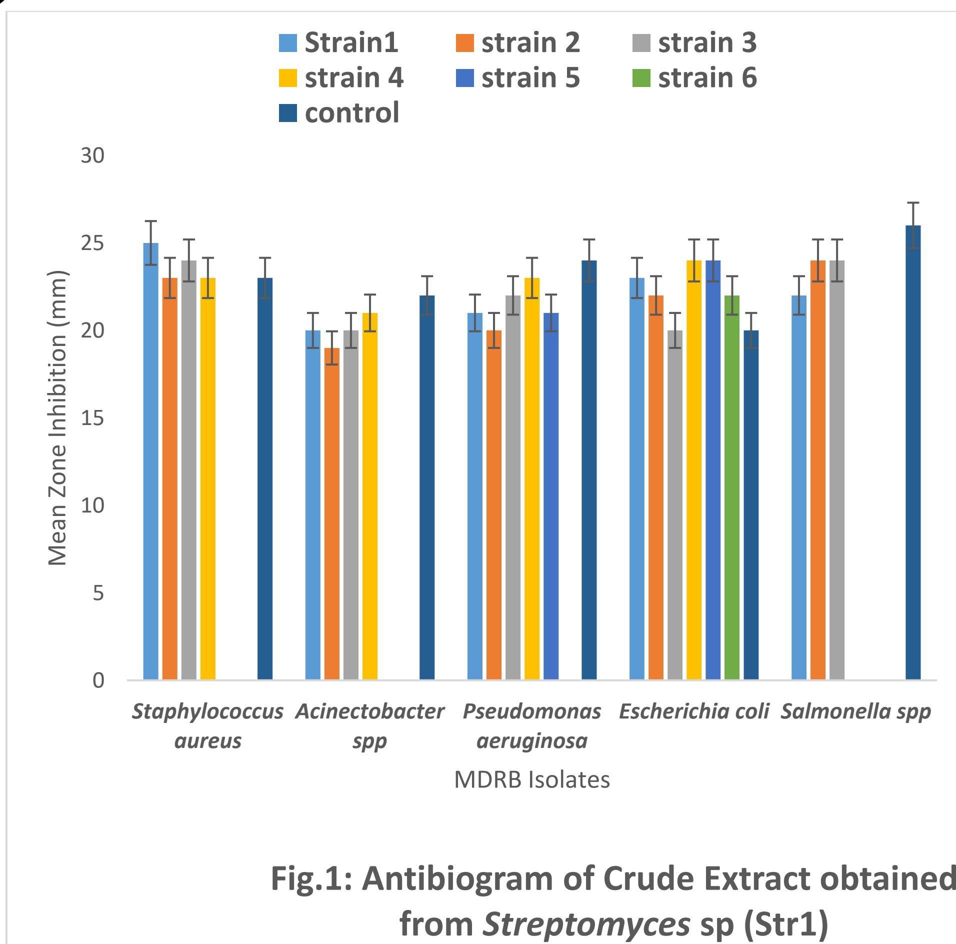

Fig.1: Antibiogram of Crude Extract obtained from *Streptomyces* sp (Str1)

Table 6: Chemical composition of compounds in ethyl acetate extract with antibacterial activity.

S/N	Compound	Retention time (mins)	Peak Height	% Correlation with compound in library	Area %
1	1,2 benzodiol,3,5-bis(1,1-dimethylethyl)-	12.984	2783	71.84	6.202
2	benzo[h]quinolone,2,4-dimethyl	14.555	2931	79.89	6.895
3	2,4,6,cycloheptatrien-1-one3,5,-bis-tri-methylsilyl	15.504	4109	95.53	8.245
4	2-methyl-7-phenyl indole	16.687	4970	100	8.630

