

GC-MS Analysis of Antibiotic-Producing Bacteria Isolated from The Soil Environment of Zamfara State

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ABSTRACT

Antibiotics produced by soil microorganisms, are crucial for treating bacterial and fungal diseases. Due to antibiotic resistance, a broad spectrum of antibiotics from bacteria is needed. Soil samples from Zamfara state were used for detecting antibiotic-producing bacteria. The study involved physico-chemical analysis, pour plate methods, biochemical tests and molecular characterization using 16s rRNA. Agar well diffusion method was employed to test isolates' ability to inhibit pathogens growth and their potential for antibiotic production. Antibiotics production was assessed through small-scale submerged fermentation condition. Compounds were extracted using solvent extraction method, thin layer chromatography and GC-MS analysis were used for separation and identification. Data analysis were done using SPSS and were presented as mean, standard error of mean, and Anova. The soil samples exhibited light brown to dark brown colors, indicating clay-loamy, loamy-sandy and sandy-loamy. Sample C had the highest pH (7.31 ± 0.03), Sample H had the highest temperature (26°C), sample D had the highest electrical conductivity ($108 \mu\text{s/m}$), and sample B had the highest moisture content (5.6). Six bacteria were identified and these include; *Bacillus cereus*, *Bacillus pumilus*, *Providencia stuartii*, *Providencia alcalificiens*, *Pseudomonas aeruginosa*, and *Micrococcus luteus*. All the organisms inhibited at least one pathogen, with *Bacillus cereus* showing the highest zone of inhibition. GC-MS analysis revealed diverse bioactive compounds namely, Carbonic acid, Glycerin, Nonadecane, 5-hydroxymethyl furfural, L-arabinitol, and Methylchloroformate. This study has demonstrated the diversity of bacterial population in the local soils and potential production of antibiotics that can be useful for many medical applications and further studies are recommended.

Keywords: Carbonic acid, Glycerin, Nonadecane, L-arabinitol, and GC-MS.

1.0 INTRODUCTION

Most people on the earth rely on natural compounds, which can be found in microorganisms, animals, marine life, and plants, for basic medical care and medication. Although these bioactive compounds have a wide range of chemical compositions, less than 10% of the world's biodiversity has had its biological activity potential evaluated (Karishna and Aparna, 2019). Accessing this naturally occurring compounds that may be beneficial remains a challenge. But research has revealed that soil is the easiest area to find antibiotic-producing microorganisms. Ananbeh *et al.* (2020) claim that a vast array of microorganisms can be found living in the extremely diverse environment, which is soil. Soils that present difficulties for the microbiota also show a great deal of diversity in both biotic and abiotic settings. Therefore, soil microbes have to adapt in order to live, and one such adaptation is the manufacture of antibiotics (Andrews, 2019). Thus, the soil has been the primary source of microorganisms that produce antibiotics (Andy and Okpo, 2018). The large concentration of

microorganisms under these conditions creates pressures that cause high levels of competition and extracellular product production among soil bacteria. Because of this, it has been discovered that soils are a substantial source of bacteria that are important for biotechnology, particularly those that produce antibiotics. Finding new producers of antibiotics is quite likely in such an environment.

Several thousands of antibiotics have been isolated from soil microorganisms since the discovery of penicillin, but unfortunately these have been limited to only fifty, most of them being too toxic to humans. According to the United state's food and drug administration approximately 80% of antibiotics produced from natural habitat are fed to the animals and only 20% of them are used to treat infections in the humans (Rafeeq *et al.*, 2019).

Despite the vast quantities of soil found in developing countries such as Nigeria, little is known about the bacteria that produce antibiotics from the soil

environment of Zamfara State. Finding any possible antibiotic-producing bacteria in the soil of Zamfara State was the aim of this investigation.

2.0 MATERIALS AND METHODS

2.1 Sample Site

Soil samples from Kotorkoshi and the surrounding communities were taken. The two most distinctive aspects of Kotorkoshi are its unspoiled natural environment and low-income populace. In the eastern section of Zamfara state, Kotorkoshi is located under Bungudu local government area and lies between latitudes 11°48'33.73"N and 8°50'39.19"E. According to estimates from the National Population Commission, Kotorkoshi had 183,162 inhabitants as of 2006. The area experiences 26 to 33°C annual temperatures and roughly 800-900 mm of rainfall on average. The primary source of income for the Kotorkoshi people is farming.

