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SCREENING FOR ANTIMICROBIAL POTENCY OF MICROORGANISMS ISOLATED FROM SOIL WITHIN THE PREMISES OF FEDERAL UNIVERSITY, OYE-EKITI, AGAINST TEST MICROORGANISMS

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ABSTRACT
The search for novel antimicrobials from soil has been on for a very long time and is still on. In a similar search, this study aimed at screening soil samples collected within the premises of Federal University, Oye-Ekiti, for microorganisms with antimicrobial potentials against test microorganisms. Eight soil samples were collected from different sites within the university and the inherent soil bacteria, fungi and actinomycete present were isolated. The isolates were cultured using Nutrient agar, Sabouraud dextrose agar and Glycerol Yeast Extract agar. They were then screened for their antimicrobial potency against selected test microorganisms: Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans using Agar well diffusion method. Seven bacterial genera: Azomonas sp, Bacillus sp, Escherichia sp, Pseudomonas sp, Gluconobacter sp, Micrococcus sp, and Staphylococcus sp; five fungal genera: Aspergillus sp, Mucor sp, Rhizopus sp, Rhodotorula sp, Trichoderma sp; and one actinomycete, Actinomyces sp, were isolated. Bacillus sp. was the only bacteria found to inhibit S. aureus and Pseudomonas aeruginosa with zones of inhibition, 14 and 12 mm, respectively. Rhizopus sp. inhibited S. aureus, with a 13 mm zone of inhibition, while the other fungal isolates did not inhibit any of the test microorganisms. Actinomyces sp. inhibited all the test microorganisms at different rates; 20 mm for S. aureus and P. aeruginosa, and 18 mm for C. albicans. The actinomycetes was seen to produce more antimicrobial potency since it inhibited the growth of all the test microorganisms and showed potential for further studies.
Keywords: soil, fungi, bacteria, actinomycetes, antimicrobial potency

INTRODUCTION

Antimicrobials are a popular and extensive research area. It describes a large group of chemical compounds, natural, synthetic or their derivatives, used to kill or inhibit microorganisms at minimal concentrations (Burnett-Boothroyd and McCarthy, 2011).

Since their discovery in the 20th century, antimicrobials have substantially reduced the threat of infectious diseases. This led to a revolution of medicine and, in combination with vaccination, steered medicine to the near eradication of diseases (Adedeji, 2016; Pfizer, 2022). In the 'golden era' of novel antimicrobial discovery, more than twenty (20) novel antimicrobial classes were discovered from a vast number of bacteria and fungi, and most of them were sourced from soil (Nicolaou and Rigol, 2018; Da Cunha *et al*., 2019). The fact that soil houses an immense number of microorganisms capable of synthesizing antimicrobials is well accepted, but the frequency with which the synthesis occurs at ecologically significant levels in nature has been much less clear, hence the need to screen for production of antimicrobials (Ahmed *et al*., 2013).

Over 5,000 antimicrobials have been identified from microbial sources but only about 100 of them have been commercially used to treat human, animal and plant diseases (Basavaraj *et al*., 2010). In history, the larger source of antimicrobials were from actinomycetes species, while much of the remaining were products of filamentous fungi and non-actinomycete bacteria (Singh and Mishra, 2013; Mast and Stegmann, 2019).

The increasing resistance of pathogenic microorganisms has led to severe forms of infection that are difficult to treat and poses great public health challenge (Peterson and Kaur, 2018). Infections caused by these resistant microorganisms result in prolonged illness, more expensive medicines and greater risk of death (WHO, 2021).

Treatment failures also lead to long periods of infectivity, which increase the number of infected people circulating in the community and thus exposes the population to the risk of contracting a multidrug-resistant strains. WHO estimates that more than 700,000 people die annually due to drug resistance and these figures could reach a whopping 10 million by the year 2050 (WHO, 2019). Most worrisome is that resistance to antibiotics is on the rise (WHO, 2021).

When microorganisms become resistant to first-line antimicrobials, treatment has to be changed to more effective antimicrobials such as second- or third- line antimicrobials (Prestinaci, *et al.*, 2015). This has necessitated deeper research into discovery of more effective antimicrobials. As the world's demand for antimicrobials grows steadily, so does intensive search for new antimicrobial due to the global scale menace of antimicrobial resistance creeping into every corner of the world, hence the need for this research. The aim of this study was to screen soils collected randomly within the university premises, for microorganisms with antimicrobial potentials against selected test microorganisms.