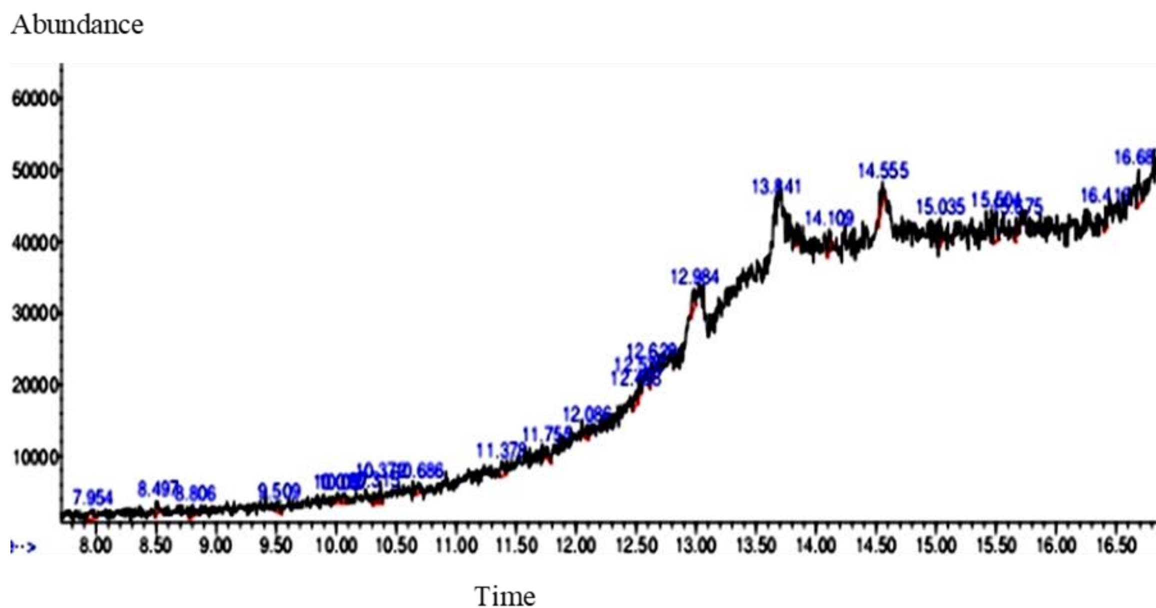

Fig.2: GC-MS chromatogram of the ethyl-acetate extract.

DISCUSSION

Multiple antibiotic resistance has been defined as the acquisition of resistance to at least any three groups or classes of antibiotics (Suganya *et* al., 2022; Bjerketorp *et* al., 2021; Exner *et al*., 2017). According to Tanwar *et al*., 2014, multiple antibiotic resistance is defined as insensitivity of microorganisms to administered antibiotics (which are structurally unrelated and have different molecular targets) despite previous sensitivity to the drugs. Therefore, the isolation of MDRB from dumpsite soil samples is alarming and may be connected to the habitual indiscriminate disposal of all kinds of materials including hospital waste, animal farm waste and the likes that probably contain unused, expired or residual antibiotics. Also, the incessant use of sub- therapeutic doses of antibiotics by the people and in livestock production as well as the problem of rampant open defecation may have contributed to the pollution of the environment by antibiotics and hence the occurrence of MDRB in the environment studied. Similar reports have been made by Mokni-Tlili *et al.,* 2023 and Nikaido, 2009. In addition, the microbial production of secondary metabolites that resemble many antibiotics currently in use in health care systems could also have promoted the observed incidence of MDRB among the environmental isolates as a direct consequence of selective pressure. Again, the dissemination of antibiotic resistance genes among the isolates in the environment can be attributed to horizontal transfer of antibiotic resistant genes among bacterial within same ecological niche (Vinayamohan *et al*., 2022). Several authors have shown the presence of plasmids and integrons (mobile genetic elements) that carry antibiotic resistance gene among and across species of bacteria in the environment (Suganya *et* al., 2022; Vinayamohan *et al*., 2022).