2.2 Collection of sample

At every sampling point, a top soil sample was obtained by means of two different sampling techniques: systematic random sampling at a minimum depth of four inches, (this is essential in order to obtain the indigenous soil bacteria who lie within the few inches of the soil and are devoid of any transient organism) and zigzag patterning. The samples were properly labeled with date, type of soil, including location and were immediately transported to Microbiology laboratory, Federal University Gusau, for determination of physicochemical parameters and isolation of antibiotic producing bacteria respectively.

2.3 Determination of Physicochemical Parameters of soil

We measured the electrical conductivity, moisture, color, texture, pH, and particle density using the method used by Isma'1 and Ahmed (2021). The mean of the three consecutive readings was then determined.

2.4 Sample preparation

Sterilized test tubes were acquired, marked, and tagged for each soil sample. After that, nine milliliters of deionized water were added. To make a soil suspension, 1g of each soil sample was put into test tubes together with 9ml of distilled water, and the mixture was vortexed. This solution was taken in one milliliter, transferred to the second test tube, vortexed, and then transferred to the third test tube. Each soil sample was so diluted one after the other.

2.5 Test Organisms

Escherichia coli and *Staphylococcus aureus*, the test organisms, were collected from stock cultures in the

Microbiology Laboratory of the Federal University of Gusau. They were produced at a density that satisfied the turbidity criteria set by McFarland (0.5).

2.6 Culturing of bacteria from the soil sample

Ten successive dilutions of the soil sample were performed. Using the pour plate method, 0.1 ml of each soil sample from the selected dilution was taken and placed onto labeled nutrient agar plates. On duplicate nutrient agar plates, the soil sample was inoculated. The Petri plates were then turned over and incubated for a full day at 37°C to produce pure colonies. The colonies obtained from the incubation were sub-cultured on freshly prepared nutrient agar plates using wire loop by streaking after a 24-hour incubation period at 37°C. To preserve each unique colony from the pure culture for additional analysis and identification, agar slants were utilized.

2.7 Identification of Bacteria from the soil sample

The method used by Ezebialu *et al.*, (2020) was used for the identification of the bacterial isolates. The identification was based on morphological description of colonies and Gram's reaction.

2.7.1 Biochemical Tests

Biochemical analysis such as, catalase test, coagulase test, sugar fermentation tests, urease test, oxidase test and citrate utilization test were performed for proper identification of bacterial isolates.

2.7.2 Molecular identification of Bacterial isolates

The isolates' genomic DNA was extracted using the Zymo Research Quick-DNA (Fungal/Bacteria Miniprep kit; catalog number D6005). This was completed in compliance with the manufacturer's guidelines. A 1% (w/v) Agarose gel electrophoresis was set up to assess the extracted DNA samples' purity. The following 36 cycles of polymerase chain reaction (PCR) amplification reactions were carried out in an Applied Biosystems GeneAmp PCR System 9700: First, denaturation at 94°C for five minutes; next, denaturation at 94°C for thirty seconds; annealing at 56°C for thirty seconds; extension at 72°C for forty-five seconds; and final extension at 72°C for seven minutes. On a 1.5% agarose gel, PCR amplicons were added and ran for 40 minutes at 100 volts. A 1 kb DNA ladder from New England Biolab was utilized.

2.8 PCR Products purification

The PCR tubes were filled with 20 µl of 100% ethanol, incubated for 15 minutes at room temperature, and then spun down for an additional 15 minutes at 10,000 rpm. The supernatant was then decanted, and 15 minutes of spinning at 10,000 rpm followed. The PCR tubes were

filled with 40 μ l of 70% ethanol, the supernatant was decanted, and the tubes were allowed to air dry. Ten microliters of ultrapure water were added to each PCR tube following air drying. Amplicon's presence was verified on a 1.5% agarose gel.

