

Hydrocarbon Degrading Potentials and Antibiotic Susceptibility Test of Bacteria Isolated from Amended Soil in Ilorin

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ABSTRACT

This study used bacteria from hydrocarbon-contaminated soil that had been treated. Organic amendments included cassava peels, plantain peels, eggshells, and poultry droppings, while inorganic amendments included NPK and Urea. In this study, three (3) bacteria were characterized and dealt with: *Bacillus amyloliquefaciens*, *Bacillus proteolyticus*, and *Providencia rettgeri*. To determine their biodegradative capabilities, these isolates were tested on a variety of hydrocarbons. In addition, the antibacterial activity of the bacteria was tested *in vitro* against extracts of *Moringa oleifera*, *Vernonia amygdalina* (Bitter leaf), *Ocimum gratissimum* (Scent leaf), and *Telfairia occidentalis* (Ugwu) at different concentrations (20%, 13%, 10%, 7%, and 3%). Using the maceration procedure, the plant materials were extracted with ethanol and methanol. The bacteria were resistant to the various concentrations of plant extracts, according to the results. The methanolic and ethanolic extracts of *Vernonia amygdalina* (bitter leaf) had the maximum inhibition zone, measuring 6.00 ± 0.00 mm. Furthermore, all isolates were resistant to crude oil, engine oil, heptane, and benzene in antimicrobial tests on hydrocarbons, with *Bacillus amyloliquefaciens* having the highest level of susceptibility (18 mm) to pyridine. However, the bacteria all demonstrated broad spectrum susceptibility to the commercial antibiotics used as a control, with the exception of *Providencia rettgeri*, which proved resistant (5 mm) to Ampiclox (APX), Zinnacef (Z) and Amoxicillin (AM).

Keywords: Antimicrobial susceptibility, Biodegradation, Hydrocarbons, Plant extracts, Soil

Introduction

Petroleum and its by-products pollute the environment as a result of inappropriate waste management, poor effluent and wastewater disposal from companies and dwellings, as well as other man-made activities (Aluko *et al.*, 2018). Oil

discovery and use, fueled by the rapid rate of industrialization and has resulted in increased pollution, which has invariably wreaked havoc on the ecology (Alam *et al.*, 2018). Contaminants build up in the soil, are absorbed by plant roots,

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seep into surface and ground water, and disperse as aerosolized pollutants into the air, all of which are harmful to human and animal health (Ghaemi *et al.*, 2015; Edyta and Dariusz, 2020). Contaminants such as petroleum hydrocarbons and heavy metals are extremely harmful to the environment, and human exposure to these dangerous substances can have serious health repercussions as well as change the socioeconomic activities of a community (Umana *et al.*, 2016; Aniefiok and Udo, 2019).

The importance of ecosystem replenishment is gaining attraction around the world. One example is the emphasis on bioremediation of contaminated sites. Microbial actions are used in this procedure, which is a multi-phased process (Adams *et al.*, 2015). Because it does not disrupt or transform the trophic chain in the de-contaminated ecosystem, bioremediation has earned global reputation as an environmentally friendly approach. Contaminants can be converted to simple chemicals by microorganisms, making them non-pollutants (Basheer, 2018). As a result, using bacteria or fungus to break down environmental contaminants has become a practical and economically acceptable strategy for successful biodegradation (Łukasz *et al.*, 2020).

Plants, man, and animals have interacted since the dawn of time as food, shelter, drugs, and oxygen are derived from plants (Hamid *et al.*, 2016; Fonmboh *et al.*, 2020). Phytochemicals such as alkaloids, steroids, flavonoids, saponins, tannins, starch, and other natural bioactive substances present in plants, particularly leaves, stems, roots, and fruits (Rungsung *et al.*, 2015) are widely used in agriculture, used as cures, and used in scientific research. As a result of their natural origins, ease of availability and accessibility in local communities, low cost of purchase and ease of administration, plants particularly those of ethno-pharmacological relevance have achieved international popularity and acceptability (Abdullahi and Mainul, 2020).

As a result, the potential of bacteria isolated from polluted soil to breakdown hydrocarbons is highlighted in this research. This research also aims to determine the significance of plants as antimicrobial agents by demonstrating the basis for their therapeutic use by analyzing antimicrobial susceptibility patterns of selected isolates.

Materials and Methods

Soil was gathered in clean polythene bags at various sites within the Department of Microbiology, University of Ilorin and sent to the laboratory for further investigation. Before being contaminated with spent engine oil, the soil sample was filtered with a 2 mm sieve to remove stones, sticks, and other debris. Organic amendments (cassava peels, plantain peels, eggshells, and poultry droppings) were also properly sun-dried, ground with a grinder, and filtered with a 2 mm sieve. Urea and NPK were used as inorganic additives. Table 1 shows the soil microcosm.

Table 1: Experimental Design of Soil Microcosm

Treatments	Details of Treatment
A	100 g soil +6 % oil + 12 % (w/w) P
B	100 g soil +6 % oil + 12 % (w/w) E
C	100 g soil +6 % oil + 12 % (w/w) C
D	100 g soil +6 % oil + 12 % (w/w) PD
E	100 g soil +6 % oil + 12 % (w/w) U
F	100 g soil +6 % oil + 12 % (w/w) N
G	100 g soil +6 % oil + 6 % (w/w) U + all organic amendments
H	100 g soil +6 % oil + 6 % (w/w) N + all organic amendments
I	100 g soil +6 % oil + 4 % (w/w) All amendments
J	100 g soil +6 % oil (Unamended soil)
K	100 g soil (Control)

Legend: P = Plantain peels, E = Eggshells, C=Cassava peels, PD= Poultry droppings, U=Urea, N = NPK

To count total heterotrophic bacteria, soil samples from each soil set-up were taken every 14 days for a total of 42 days.

Plant Collection

The plant samples (*Telfairia occidentalis* – Ugwu, *Ocimum gratissimum* – Scent Leaf, *Vernonia amygdalina* – Bitter Leaf, and *Moringa oleifera*) were collected in Ilorin, Kwara State and identified at the Department of Plant Biology, University of Ilorin. The leaves were washed to eliminate contaminants before being air-dried for 5 days at room temperature and then pulverized using a blender.