Further results obtained in this study, confirms the abundance of *Streptomyces* spp in the rhizosphere of lemon grass as it was the predominant species of Actinomycetes in samples collected from different stations. Several authors including Chaudhary *et al*., 2013, Ramazani *et al*., 2013 and Alimuddin *et al*., 2011 have reported the predominance of Streptomyces among other Actinomycetes in soils and hence rhizospheres. This is usually linked to soils with near neutral and alkaline pH, high humic and moderate moisture contents (Sapkota *et al*., 2020; Ramazani *et al*., 2013; Nanjwade *et al*., 2010; Hayakawa, 2008). The type of culture medium used in isolation can also favour a particular species of Actinomycetes (Rammali *et al*., 2022; Promnuan *et al*., 2020). In this case starch casein agar was used.

Among the Streptomyces, the rate of isolation of species with antibacterial property was 12.24%, although, only 2.04% demonstrated activity against all the MDRB used in the assay (ie broad spectrum activity). Both higher and lower percentages have been reported in previous studies (Promnuan *et al*., 2020; Manimaran *et al*., 2017; Ramazani *et al*., 2013). Moreover, the lower susceptibility of Gram-negative test bacteria than the Gram-positive bacterium to the antibacterial activity of majority (83.3%) of *Streptomyces* spp. assayed in the preliminary screening probably, relates to the nature of their cell walls. The Gram negative bacterial cell wall contain phospholipids and lipopolysaccharides which make it impermeable to hydrophilic substances (Rammali *et al*., 2022). The extent of the antimicrobial capabilities of organisms is dependent on the kind of secondary metabolite produced and relies ultimately, on its genetic machinery (Bjerketorp *et al*., 2021; Shukla, 2015). This may explain the variability noted in the antagonistic activities of the various Streptomyces species against the test bacteria.

Interestingly, the ethyl acetate extract of the metabolites of the promising Streptomyces strain, demonstrated better inhibitory property to the MDRB than sceptrin, chloramphenicol, ciprofloxacin, sparfloxacin gentamycin and streptomycin. This is most likely due to the occurrence of compounds in the extract with different chemical structure than these antibiotics. The extract was shown to contain 2-methyl-7-phenyl indole, 2,4,6, cycloheptatrien-1-one3,5, -bis-tri-methylsilyl 1,2 benzodiol,3,5-bis(1,1-dimethylethyl)- and benzo[h]quinolone,2,4-dimethyl. This result is consistent with those of other investigators who have reported a wide array of secondary metabolites with antimicrobial activities, produced by different species of Streptomyces (Ghanem *et al.,* 2022; Promnuan *et al*., 2020; Al-Ansari *et al*., 2019; Manimaran *et al*., 2017).

Literature survey has demonstrated that indole derivatives have diverse biological activities including anti-bacterial, anti-viral, anti-plasmodium, antioxidant, anti-inflammatory and anti-cancer (Seenivasan *et al*., 2022; Vicham *et al*., 2022; Kumar and Ritika, 2020; Arora *et al*., 2018; Ambrus *et al*., 2009). Therefore, the production of 2-methyl-7-phenyl indole as one of its secondary metabolites, may have contributed to the anti-bacterial tendency displayed by the *Streptomyces* sp. Str1. Also, some authors have demonstrated the effectiveness of synthesized 2,4,6, cycloheptatrien-1-one3,5,-bis-tri-methylsilyl as well as benzoquinoline and its derivatives against both Gram positive and Gram negative bacteria (Sani, 2022; Antoc *et al*., 2021; Haiba *et al*., 2016). Probably, these compounds which are more or less aromatic are likely responsible for

the broad-spectrum activity of the strain of *Streptomyces* assayed and its extract. Although, these compounds have been chemically synthesized and their antibacterial properties elucidated, they have not been previously reported in *Streptomyces* from lemon grass rhizosphere.

Finally, the study concludes that the *Streptomyces* sp. Str1 produced compounds with antibacterial properties against MDRB and thus is a potential candidate for the development of promising antibacterial agents. However, there is need for further research on the purification of these compounds for the determination of their mechanisms of action, *in vivo* activities including their pharmacokinetics, pharmacodynamics and cytotoxicity prior to their inclusion in the repertoire of bioactive substances produced by *Streptomyces* species.

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