2.9 Sequencing

From the amplified products, the amplicons with a single band were chosen, and they were purified according to the manufacturer's instructions (QIAquick PCR Purification Kit, cat. No. 28106). Big Dye terminator sequencing kit (Applied BioSystems) was used for the sequencing process. Ethanol EDTA solution was used to purify and precipitate the unincorporated dye terminators. Next, the pellets were again dissolved in Applied Biosystems Cat. No. 4311320 HiDiformamide buffer. This sequencing was done with a 3130xl Genetic Analyzer.

2.10 Analysis of 16S rRNA

The following universal primers were used for the reaction 27F (16SF) – AGAGTTTGATCMTGGCTCAG and 1492R (16SR) +- CGGTTACCTTGTTACGACTT for both forward and reverse primers.

In order to find identical matches with already defined reference sequences, the 16S rRNA sequence was examined using the Basic local alignment search tool (BLAST), which is accessible from the National Center for Biotechnology Information website (<https://blast.ncbi.nlm.nih.gov>).

2.10.1 Protocol for PCR reaction composition

PCR Protocol with Master-mix (MO486S, New England Biolabs-NEB). All reaction components were assembled on ice and were quickly transferred to a preheated thermo-cycler to a denaturation temperature of (94°C).

Table 1: PCR Reaction Composition

Components	12.5 μ l	Final concentration
Master mix with standard buffer	6.25 μ l	1 \times
Forward primer (10 μ l)	0.25 μ l	0.2 μ m
Reverse primer (10 μ l)	0.25 μ l	0.2 μ m
Template	variable (2 μ l should contain appropriate conc.)	<1,000ng
Nuclease free water	up to 12.5 μ l.	

The reaction was gently mixed. The liquid was collected at the bottom of the tube after a quick spin. The PCR tubes were transferred to a PCR machine and thermo-cycling was carried out.

2.11 Antibacterial Susceptibility Testing

The agar well diffusion method was used to assess the antibacterial activity of the isolates. A comparison was made between the turbidity of the inoculum and the reference 0.5 McFarland solution. A sterile cotton swab was dipped into the suspension and streaked across the whole surface of the plate medium three times by rotating the plate at about 60°C in order to obtain equal dispersion of the inoculums (Sapkota *et al.*, 2020). A sterile cork borer (6 mm in diameter) was used to create the wells on a Mueller Hinton agar (MHA, India) plate. Ten microliters of the bacterial isolates were carefully added to each well, and after a 24-hour diffusion period, the wells were incubated at 37°C. After incubation, each well's surrounding inhibitory zones were observed (Sapkota *et al.*, 2022).

2.12 Antibacterial Compounds Production

To assess the potential for producing antibiotics, isolates exhibiting antibacterial activity after the first screening stage were placed in a small-scale

submerged fermentation condition. Bacterial cultures were suspended in a 250 milliliter Nutrient Broth. Subsequently, the broth cultures were agitated (inside an incubator machine) at 2,000 rpm for 14 days at 37°C (Seerivasan *et al.*, 2020).

2.13 Extraction of metabolite using solvent extraction method

After various changes, the metabolites were extracted for gas chromatography (GC) analysis using the technique used by Muhammed *et al.* (2012). The extraction process involved transferring the broth culture of bacterial isolates that had grown for 14 days into individual 500 milliliter Erlenmeyer flasks. After adding the culture filtrates to n-hexane and ethanol in an equal ratio (1:1), a vigorous shaking period of one hour was observed. Using Whatman No. 1 filter paper on a separator funnel, the contents of the flasks were filtered, separating the solvent phase from the aqueous phase which is assumed to contain antibiotic compounds. The antibiotics were then concentrated in the aqueous phase using a rotary evaporator (Seerivasan *et al.*, 2020).

2.14 Thin Layer Chromatography and Gas Chromatography Mass Spectrophotometry

The analysis of Thin Layer Chromatography and Gas Chromatograph-Mass Spectrophotometry were carried out at Multi-user Research Laboratory Ahmadu Bello University Zaria, Kaduna State, Nigeria.