Plant Extract Preparation

A mass of 25 g of each pulverized plant material was weighed into sterile stoppered volumetric flasks containing 200 ml of 98 % ethanol. The flasks were adequately covered and shaken for five (5) days to ensure continual agitation and homogenization of the contents. The resulting extractant/formulation was filtered with a Whatman No.1 filter paper after passing through a muslin/cheese cloth. The residue was then filtered after being reconstituted in 100 ml of 98 % ethanol. The extract was concentrated in a water bath at 50 °C before being used in the experiment as described by Awe and Omojasola (2003) with slight modifications.

Likewise, the methanolic extraction was also prepared using the aforesaid approach and as explained by Mashiar *et al.* (2009).

Determination of pH and Phytochemical Screening of Plant Extracts

The pH of the extracts was evaluated by calibrating a pH meter with pH buffers 4.0 and 7.0 before dipping the pH probe into the extracts. The average values were recorded after taking duplicate readings. In addition, qualitative screening of both methanolic and ethanolic plant extracts for various phytochemicals was carried out using the methodologies of Mohammed *et al.* (2014) and Dixon and Jeena (2017).

Isolation, Enumeration and Characterization of Isolates

A volume of 100 µl was measured from the 10⁻⁴ serially diluted soil samples and placed into correctly labeled Petri dishes followed by the addition of sterile molten Nutrient Agar. The plates were allowed to solidify before being inverted and incubated for 24 hours at 37 °C. Following that, using Bergey's Manual of Determinative Bacteriology, the colonies were counted and isolates were properly identified and characterized (Holt *et al.*, 1994). Gram staining was carried out according to the protocol of Fawole and Oso (2007) with minor modifications. Molecular analysis was carried out for proper identification by analyzing the nucleotide sequence codes of the isolates in the National Center for Biotechnology Information (NCBI) database using the BLASTn algorithm.

Assay of Plant Extracts, Hydrocarbon and Antibiotics

The antibacterial activity of the extracts and the antimicrobial susceptibility of the isolates were evaluated using the agar diffusion method on Mueller Hinton Agar. Using sterile swab sticks, overnight broth cultures of the test organisms were swabbed on solidified sterile Mueller Hinton Agar on Petri dishes. A sterile cork borer was used to cut five (5) circular wells (10 mm in diameter) in the media. 100 µl of the relevant leaf extract concentrations (20%, 13%, 10%, 7%, and 3%) were measured and dispensed into correctly labeled Petri dishes using a sterile micropipette. To allow the extracts to thoroughly diffuse into the agar, the plates were left on the workbench for 1 hour. Plates were incubated upright for 24 hours at 37°C following which the zones of inhibition were measured to the nearest diameter along two axes of 90° to each other and average values were recorded (Omotayo, 1998).

Using sterile swab sticks, overnight broth cultures of the test organisms were swabbed on sterile Mueller Hinton Agar in Petri dishes. A circular

ditch was cut in the middle of the plates with a sterile cork borer of 10 mm diameter. A volume of 70 μl of the various hydrocarbons was measured into correctly designated Petri dishes using a sterile micropipette. To allow the hydrocarbons to diffuse properly into the agar, the plates were left on the workbench for 1 hour. Plates were incubated upright for 24 hours at 37 $^{\circ}\text{C}$, following which the zones of inhibition were measured to the nearest diameter along two axes of 90 $^{\circ}\text{C}$ each, and the mean of the results was calculated. The plating was done twice and the average readings were taken.

Standard antibiotic discs impregnated with various antibiotics were used to conduct an antibiotic susceptibility test. Using sterile swab sticks, overnight broth cultures of the test organisms were swabbed on sterile Mueller Hinton Agar in Petri dishes. A ditch was formed in the centre of plates using a sterile cork borer of 10 mm diameter and 100 μl of the extraction solvent (methanol and ethanol) was put into correctly labeled ditches using sterile micropipettes. To allow the extracts to thoroughly diffuse into the agar, the plates were left on the workbench for 1 hour. The antibiotic-impregnated discs were then placed on the agar surface with sterile forceps to ensure complete contact with the agar before being incubated at 37 $^{\circ}\text{C}$ for 24 hours. Sensitive (S), moderately sensitive (MS), and resistant (R) inhibition zones were measured to the closest millimeters and recorded.

Statistical Analysis

Using SPSS 16 software, the group mean and standard deviation (SD) were calculated and significant differences between the groups were evaluated. The statistical significance of the difference between the groups was determined using one-way analysis of variance (ANOVA) and Duncan's multiple range test at a 95 % confidence level for all measurements. P values < 0.05 were considered significant.

Results

Prior to contamination with spent engine oil, physiochemical analysis of the experimental soil and organic amendments revealed that the soil had a pH of 6.5 and a sandy loam texture when assessed on the textural triangle. The pH of the organic amendments on the other hand varied depending on their individual contents as shown in Table 2.

As indicated in Table 3, the study counted the number of bacterial colonies obtained from the microcosm setup. On Day 14, plantain peels yielded the greatest bacterial count of 6.11 ± 0.08 cfu ml^{-1} while unamended soil yielded the lowest value of 2.70 ± 2.36 cfu ml^{-1} on Day 0. In addition, soil control values rose from 2.87 ± 2.50 cfu ml^{-1} at the end of Day 0 to 4.91 ± 0.40 cfu ml^{-1} at the end of Day 42. From the result, the biodegradation of the soil containing plantain peels revealed that the values obtained on Day 0, Day 14, and Day 42 were not significantly different. On Day 28, however, there was a significant difference of 4.63 ± 0.51 cfu ml^{-1} . Microbial counts derived from NPK amendment increased from 4.91 ± 0.38 cfu ml^{-1} on Day 0 to 5.94 ± 0.21 cfu ml^{-1} on Day 28, but then decreased to 4.57 ± 0.26 cfu ml^{-1} on Day 42.

Figure 1 depicts the pH readings of the plant extracts. The methanolic extract of ugwu (U_M) had the highest reading of 6.7, while the ethanolic extract of bitter leaf (B_E) had the lowest reading of 6.4. Also, Tables 4 and 5 show the findings of the phytochemical screening of methanolic and ethanolic plant extracts. Only *Moringa oleifera* extracts contained all phytochemicals (saponins, alkaloids, steroids, phlobatanins, and tannins) whereas, other plant extracts had different phytochemicals.