MATERIALS AND METHODS

Collection of soil samples

Eight soil samples, (presumed to be clayey, loamy, sandy and humus), were collected at selected locations from depths of 10cm using a sterile soil auger. One hundred grams of each soil samples, collected from different sites, were placed in labelled, sterile zip-lock bags and transported immediately to the laboratory.

Physical analysis

The pH of the various soil samples were determined using pH meter (Hanna HI 98107) and recorded. The moisture content was determined according to the method of Pepper and Gerba (2005). Soil identification was carried out as described by FAO (2020).

Isolation of microflora from soil sample

Serial dilution was carried out for the eight soil samples collected, according to the method of Sapkota *et al.*, (2020), and 0.1ml of dilutions were inoculated into petri dishes in triplicates using pour plate method. Media used were Nutrient agar, Sabouraud Dextrose agar and Glycerol Yeast Extract agar, prepared according to the manufacturer's specifications, for bacteria, fungi and actinomyces, respectively. The inoculated plates were incubated thus: at 37˚C for 24 hours for bacteria, at 25°C for 120 hours for fungi and at 30˚C for 48-72 hours for actinomycetes. Pure colonies were isolated by continuous culturing, stocked in double strength media and stored at 4°C till required.

Identification of Isolates

The bacterial and actinomyces isolates were characterized using colonial and morphological characteristics, and standard biochemical tests. The tests employed include motility, catalase, methyl red, Voges Proskaeur, urease, starch hydrolysis and sugar fermentation. The fungal isolates were identified using their morphological and microscopic characteristics. Lactophenol cotton blue stain was employed for microscopy, with reference to standard literature on fungal identification (Beneke and Rogers, 1980; Collins *et al.*, 1991).

Test microorganisms

The test microorganisms, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*, were obtained from the Drug Discovery Unit, Pharmaceutical Microbiology Division, Department of Microbiology, Federal University, Oye-Ekiti, Ekiti State. They were activated by growth in appropriate media for 24 hours.

Screening for inhibition by Agar well diffusion method

Assessment of the inhibitory effects of the isolates on test microorganisms was performed using Agar well diffusion method according to the method of Yilmaz *et al.* (2006). The identified microorganisms were investigated against test microorganisms: *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans.* All the isolates were grown in sterile Muller Hinton broth prepared in petri dishes under aseptic conditions. Two 8 mm wells were bored into solidified Muller Hinton agar in petri dishes, and 100 µl of crude extracts from isolates, grown in 24 hours culture broth, were gently introduced into the agar wells. The crude extract, obtained after centrifugation of culture broth (Muller Hinton broth), were loaded with micropipette into the wells.

The petri plates were incubated overnight without inverting, at optimum temperature of 37°C for 24 hours for bacteria, at 25°C for 120 hours for fungi and at 30˚C for 48-72 hours for actinomycetes. Standard antimicrobials were used as control for the experiment.

Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacet (20 µg), Amoxicillin (30 µg), Rocephin (30 µg), Tanvid (10 µg), Ciprofloxacin (10 µg), Streptomycin (30 µg), Septrin (30 µg), Augmentin (10 µg), Erythromycin (19 µg), Chloramphenicol (30 µg) and Sparfloxacin (10 µg) were used as control for bacteria and actinomycetes; Ketoconazole (10 µg) and Nystatin (50 µg) were used as control for fungi.