2.14.1 Separation of Extract Using TLC

In the lab, thin layer chromatography plates of 20 cm by 20 cm were manually manufactured. It was possible to obtain solvent systems for the analytical TLC. Using the capillary tubes, microbial crude extracts were identified on the TLC plates. Following that, the spotted plates were put in the developing chamber, spotted end down. The solvent level was then adjusted to be below the spots, precisely where the pencil line was drawn. Through capillary action, the solvent then gradually ascended within the adsorbent. The TLC plates were taken out, and the location of the traveled solvent front was noted, once the solvent front had moved to within roughly 1 cm of the adsorbent's top end. After that, the TLC plates were observed using ultraviolet light with a 254 nm and 365 nm wave length.

2.15 Gas Chromatograph-Mass Spectrometry Analysis (GC-MS)

Using the agar overlay method, this machine was used to determine which of the separated compounds' active ingredients were present. The bioactive elements of the TLC fractions of each extracted extract were analyzed using a mass spectrophotometer using gas chromatography. The following parameters were used for the GC-MS analysis of the bioactive volatile compounds: pressure of 108.0 kPa, total flow of 6.2 Ml/min, column flow of 1.58 Ml/min, linear velocity

of 46.3 cm/sec, purge flow of 3.0 ML, split ratio of 1.0, column over temperature of 80.0 OC, injection temperature of 25.00 OC, injection mode split, and linear velocity control mode. Analysis of the mass spectrum The National Institute of Standard and Technology (NIST) database, which contains more than 62,000 patterns, was used for GC-MS analysis. The spectrum of the unknown components was compared with the spectrum of the known component stored in the NIST library.

2.16 Statistical analysis

Data was analyzed using SPSS version 25.0 software. While one way ANOVA was used to analyzed the variables. The statistical significant threshold was fixed at $P < 0.05$.

3.0 RESULTS

Table 2 showed results for the physicochemical parameters of soil samples. The color ranged from light brown to dark brown. The texture ranged from sandy loamy, clay and loamy sandy. Samples C, I, and F were found to have the highest mean pH, while sample H and B had the least mean pH. Samples B, H, and J had the highest mean temperature of 26, while, the least temperature was 25 which was recorded in sample A, C and E respectively. Mean electrical conductivities of 7.46 μ s/cm, 7.37 μ s/cm, and 105.90 μ s/cm, were seen in samples J, D, and I respectively. While, sample E had the least mean electrical conductivity. Samples B and E had the highest moisture contents of 14.69%, while the least mean of moisture content was seen in sample F with a mean of 2.231%.

Table 2: Physicochemical Properties of Soil Sample of the Different Locations of Zamfara State

Locations	Color	Texture	pH	Temp (°C)	EC (g/cm ³)	MC (%)
Kotorkoshi	Light brown	Sandy-loamy	5.43 \pm .45	25.00 \pm 1.00	79.20	4.384
Furfuri	Light brown	Sandy-loamy	5.41 \pm .38	25.66 \pm .57	73.11	5.679
Tsafe	Dark brown	Loamy-sandy	7.31 \pm .32	25.00 \pm .00	101.40	2.249
Mada	Light brown	Sandy-loamy	6.93 \pm .08	25.33 \pm 1.52	105.80	4.311
Jabaka	Dark brown	Sandy-loamy	7.05 \pm .14	25.00 \pm 2.00	72.90	5.62
Bela	Dark brown	Loamy-sandy	7.15 \pm .27	25.00 \pm 3.00	89.21	2.231
Dauran	Dark brown	Loamy-sandy	6.09 \pm .44	25.00 \pm 1.00	101.0	4.311
Dosara	Light brown	Sandy-loamy	5.30 \pm .40	26.00 \pm 2.00	71.11	2.231
Danfarko	Light brown	Sandy-loamy	7.12 \pm .22	25.33 \pm .57	101.23	4.678
Daki-takwas	Light brown	Sandy-loamy	7.12 \pm .12	25.31 \pm .33	108.90	2.571

pH =Hydrogen potential, EC = Electrical Conductivity, MC = Moisture Content, Anova P-Value=000.1
P<0.05

3.1 Morphological and biochemical identification of the bacterial isolates

Results for the morphological and biochemical identification of bacterial isolates is presented in Table 3. Six bacterial species were isolated and identified based on morphological description, gram's reaction and standard biochemical

tests such as catalase, coagulase, methyl red, Voges-proskauer, sugar fermentation and motility tests. *Bacillus cereus*, *Bacillus pumilus*, *Micrococcus luteus*, *Providencia stuartii*, *Providencia alcalifeciens*, and *Pseudomonas aeruginosa* were obtained.