Table 2: Physiochemical Analysis of the Experimental Soil and Organic Amendments prior to Contamination

Sample	pH	N (%)	Organic carbon (%)	Organic matter (%)	Sand (%)	Silt (%)	Clay (%)	Ca (Cmol/kg)	Mg (Cmol/kg)	Na (Cmol/kg)	K (Cmol/kg)	Acidity (Cmol/kg)	P (mg/kg)	C.E.C
Soil	6.5	0.63	2.19	3.78	79.96	10.00	10.04	2.60	1.20	0.16	0.31	0.1	3.01	4.37
Cassava peels	6.2	1.05	4.28	7.40	NA	NA	NA	1.24	0.37	0.59	3.42	0.4	11.5	9.16
Plantain peels	4.5	1.47	4.37	7.55	NA	NA	NA	4.38	1.49	0.46	2.18	0.3	4.8	10.58
Egg shells	7.1	0.98	3.01	5.20	NA	NA	NA	6.15	NA	NA	1.22	0.1	4.6	2.56
Poultry Droppings	6.7	1.05	4.52	7.81	NA	NA	NA	4.11	2.17	1.28	2.66	0.5	7.2	10.72

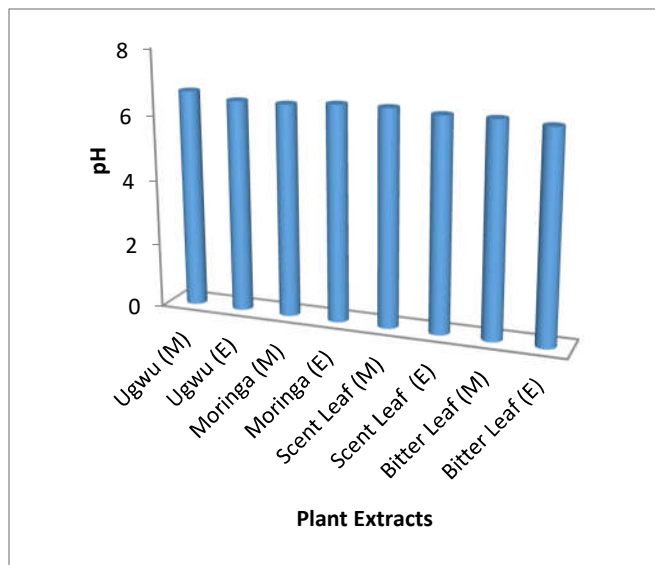
Legend: NA = Not Applicable

Table 3: Enumeration of bacterial counts (Cfu ml⁻¹ in Log₁₀) from soil microcosm

Samples	Day 0	Day 14	Day 28	Day 42
E	5.20 ± 0.41 ^a	5.69 ± 0.44 ^{a,b}	5.02 ± 0.33 ^{a,b,c}	4.61 ± 0.19 ^{c,d}
P	5.15 ± 0.53 ^a	6.11 ± 0.08 ^a	4.63 ± 0.51 ^c	5.53 ± 0.62 ^a
C	5.07 ± 0.37 ^a	4.13 ± 2.91 ^b	5.46 ± 0.49 ^{a,b,c}	5.02 ± 0.51 ^{a,b,c,d}
PD	5.82 ± 0.34 ^a	6.10 ± 0.05 ^a	4.71 ± 0.31 ^c	5.39 ± 0.33 ^{a,b}
U	5.47 ± 0.27 ^a	5.18 ± 0.68 ^{a,b}	4.93 ± 0.33 ^{a,b,c}	5.50 ± 0.09 ^a
N	4.91 ± 0.38 ^a	5.25 ± 0.14 ^{a,b}	5.94 ± 0.21 ^a	4.57 ± 0.26 ^d
UPE	5.05 ± 0.67 ^a	5.90 ± 0.15 ^{a,b}	5.29 ± 0.62 ^{a,b,c}	5.35 ± 0.54 ^{a,b}
NPE	4.02 ± 0.28 ^{a,b}	6.07 ± 0.19 ^a	5.25 ± 0.93 ^{a,b,c}	5.27 ± 0.19 ^{a,b,c}
AA	4.10 ± 0.46 ^{a,b}	5.58 ± 0.43 ^{a,b}	5.39 ± 1.08 ^{a,b,c}	4.73 ± 0.25 ^{b,c,d}
US	2.70 ± 2.36 ^b	5.42 ± 0.02 ^{a,b}	5.81 ± 0.12 ^{a,b}	4.46 ± 0.25 ^d
CO	2.87 ± 2.50 ^b	4.89 ± 0.34 ^{a,b}	4.84 ± 0.38 ^c	4.91 ± 0.40 ^{a,b,c,d}

Values represented are means (n=3) ± SD; Values with the same superscript across rows are not significantly different from each other (P < 0.05).

Legend: E = Eggshells, P = Plantain peels, C = Cassava peels, PD = Poultry droppings, U = Urea, N = NPK, UPE = Urea + All organic amendments, NPE = NPK + All organic amendments, AA = All amendments, US = Unamended soil, CO = Control soil



Legend: M= Methanol; E =Ethanol

Figure 1: pH of Plant Extracts after Methanolic and Ethanolic Extraction

Table 4: Phytochemical Screening of Methanolic Plant Extracts

Phytochemicals	Moringa	Ugwu	Scent Leaf	Bitter Leaf
Saponins	+	+	+	+
Alkaloids	+	+	+	-
Steroids	+	+	-	-
Phlobatannins	+	-	-	-
Tannins	+	+	+	+

Legend: + = Present; - = Absent

Table 5: Phytochemical Screening of Ethanolic Plant Extracts

Phytochemicals	Moringa	Ugwu	Scent Leaf	Bitter Leaf
Saponins	+	+	+	+
Alkaloids	+	+	-	-
Steroids	+	+	-	-
Phlobatannins	+	-	-	-
Tannins	+	+	+	+

Legend: + = Present; - = Absent

The results as shown in Figures 2-4, suggested that the plant extracts had low antibacterial activity. *Bacillus amyloliquefaciens* had the largest zone of inhibition of 5.50 ± 0.71 mm in the methanolic and ethanolic extracts of *Vernonia amygdalina* (bitter leaf) whereas, *Bacillus proteolyticus* had the highest zone of inhibition of 6.00 ± 0.00 mm in the ethanol extract of bitter leaf. Similarly, the methanolic bitter leaf extract inhibited *Providencia rettgeri* the most with a zone of inhibition of 6.00 ± 0.00 mm.