RESULTS

The soil types and locations of collection of soil samples, as well as the physical characteristics of the soil samples are shown in Table 1. Soil pH ranged from 6.20-9.81 while the moisture content was between 9.63 and 62.5%. Table 2 is a display of the biochemical and morphological properties of the bacterial and actinomycetes isolates. These characteristics aided in their identification. The different microorganisms and soil samples types from which they were isolated, are shown in Table 3. A greater number of isolates were found in humus soil than in other soil types. Assessment of the standard antimicrobials used as control against the test microorganisms are displayed in Table 4. The test microorganisms were seen to have multiple antimicrobial resistance to the controls. The assessment of the antimicrobial potency of the isolates against the test microorganisms is shown in Table 5. *Bacillus* sp was seen to inhibit the growth of both *P. aeruginosa* and *S. aureus,* with zones of inhibition, 14 and 12 mm, respectively*. Actinomyces* sp. inhibited all the test microorganisms at different rates; 20 mm for *P. aeruginosa* and *S. aureus*, and 18 mm for *C. albicans. Rhizopus* sp was the only fungi that inhibited the growth of *S. aureus,* with a 13 mm zone of inhibition, but it did not inhibit the growth of *P. aeruginosa* or *C. albicans.*

Table 1: Sites of sample collection and soil physical properties

Soil identification	Sites	Soil pH	Moisture Content (%)
Clay	School auditorium (SA)	7.00	9. 63
	Faculty of Science (FS)1	7.10	9. 61
Loamy	Faculty of Science (FS) 2	9.79	43.3
	Administrative building (AB)	9.81	43.0
Sandy	Faculty of Science (FS) 3	6.20	42.3
	Faculty of Social Science (FSS) 1	6.27	42.5
Humus	Faculty of Science dump site (FSD)	7.71	19.9
	Daycare centre dump site (DCD)	7.69	23.7

Table 2: Morphological and Biochemical characteristics of the bacterial and actinomyces isolates from soil

Morphological characteristics							Biochemical characteristics											
							Sugar Fermentation tests											
Shape	Elevation	Optical view	Motility	Spore test	Colour pigment	Gram reaction	Glucose	Lactose	Maltose	Inositol	Xylose	Catalase	Starch hydrolysis	Nitrate reduction	Methyl red	Voges Proskauer	Urease	Probable bacteria
ovoid	F	T	+	-	Yellowy-green	-	A	-	-	-	-	+	+	-	+	-	+	*Azomonas* sp
rod	R	O	+	-	pink	-	A	-	-	-	-	+	-	-	-	-	+	*Gluconobacter* sp
rod	R	T	-	+	White	+	AG	A	A	-	A	+	+	+	-	-	-	*Bacillus* sp
rod	R	T	-	-	White	-	AG	AG	A	AG	NA	-	+	+	+	-	-	*Escherichia* sp
cocci	R	T	-	-	Yellow	+	A	AG	A	NA	A	+	+	-	-	-	+	*Micrococcus* sp
rod	F	T	+	-	Bluish green	-	A	-	-	-	-	+	+	-	-	-	-	*Pseudomonas* sp
cocci	F	O	-	-	Yellow	+	A	A	A	NA	-	+	+	+	+	+	-	*Staphylococcus* sp
rod	R	O	-	-	Cream	+	A	+	+	A	+	-	-	+	+	-	-	*Actinomyces* sp

KEY

AG = Acid and gas production; A = Acid production only; G = Gas production only; - = No production of acid or gas, Negative reaction; + = Positive reaction; T – Transparent; O – Opaque; NA - Not available; F = Flat; R = Raised

Table 3: Isolates from the different soil types

Soil Type	Bacterial isolates	Fungal isolates	Actinomycetes isolates
Clay	*Azomonas* sp, *Gluconobacter* sp	*Rhizopus* sp, *Trichoderma* sp (C)	Nil
Loamy	*Bacillus* sp, *Escherichia* sp , *Micrococcus* sp	*Aspergillus* sp, *Mucor* sp (L), *Rhodotorula* sp (L)	Nil
Sandy	*Bacillus* sp, *Pseudomonas* sp, *Azomonas* sp	*Mucor* sp (S), *Rhodotorula* sp (S),	Nil
Humus	*Staphylococcus* sp I, *Escherichia* sp, *Bacillus* sp, *Staphylococcus* sp. II	*Rhodotorula* sp (H), *Mucor* sp (H), *Trichoderma* sp (H)	*Actinomyces* sp

Table 4: Standard antimicrobials control against test microorganisms

TEST MICRO-ORGANISMS	**Zones of inhibition (mm)**									
	PEF	**SXT**	**AM**	**CPX**	**S**	**R**	**Z**	**E**	**GN**	**APX**
S. aureus	14	8	8	15	8	11	8	10	8	8
	PEF	**SXT**	**AM**	**CPX**	**S**	**AU**	**GN**	**CH**	**OFX**	**SP**
P. aeruginosa	8	8	8	8	8	8	8	8	8	8
	KETOCONAZOLE					**NYSTATIN**				
Candida albicans	**8**					25				