Table 3: Morphological and Biochemical Identification of Bacterial Isolates

S/N	Morphology of the colony	Gram's reaction	CAT.	COA.	CIT.	MR.	VP.	MOT.	Indole	OX.	Urease	Presumptive organisms
1.	Irregular, rough, whitish colony	+ rods	+	-	-	+	+	+	-	-	+	<i>Bacillus cereus</i>
2.	Curator, pinhead, smooth	+ in chain	+	-	-	+	-	+	-	-	-	<i>Micrococcus luteus</i>
3.	Dull, circular, smooth	- small rods	+	-	-	-	+	+	-	-	+	<i>Providencia stuartii</i>
4.	Irregular, whitish to creamy	+rods	+	-	-	+	+	+	-	-	+	<i>Bacillus pumilus</i>
5.	Oval, greenish, mucoid	- rods	+	-	+	-	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
6.	Circular, smooth, creamy	-small rods	+	-	-	+	-	+	-	+	-	<i>Providencia alcalifeciens</i>

CAT = Catalase, OX = Oxidase, MOT= Motility, MR = Methyl red, COA=Coagulase, Cit= Citrate, VP=Voges-Proskauer

3.2 Molecular Characterization of bacterial isolates

Table 4 shows the blast Analysis of the sequences generated from the bacterial isolates. Six (6) different bacterial isolates were used for the molecular characterization of the isolated bacteria. The DNA was successfully isolated from the suspected isolates after which the extracted DNA was used for polymerase chain reaction (PCR). The success of the PCR was confirmed by agarose gel electrophoresis and all amplicons produced a band of approximately 1000 bp which is expected for the amplified gene. The result of the sequence analysis showed that the six samples were of high quality (*Providencia stuartii*, *Providencia alcalifeciens*, *Bacillus cereus*, *Bacillus pumilus*, *Micrococcus luteus*, and *Pseudomonas aeruginosa*).

Table 4: Blast Outputs of Total Score, Percentage Identity, and Accession Number Obtained From the Isolate Sequence

Isolate codes	Top hit from the NCBI Data base	Accession No.	% Identity	Total hit score
NS ₁	<i>Bacillus cereus</i> strain Kks1	PP858083	100.00	2220
NS ₂	<i>Bacillus pumilus</i> strain Kks2	PP858086	100.00	101
NS ₃	<i>Providencia stuartii</i> strain Kks3	PP858089	100.00	1167
NS ₄	<i>Providencia alcalifeciens</i> strain Kks4	PP858093	99.00	110
NS ₅	<i>Pseudomonas aeruginosa</i> strain Kks5	PP783530	100.00	2220
NS ₆	<i>Micrococcus luteus</i> strain Kks6	PP858095	100.00	1101

3.3 Antibacterial Activity of the bacterial isolates against some test pathogens

The bacteria isolated were tested against some test pathogens viz: *Staphylococcus aureus* and *Escherichia coli* to determine their sensitivity and resistance pattern using agar well diffusion method. *E. coli* had the highest (18mm) susceptibility to metabolite produce by *Bacillus cereus* and the least susceptibility to metabolites produced by *Pseudomonas aeruginosa* with inhibition zone of (13mm). Metabolites produced by *Providencia stuartii* had the second highest zone of inhibition of (17mm) against *Staphylococcus aureus*. While, the control, Ciprofloxacin (250mg/ml) produced the inhibition zones of 20mm and 18mm respectively.