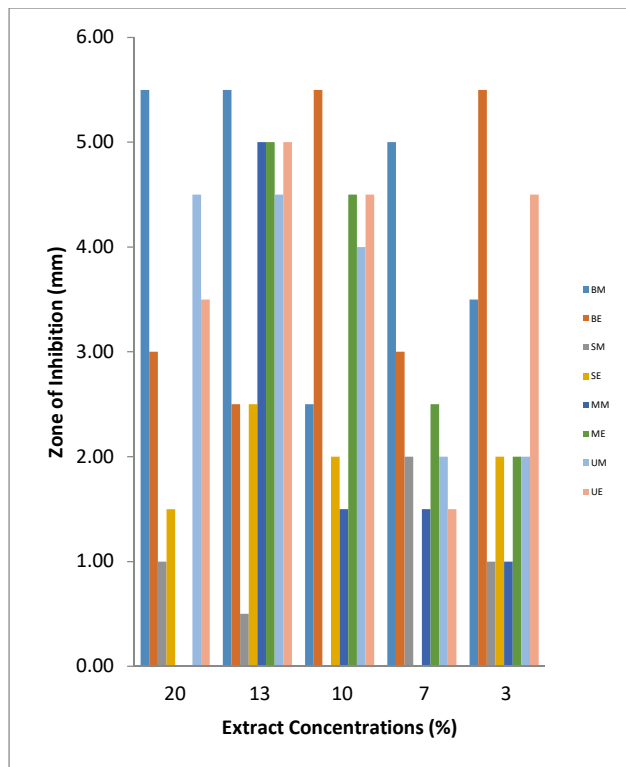


Figure 2: Zone of inhibition of *Bacillus amyloliquefaciens* on the plant extracts

Legend: 0.0 mm: No Zone of Inhibition; >11 mm = Sensitive (S); 7 mm-10 mm = Moderately Sensitive (MS); 2 mm-6 mm = Resistant (R)
 BM – Bitter Leaf (Methanol) MM – Moringa (Methanol)
 BE – Bitter Leaf (Ethanol) ME – Moringa (Ethanol)
 SM – Scent Leaf (Methanol) UM – Ugwu (Methanol)
 SE – Scent Leaf (Ethanol) UE – Ugwu (Ethanol)

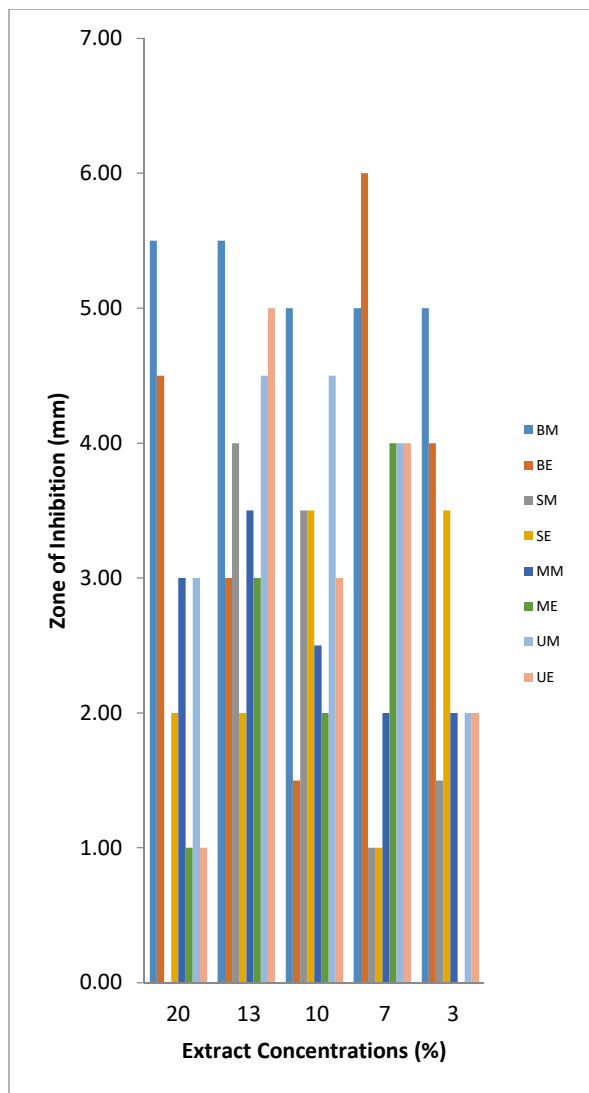


Figure 3: Zone of inhibition of *Bacillus proteolyticus* on the plant extracts

Legend:

0.0mm: No Zone of Inhibition; >11mm = Sensitive (S); 7mm-10mm = Moderately Sensitive (MS); 2mm-6mm = Resistant (R)

- BM – Bitter Leaf (Methanol) MM – Moringa (Methanol)
- BE – Bitter Leaf (Ethanol) ME – Moringa (Ethanol)
- SM – Scent Leaf (Methanol) UM – Ugwu (Methanol)
- SE – Scent Leaf (Ethanol) UE – Ugwu (Ethanol)

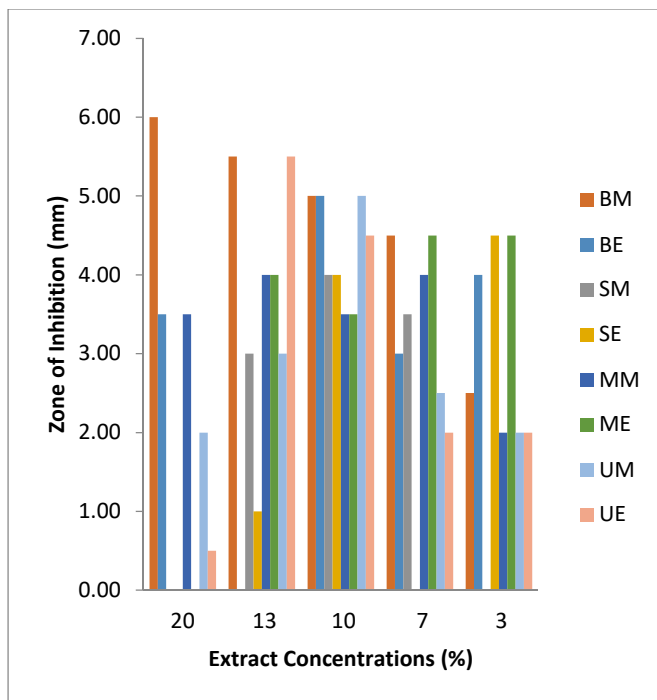


Figure 4: Zone of inhibition of *Providencia rettgeri* on the plant extracts

Legend:

0.0mm: No Zone of Inhibition; >11mm = Sensitive (S); 7mm-10mm = Moderately Sensitive (MS); 2mm-6mm = Resistant (R)

- BM – Bitter Leaf (Methanol) MM – Moringa (Methanol)
- BE – Bitter Leaf (Ethanol) ME – Moringa (Ethanol)
- SM – Scent Leaf (Methanol) UM – Ugwu (Methanol)
- SE – Scent Leaf (Ethanol) UE – Ugwu (Ethanol)

Bacillus amyloliquefaciens, *Bacillus proteolyticus* and *Providencia rettgeri* were resistant to crude oil, engine oil, heptane, and benzene as shown in Figure 5. However, *Bacillus amyloliquefaciens* was highly susceptible to pyridine with an inhibition zone of 18 mm, while *Bacillus proteolyticus* was moderately susceptible to n-hexadecane and pyridine with clear zones of 9 mm and 7 mm respectively. *Providencia rettgeri* was moderately susceptible to pyridine with an inhibition zone of 8 mm.

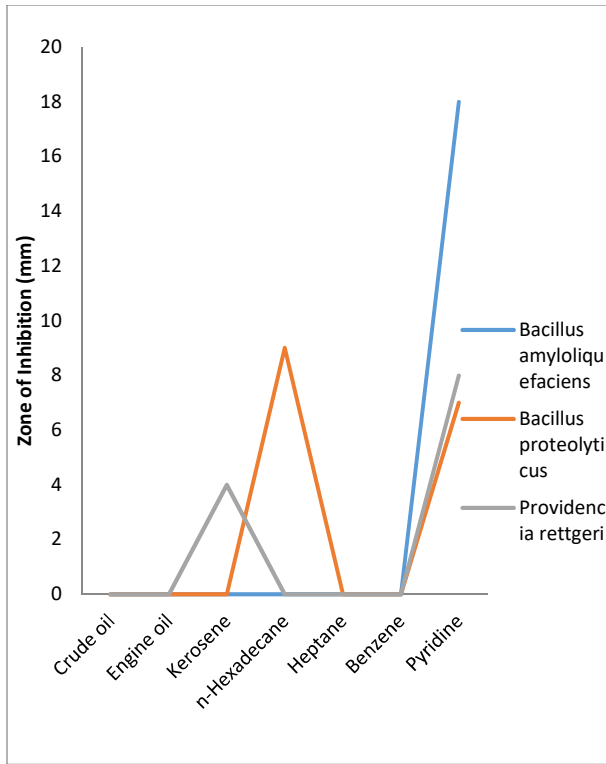


Figure 5: Inhibition zones of isolates on various hydrocarbons

Bacillus amyloliquefaciens and *Bacillus proteolyticus* were found to be susceptible to Septrin (SXT), Streptomycin (S), Tarivid (OFX), Gentamycin (CN), and Augmentin (AU), among others on Gram-negative discs with a broad spectrum of 20 mm. On Gram-positive discs, they were likewise susceptible to Ciprofloxacin (CPX), Streptomycin (S), Septrin (SXT), Erythromycin (E) and Pefloxacin (PEF) with a broad spectrum of 20 mm. Furthermore, *Providencia rettgeri* was found to be highly susceptible to all Gram-negative antibiotics, including Chloranphenicol (CH), Sparfloxacin (SP), Amoxicillin (AM), Tarivid (OFX), Gentamycin (CN), Augmentin (AU), Ciprofloxacin (CPX) and others, but showed resistance to antibiotics like Ampiclox (APX), Zinnacef (Z) and Amoxicillin as illustrated in Figures 6-9.

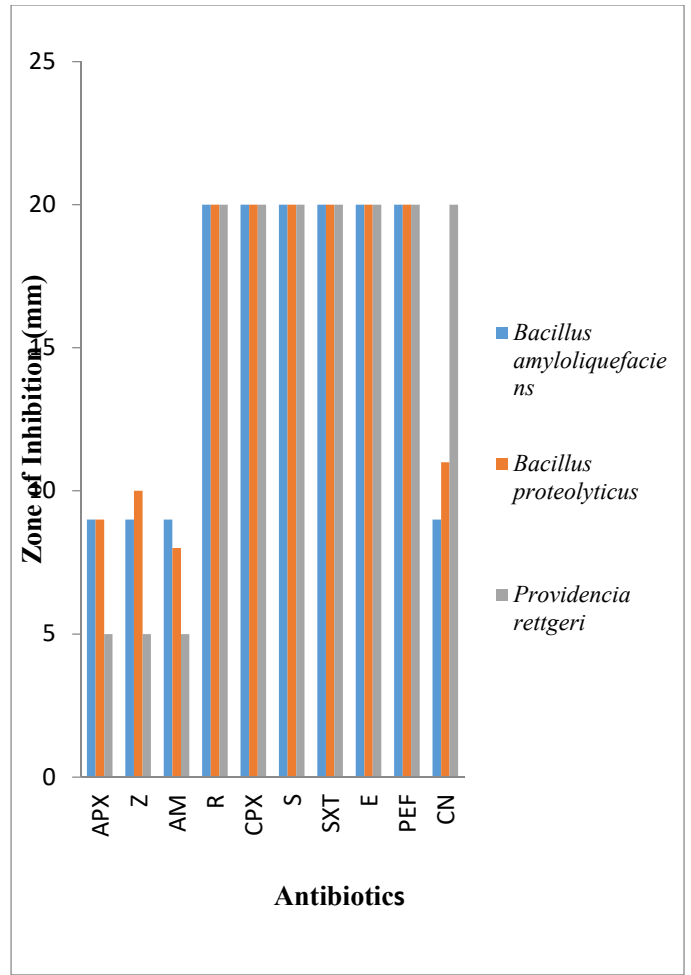


Figure 6: Zone of Inhibition of isolates on Gram-positive disc (Methanol)