KEY: 8 cm = No inhibition

PEF= Pefloxacin (10µg), GN= Gentamycin (10µg), APX= Ampiclox (30µg), Z= Zinnacet (20µg), AM= Amoxicillin (30µg), R= Rocephin (30µg), OFX= Tanvid (10µg), CPX= Ciprofloxacin (10µg), S= Streptomycin (30µg), SXT= Septrin (30µg), AU= Augmentin (10µg), E= Erythromycin (19µg), CH= Chloramphenicol (30µg), SP= Sparfloxacin (10µg), Ketoconazole (10 µg) and Nystatin (50 µg)

Table 5: Antimicrobial potency of isolates against test microorganisms

		Test microorganisms (mm)		
	Isolates from soil	*P. aeruginosa*	*S. aureus*	*C. albicans*
Bacterial isolates	*Azomonas* sp	8	8	8
	Gluconobacter sp	8	8	8
	Bacillus sp FS	11	12	8
	Escherichia sp	8	8	8
	Micrococcus sp	8	8	8
	Bacillus sp FSS	13	15	8
	Pseudomonas sp	8	8	8
	Azomonas sp	8	8	8
	Staphylococcus sp I	8	8	8
	Escherichia sp	8	8	8
	Bacillus sp DCD	12	14	8
	Staphylococcus sp II	8	8	8
Actinomycetes isolate	*Actinomyces* sp	20	20	18
Fungal isolates	*Rhizopus* sp	8	13	8
	Trichoderma sp	8	8	8
	Mucor sp (S)	8	8	8
	Rhodotorula sp (L)	8	8	8
	Mucor sp (L)	8	8	8
	Aspergillus sp	8	8	8
	Mucor sp (H)	8	8	8
	Rhodotorula sp (S)	8	8	8
	Trichoderma sp	8	8	8
	Rhodotorula sp (H)	8	8	8

Key: S=Sandy L=Loamy H=Humus 8 cm = No inhibition

DISCUSSION

Soil pH is influenced by mineral content, climate and soil texture and affects the activities within the soil including soil microorganism types and number and soil nutrients (USDA, 2014). The soil pH range in this study bordered around neutral values (6.20 - 9.81). This may have affected the types and number of microorganisms found therein, as bacteria and fungi thrive best at pH ranges of between 5.5 and 7.0 while a class of acid tolerant actinomycetes, like the species isolated in this study, grow well at pH 5.0 -8.0 (Tables 1 and 3) (Perry, 2003; Guo *et al*., 2015; Vylkova, 2017).

Percentage moisture content of soil was 9.63 – 62.5%. Soil moisture content also influence the type of microorganisms inherent in soils as well moist soils are known to hold a more diverse microbial community (Walker *et al*., 2003). In this study, the soil moisture content was highest with loamy soil and least with the clay soil (Table 1). This may account for the lower number of microorganisms found in clay soil (4), as against the other soil types (loamy-6, sandy-5 and humus-8) as excessively dry soils leads to lower number of microbes (Borowik and Wyszkowska, 2016).

The bacteria genera isolated from the different soils were *Azomonas, Bacillus, Escherichia, Gluconobacter, Pseudomonas* and *Staphylococcus* species (Table 2). *Azomonas, Escherichia* and *Staphylococcus* were isolated from twice from different locations while *Bacillus* was isolated three times. *Bacillus* sp. is a common soil bacterium that has been isolated from many different soils, as seen in different works (Salih *et al*., 2009; Yahya *et al*., 2021). Their possession of endospores enables them survive several adverse environments and spans of many years. Thus, their resilience accounts for their presence in a variety of soils (Nicholson, 2002; Nicholson *et al*., 2000). *Pseudomonas* and *Escherichia* spp. have also been isolated from many soils (Salih *et al*., 2009; Montealegere *et al*., 2018; Agarwal *et al*., 2021).