Table 5: Zones of Inhibition by Different Bacterial Isolates against the Test Organism

Isolates	Zones of inhibitions (mm) of the isolates against the test bacteria		
	<i>S. aureus</i> (%)	<i>E. Coli</i> (%)	Anova p-value

<i>Bacillus cereus</i>	18.01±1.0	16.00±1.0	0.000
<i>Providencia stuartii</i>	17.01±1.0	14.63±06	
<i>Micrococcus luteus</i>	15.00±1.0	16.03±0.5	
<i>Bacillus pumilus</i>	16.01±1.0	15.33±1.5	
<i>Pseudomonas aeruginosa</i>	16.00±1.0	13.00±1.05	
<i>Providencia alcalifeciens</i>	16.00±1.0	13.67±0.5	
Positive control	20.00±1.0	18.00±0.1	
Negative control	00.00±0.0	00.00±0.0	

p≥0.05, Positive control= Ciprofloxacin, Negative control= Dimethyl sulfoxide

3.4 Gas Chromatography-Mass Spectrophotometry Analysis of Bacterial Extracts

Table 6 showed the result of GC-MS analysis of N-hexane extracts of Bacterial isolates exhibiting the presence of bioactive compounds. The presence of several compounds with known biological activity was found in the bacterial extracts. The identification of bioactive chemical compounds is based on the peak area, retention time, molecular weight and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of bacterial isolates revealed the presence of Carbonic acid, Glycerin, Nonadecane, 5-hydroxymethyl furfural, L-arabinitol, and Methylchloroformate.

Table 6: Compounds Present in Fractions Produced by Bacterial Isolates Analyzed by GC-MS

Isolates	Peak area %	Retention time	Compound name	Molecular formula	Molecular structure
<i>Bacillus cereus</i>	0.13	6.534	Carbonic Acid	H ₂ CO ₃	
<i>Providencia stuartii</i>	4.36	8.503	Glycerin	C ₃ H ₈ O ₃	<p>Glycerol also referred to as Glycerin</p>
<i>Micrococcus luteus</i>	0.13	9.0902	Nonadecane	C ₁₉ H ₄₀	
<i>Bacillus pumilus</i>	7.0	9.392	5-Hydroxymethyl Furfural	C ₆ H ₆ O ₃	
<i>Pseudomonas aeruginosa</i>	2.267	6.267	L-Arabinitol	C ₅ H ₁₂ O ₅	

4.0 DISCUSSION

The microorganisms present in soil are the most significant group on Earth, because they generate biological components that are active and useful to humanity in a variety of ways (Niazi *et al.*, 2022). The results of the physicochemical properties of the soil

samples in this research region show that there is a notable variation between the soil samples in several parts of Zamfara state. Soil structure is the arrangement of soil particles into aggregates. These could come in various shapes, sizes, and developmental or expression stages (Kekane, 2015).

This research site's diverse range of soil types and textures could be the reason behind variations in temperature, pH, electrical conductivity, moisture content, and porosity. In the present study, the pH values of the soil samples showed that sample A was the most acidic with pH of 5.8, followed by sample H with a pH of 5.7. While sample B had the least acidic pH of 5.6. Sample G had a normal pH of 6.5, followed by sample J with a normal pH of 6.7, and sample D with a normal pH of 6.9. Samples E, I, C and F all had a normal soil pH of 7.0, 7.31, 7.4, and 7.4 respectively. This finding is in agreement with the study of Niaz *et al.* (2022) who reported that normal to alkaline pH are the favorite conditions for bacterial growth, and mostly in normal pH, bacterial growth has positive correlation with diversity, richness, bacterial community, and composition of soil bacteria. There are many factors that influence the soil microbial population and activity, viz; soil pH, temperature, color and texture are the most significant properties of the soil which affect soil electrical conductivities, particle density, moisture content and organic contents (Isma'il, and Ahmed 2021).

During the rainy season, when this study was conducted, the temperature of the soil environs in Zamfara was found to range between 25⁰ and 26⁰ degrees Celsius. The findings of Kekane (2015), who reported that soil temperature varies with season, time of day, and local climate conditions, support this conclusion. One of the most significant characteristics of soil is its temperature, which affects the physical, chemical, and biological processes involved in the development of native microorganisms (Andrews, 2019).