Legend: 0.0 mm: No Zone of Inhibition, >11mm = Sensitive (S); 7mm-10mm = Moderately Sensitive (MS); 2 mm-6 mm = Resistant (R)
 APX - Ampiclox (30µg) S – Streptomycin (30µg)
 Z – Zinnacef (20µg) SXT – Septrin (30µg)
 AM – Amoxicillin (30µg) E – Erythromycin (30µg)
 R – Rocephin (25µg) PEF – Pefloxacin (10µg)
 CPX – Ciprofloxacin (10µg) CN – Gentamycin (10µg)

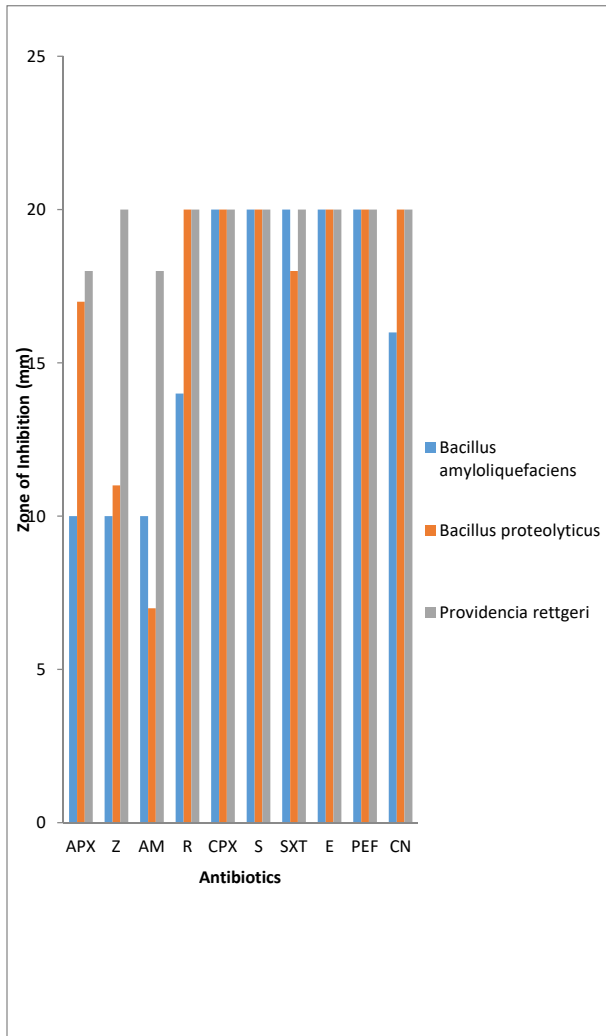


Figure 7: Zone of Inhibition of isolates on Gram-positive disc (Ethanol)

Legend: 0.0 mm: No Zone of Inhibition, >11mm = Sensitive (S); 7mm-10mm = Moderately Sensitive (MS); 2 mm-6 mm = Resistant (R)

APX - Ampiclox (30µg) S - Streptomycin (30µg)
 Z - Zinnacef (20µg) SXT - Septrin (30µg)
 AM - Amoxicillin (30µg) E - Erythromycin (30µg)
 R - Rocephin (25µg) PEF - Pefloxacin (10µg)
 CPX - Ciprofloxacin (10µg) CN - Gentamycin (10µg)

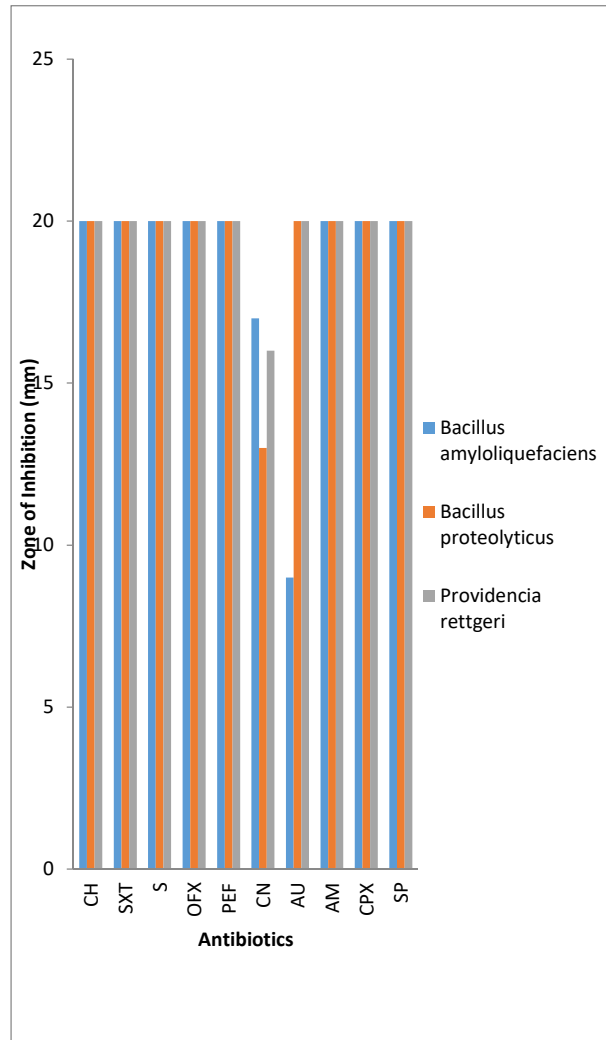


Figure 8: Zone of Inhibition of isolates on Gram-negative disc (Methanol)

Legend: 0.0 mm: No Zone of Inhibition, >11mm = Sensitive (S); 7mm-10mm = Moderately Sensitive (MS); 2 mm-6 mm = Resistant (R)

CH - Chloranphenicol (30µg) CN - Gentamycin (30µg)
 SXT - Septrin (30µg) AU - Augmentin (10µg)
 S - Streptomycin (30µg) AM - Amoxicillin (30µg)
 OFX - Tarivid (10µg) CPX - Ciprofloxacin (30µg)
 PEF - Pefloxacin (30µg) SP - Sparfloxacin (10µg)