The fungal isolates, as shown in Table 3, were *Aspergillus, Mucor, Rhizopus, Rhodotorula* and *Trichoderma* species. *Mucor* and *Rhodotorula* were isolated three times from different locations while *Trichoderma* was isolated twice. Environmental fungi such as those obtained in this study, are commonly isolated from soils especially those with relatively high anthropogenic activities. Fungi flourish on organic matter in these soils, helped by their distinctive possession of degradative enzymes. They also form structures that help them thrive under adverse conditions like spores and sclerotia (Willets, 1971; Garcia-Rubio *et al*., 2015). These features help them survive long in favourable and unfavourable conditions.

One actinomycete was isolated from soil in this study (Table 2 and 3). Actinomycetes are usually found in soil, where they contribute to nutrient and organic matter cycling, inhibition of some plant pathogens and decomposition of complex polymers. Their general role in soil helps to improve soil health (Bhatti *et al*., 2017). The isolation of only one actinomycete in this study may be as a result of the type of soil, geographical location or organic matter content of the soil, as these factors affect the abundance and diversity of actinomycetes in the soil. Their relative availability in soils is also attributed to salinity, relative moisture, temperature, pH and vegetation in the soils (Ghorbani-Nasrabadi *et al*., 2013). Their resilient properties such as spore formation and adaptability to wide range of environmental conditions help them thrive in a large variety of soils (Trenozhinikova and Azizan, 2018).

The result of the standard antimicrobials against test bacteria revealed that the bacteria are multi-drug resistant species, given that they pose resistance to more than one antimicrobial (Magiorakos *et al*., 2012). This is part of the problem that must be tackled in the nearest future (WHO, 2021).Of the 12 bacteria isolated, only *Bacillus* sp showed any significant inhibition against test bacteria. *Bacillus* spp. have been recorded to produce antimicrobial effects against other bacteria (Yilmaz *et al*., 2006; Yahya *et al*., 2021). In this study, it inhibited *P. aeruginosa* and *S. aureus* but not the fungus, *C. albicans.* Yahya *et al*., (2021) had isolated a *Bacillus* strain that could inhibit *S. aureus, P. fluorecens* and *C. albicans.* The inhibitory effects of *Bacillus* has been attributed to its production of polypeptides which have been utilized in antibiotics like bacitracin and polymyxin (Yilmaz *et al*., 2006).

The fungus *Rhizopus,* showed inhibitory effect against *Staphylococcus*. *Rhizopus* species have been noted to show inhibition against some fungi and bacteria, including *Staphylococcus* and their inhibitory effects stem from production of metabolites including mycotoxins, aflatoxins B1, B2, G1 and G2 (Sohail *et al*., 2014, Yahaya, *et al*., 2017).

The only actinomycete isolated, *Actinomyces* sp., showed marked inhibitory effect against all test organisms. *Actinomyces* isolated was able to inhibit the growth of the test bacteria and fungus, more significantly than other isolates. Actinomycetes are regarded as an inexhaustible source of antimicrobials because many antimicrobials have been isolated from them (Mast and Stegmann, 2019). The antimicrobials derived from actinomycetes have a wide variety of active constituents classified as macrolides, bate-lactams, aminoglycosides, chloramphenicol, tetracyclines, rifamycins, ivermectins, among others (Raja and Prabakarana, 2011). Genome mining has discovered a large amount of secondary metabolite biosynthetic gene clusters (smBGCs) in actinomycetes, many of which are silent during their growth in the lab. This may account for their being a rich reservoir of antimicrobials (Kim *et al*., 2021).

CONCLUSION

Of all the bacteria and fungi isolated, *Bacillus, Rhizopus* and *Actinomyces* produced antimicrobial activities against the test microorganisms: *S. aureus, P. aeruginosa*, and *C. albicans,* at different rates. *Actinomyces*, which is an actinomycetes, produced a higher inhibition zone, indicating more effectiveness against the test microorganisms. The *Actinomyces* sp. in this study shows potential as a potent antimicrobial producer and is proposed as a candidate for further studies.

AUTHOR'S CONTRIBUTIONS:

NCI, FOF conceived and designed the study.
FOF, CJO conducted the experiments.
FOF wrote the first draft of the manuscript,
which was edited and approved by all authors.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

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