Sample D had the highest electrical conductivity of 108, sample J had the second highest at 105, sample G and I both had 101, and sample H had the lowest at 71. This study's report concurs with Ismail and Ahmed's (2021) findings, which indicated a maximum electrical conductivity of 108. The two investigations were conducted in the same geographic location (Northwestern Nigeria) and during the same season (the rainy season), which may account for the parallels between them.

The study's findings on moisture content vary depending on the location; however, samples B, E, and I had higher moisture content (5.67%, 5.62%, and 4.67%, respectively). However, the rate was only approximately 2% in samples H, F, and C. The clay-loamy soil types found in these areas may be the cause of the greatest moisture contents found in the aforementioned samples. Unlike sandy soil, which has a weak water holding capacity and consequently reduced moisture content, clay

soil has a good water holding capacity due to the aggregates of particles that make up the soil.

The findings in this study are similar to the findings reported by Nadia *et al.*, (2015) in Algeria. The similarities observed among the two studies may also be related to the same vegetation cover in these areas. Because, the roots play an important role in the exchange and maintenance of plants-animal symbiosis in the soil. Morphological and biochemical characterization of isolates were carried out as a tool for preliminary identification of bacteria and it is a conventional method followed by Microbiologists all over the world (Sitotaw *et al.* 2021).

Following their isolation, the bacteria strains were identified by biochemical characterization tests and methods such as gram staining. *Bacillus cereus*, *Bacillus pumilus*, *Providencia stuartii*, *Providencia alcalifaciens*, *Pseudomonas aeruginosa*, and *Micrococcus luteus* were identified using molecular analysis and sequence alignment of the isolates (Adelrafeeq *et al.*, 2019; Ezebialu *et al.*, 2020). This is also consistent with the well-known soil dwellers, *Bacillus* species (Sitotaw *et al.*, 2021). There are several explanations for the soil's prevalence of *Bacillus* species. Because of their endospores, *Bacillus* species can withstand harsh chemical and physical conditions and produce compounds that have antagonizing effects on other microorganisms. They also exhibit a wide range of physiological capabilities (Camides *et al.*, 2020).

These characteristics enable the organism to survive and thrive in a wide range of environments, including the selection pressure that pollution exerts on some soil bacterial species. In a similar vein, *Pseudomonas* is a widespread genus that inhabits a range of habitats.

Providencia sp. members are widely distributed in the environment and are part of the typical bacterial flora of the intestinal tracts of people and animals (Ezebialu *et al.*, 2020). *Providencia* species, which belong to the enterobacteriaceae family, have been detected in soil, feces, and urine. They are known to invade the intestine (Ananbeh, 2020). The presence of these microorganisms in the soil, as revealed by earlier and ongoing investigations, may be related to the municipal waste dumping site's discharge of complex wastes from multiple sources (Camides *et al.*, 2020). Additionally, *Bacillus* spp., *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *S. aureus*, and *Streptococcus* spp. were isolated from dumpsites in the Nigerian city of Port Harcourt by Williams and Hakam (2016). The isolates exhibited antibacterial activity against both Gram-positive and Gram-negative test bacteria, according to the findings of the first screening stage. With some variations in culture traits, the isolates exhibiting

antimicrobial activity are *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Providencia stuartii*, *Bacillus cereus*, and *Providencia pumilus*. The isolates demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, as shown in Table 5. These varied antagonistic characteristics in vitro might result from the isolates' various mechanisms of action against test microorganisms. The fact that the bacterial isolates exhibited antimicrobial activity against every pathogen suggests that these bacteria secrete some kind of antimicrobial materials or substances that inhibit the test organisms.