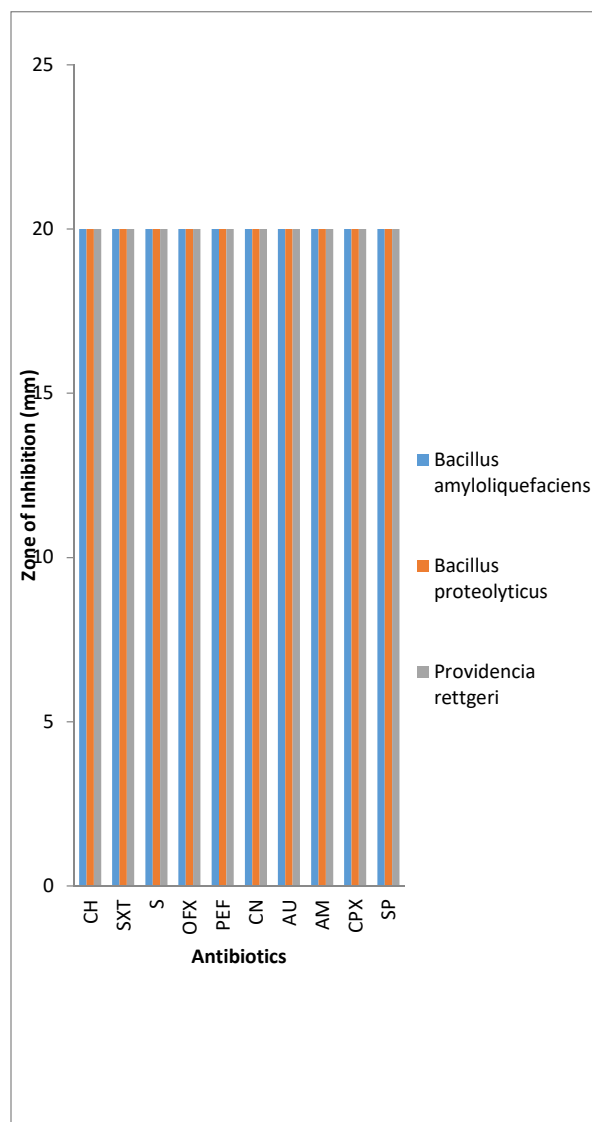


Figure 9: Zone of Inhibition of isolates on Gram-negative disc (Ethanol)

Legend: 0.0 mm: No Zone of Inhibition, >11mm = Sensitive (S); 7mm-10mm = Moderately Sensitive (MS); 2 mm-6 mm = Resistant (R)

CH - Chloranphenicol (30µg)	CN - Gentamycin (30µg)
SXT - Septrin (30µg)	AU - Augmentin (10µg)
S - Streptomycin (30µg)	AM - Amoxicillin (30µg)
OFX - Tarivid(10µg)	CPX - Ciprofloxacin (30µg)
PEF -Pefloxacin (30µg)	SP - Sparfloxacin (10µg)

Discussion

Mineral nutrients must be available for bioremediation processes to take place, thereby, necessitating the use of biostimulation. Bacterial growth must have been aided by the additional nutrients included in the amendments utilized in this study. When the results from Table 2 were compared to those from earlier studies, they revealed some similarities. Ogunjobi and Ekanem (2017) measured soil pH (6.9), eggshell pH (7.4), and plantain peel pH (6.9) while Agamuthu *et al.* (2013) measured soil pH (5.75) and cow dung pH (7.00). Vidali (2001) recommended a pH range of 6.5 to 8 for the breakdown of hydrocarbon oils. For Nitrogen, Phosphorus, and Carbon, Ogunjobi and Ekanem (2017) found similar results of 0.81 %, 0.67 %, and 1.22 % respectively. Agamuthu *et al.* (2013) on the other hand, achieved varied concentrations of 0.15 %, 8.02 %, and 4.33 % for these parameters. The results of the nitrogen content of plantain peels and eggshells is in accordance with the observation of Ogunjobi and Ekanem (2017) who recorded 1.22 % and 0.62 % respectively while Agamuthu *et al.* (2013) recorded 0.54 % as nitrogen content in cow dung. The presence of various nutritional components in the amendments facilitated the enrichment of the soil and might have prompted the biodegradative abilities of the isolates. The microbial counts derived from NPK rose from Day 0 to Day 28 according to the enumerated bacterial colonies obtained from the microcosm set up displayed in Table 3. A related work carried out by Ogunjobi and Ekanem (2017) reported NPK to exhibit higher biodegradation efficiency. In contrast, Agarry and Jimoda (2013) had a lower NPK fertilizer efficiency than a combination of all organic amendments (plant residues and animal dung) applied. The capacity of aboriginal microorganisms to degrade crude oil polluted soil by biostimulation was established in a study by Hamoudi-Belarbi *et al.* (2018) using carrot peels. Furthermore, Ofoegbu *et al.* (2014) discovered that combining inorganic fertilizers with cow

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fungus additives improved biodegradation. Agbor *et al.* (2019) and Ekpobari *et al.* (2019) conducted further studies that corroborated these findings. It was concluded from this investigation that certain amendments when employed singly or in combination, might greatly improve bioremediation. Rather than being disposed of indiscriminately causing an ecological nuisance and harming the aesthetics of the environment, numerous organic amendments can be studied and exploited for bioremediation.

According to Figure 1, the pH values obtained from the methanolic and ethanolic plant extracts were similar with the highest value of 6.7 recorded in the methanolic extraction of Ugwu (U_M) and the lowest value of 6.4 in the ethanolic extraction of Bitter leaf (B_E). The influence of acidity or alkalinity on the efficiency of popular antiseptic compounds was examined by Wiegand *et al.* (2015). The result obtained revealed a low pH range of 5.0 to 9.0 for chlorhexidine and octenidine. Also, a low pH effectively inhibited microbial growth while antimicrobial effects reduced with a rise in pH. This shows that the acidic pH obtained in this study may be responsible for the presence and stability of phytochemicals in plant extracts (Mohamed *et al.*, 2015).