Bacillus pumilus was found to inhibit *Staphylococcus aureus* and *E. coli* with inhibition zones of 16 mm and 15 mm, while *Bacillus cereus* was found to inhibit both bacteria with inhibition zones of 18 mm and 16 mm. The two *Providencia* species displayed differing degrees of inhibition. It was discovered that *Providencia alcalifeciens* inhibited *E. coli* and *Staphylococcus aureus* with inhibition zones of 16 mm and 13 mm, respectively, whereas *Providencia stuartii* produced inhibition zones of 17 mm and 13 mm. A 15-millimeter zone of inhibition was produced by *Micrococcus luteus* against *Escherichia coli* and 16-millimeter zone of inhibition against *Staphylococcus aureus*. *Pseudomonas aeruginosa* produced inhibition zones measuring 16 mm and 15 mm, respectively, against *Escherichia coli* and *Staphylococcus aureus*. According to the current investigation, *Bacillus* species were predominant in demonstrating antibiotic action against *Staphylococcus aureus* and *Escherichia coli*. The results of Ahmad and Isma'il (2021), who examined soil microorganisms for the production of antibiotics, support this conclusion. Given that numerous dynamic systems influence the growth inhibition zone's size and location. According to Kaur *et al.*, (2020), comparing antibiotic agents based solely on the extent of their inhibition zones is dubious.

The presence of carbonic acid, glycerin, nonadecane, 5-hydroxymethyl furfural, L-arabinitol, methyl chloroformate, and other derivatives was shown by the GC-MS analysis, which was carried out to determine the bioactive chemicals of the isolated bacterial extracts. According to earlier research, the aforementioned substances have antibacterial properties (Kaur *et al.*, 2020). The chemical formulas and structures of some significant substances included in bacterial samples were shown in Table 6.

The retention time and peak area of the GC-MS measurement were used to identify the chemicals. It is commonly known that *Bacillus* species have the ability to produce secondary metabolites that are

antibiotics. The results of this investigation are consistent with multiple prior scientific publications stating that *Bacillus* species generate over 800 bioactive secondary metabolites, some of which are employed as pharmaceutical drugs (De Simeis and Serra, 2021).

According to reports, genes involved in the synthesis of antibiotics and the degradation of various carbon sources are carried by *Bacillus* species and other spore-forming bacteria (Prescott *et al.*, 2008). *Bacillus* species are widely recognized for their extraordinary diversity in structure and function when it comes to the synthesis of secondary metabolites (De Simeis and Serra, 2021).

Although *Providencia* species are not known to produce antibiotics (Ezebialu *et al.*, 2020), they demonstrated antibacterial activity in the current investigation by generating substances that give them these potentials. Geographical location and a number of other factors have been shown to vary antibiotic-producing bacterial strains with different bioactive chemicals (Adelrafeeq *et al.*, 2019).

Pseudomonas aeruginosa and *Micrococcus luteus* were also found to have antibiotic action in the current investigation. These bacteria produced secondary metabolites that prevented the growth of certain test pathogens. This result is consistent with that of Sapkota *et al.*, (2020), who in his work isolated and identified the previously described bacteria. The findings of Isma'il and Ahmad (2021), who tested soil for microbes that produced antibiotics, further support this conclusion. The findings may contribute to a better understanding of the differences in antibiotic-producing bacterial strains and their varied bioactive secondary metabolites resulting from geographic variances and nutritional availability. The current research's findings demonstrated that *Bacillus* species have the capacity to generate a range of bioactive secondary metabolites that are effective against a wide spectrum of microbial growth in different conditions.

5.0 CONCLUSION

The results of the screening analysis carried out in this study, have shown that soils of Kotorkoshi and its environs do harbor antibiotic producing bacteria capable of producing antibiotics against some major human pathogens. This study has demonstrated, that both narrow and broad spectrum antibiotic-producing bacteria may be recovered from the soils environment of Zamfara State. Morphological, biochemical and molecular analysis revealed them as *Bacillus cereus*, *Bacillus pumilus*, *Providencia stuartii*, *Providencia alcalifeciens*, *Micrococcus luteus* and *Pseudomonas aeruginosa*. This study has demonstrated that production of antibiotics is widespread among these bacteria, almost 80% of the

isolates exhibited antibacterial activity against one or more indicator organisms. Furthermore, the GC-MS analysis hinted at the presence of bioactive metabolites such as Carbonic acid, Glycerin, Nonadecane, 5-hydroxymethyl furfural, L-arabinitol, Methyl chloroformate, which are known for various pharmaceutical applications.

6.0 RECOMMENDATION

New magnetic resonance techniques should be employed for further purification of these compounds with antibiotic activities produced by these bacteria.

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