The existence of bioactive substances of therapeutic importance was discovered in the phytochemical screening of the plant extracts (Tables 4 and 5). Ethanol and methanol, according to Alara *et al.* (2020) are organic solvents capable of freeing phytochemicals such as tannins, alkaloids, saponins, flavonoids and other compounds. The findings of this study revealed that the extraction solvents were unable to liberate some of the screened phytochemicals as steroids were absent in both the scent and bitter leaf extracts, phlobatannins were absent in all leaf extracts except *Moringa* and alkaloids were absent in both the bitter and scent leaf extracts (ethanol extraction). Adetitun *et al.* (2013)

discovered saponins, tannins, flavonoids, phlobatannins, terpenoids, and alkaloids in *Moringa oleifera* leaves. Also, saponins, tannins, and alkaloids were discovered in scent leaf methanolic extracts but only flavonoids were present in the chloroform extracts while other phytochemicals were lacking according to the study of Umar *et al.* (2019). Similarly, Oladosu-Ajayi *et al.* (2017) discovered that *Vernonia amygdalina* and *Ocimum gratissimum* contained different phytochemical substances when they were exposed to different extraction solvents. Plant species, plant part, plant growth conditions (soil, water, and temperature), geographical location, season of plant collection, polarity of the solvent and absorbent nature of the plant matrix may all influence the presence or lack of particular phytochemicals in plants (Umar *et al.*, 2019).

The results in Figures 2-4 showed that the plant extracts had little antibacterial activity. This explains why Gram-positive bacteria (*Bacillus amyloliquefaciens* and *Bacillus proteolyticus*) and Gram-negative bacteria (*Providencia rettgeri*) were all resistant. The limited effectiveness of the plant extracts in influencing the zones of inhibition of *Bacillus amylolequefaciens* and *Bacillus proteolyticus* could be related to *Bacillus* spp. resistant endospores. Also, the capacity of *Providencia rettgeri* to display resistance to antimicrobial compounds in the plants may be due to the formation of lipase from fatty carbon sources which may have hampered the entry of antimicrobial contents into the cell wall. Masud Rana *et al.* (2014) investigated the antibacterial susceptibility of methanolic extracts of *Terminalia chebula* (bark), *Phyllanthus acidus* (fruits), *Sarcochlamys pulcherrima* (leaves), and *Abelmoschus esculentus* (fruits) all of which had low activity against Gram-negative bacteria but were effective against Gram-positive bacteria. Similarly, Nigussie *et al.* (2021) found that the methanol extract of *Lawsonia inermis* leaves had high antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*,

Pseudomonas aeruginosa, *Shemanella alage* and *Streptococcus pyogenes* while the same methanol extract of *Achyranthus aspera* revealed low antimicrobial activity on all tested isolates except *Streptococcus pyogenes*.

Gram-negative bacteria are known to be more resistant to antibiotics than Gram-positive bacteria. This is due to differences in cell wall composition which prevent many ambient chemicals from entering. Gram-negative bacteria when compared to Gram-positive bacteria have higher antibiotic resistance due to the existence of phospholipid membranes with lipopolysaccharide components. This prevents antibacterial compounds from passing through (Taye *et al.*, 2011; Leach, 2015). Low antimicrobial activity of plant extracts can be caused by the inability of the antimicrobial compounds to diffuse adequately into the agar which can be caused by the volume of inoculum in the agar well or the duration of the diffusion phase prior to incubation (Eloff, 2019).

The antimicrobial susceptibility test on hydrocarbons revealed that the isolates were resistant and susceptible to the various hydrocarbons tested as shown in Figure 5. Their ability to grow well on hydrocarbons and use them as carbon and energy sources demonstrates their resistance. When compared to the findings of Thamer *et al.* (2013), it was discovered that resistant endospores of *Bacillus* spp. allow them to tolerate high levels of oils which explains why *Bacillus amyloliquefaciens* and *Bacillus proteolyticus* grew well on crude oil, engine oil, kerosene, n-hexadecane, heptane, and benzene. Furthermore, many hydrocarbon-degrading bacteria have been found to make the best use of hydrocarbons by creating biosurfactants. *Bacillus* spp. have shown to be capable of producing lipopeptide-derived surfactants, making them useful in the cleanup of hydrocarbon-contaminated locations (Chen *et al.*, 2015).

Furthermore, *Providencia* spp. are known to produce lipase from lipid carbon sources (usually

natural oils), which stimulates their enzymatic degradation of petroleum hydrocarbons (Osho and Alabi, 2020). This could have influenced the tolerance of *Providencia rettgeri* to crude oil, engine oil, n-hexadecane, heptane, and benzene (Osho and Alabi, 2020). Adetitun *et al.* (2019) reported that *Alcaligenes* sp. strain 3k could grow effectively on kerosene and hexadecane and use them as carbon sources but, that it could not break down hexane. *Rhodococcus opacus* R7 was also described by Zampolli *et al.* (2014) as having the ability to biodegrade decane and hexadecane but not hexane or octane.

Genes, antibiotic strength, antibiotic concentration and other factors can influence the sensitivity or resistance of bacteria to antibiotics. The organisms had extensive clearance zones to the majority of antibiotics according to the results of the zones of inhibition shown in Figures 6 - 9. Fiedler *et al.* (2019) discovered that *Bacillus cereus* strains were sensitive to Ciprofloxacin, Erythromycin, Gentamycin, Tetracycline, and Chloramphenicol among other antibiotics. *Bacillus cereus* was clearly resistant to Erythromycin, Tetracycline and β -lactamase antibiotics according to a divergent view on susceptibility explained by Luna *et al.* (2007) and Kim *et al.* (2015) whereas, *Bacillus cereus* was clearly resistant to Amoxil, Streptomycin and Ampiclox according to Ataikiru *et al.* (2020). BamHI is a restriction enzyme produced by *Bacillus amyloliquefaciens*, Barnase - a protein antibiotic is also produced by it (Molohon *et al.*, 2011). *Bacillus amyloliquefaciens* has a symbiotic relationship with plants and has been found to produce bacillaene and macrolactin among other antibiotics. *Bacillus amyloliquefaciens* is beneficial as a biological pest-control agent because these compounds protect the host plant from infection by other microbes (Chowdhury *et al.*, 2015; Fan *et al.*, 2017).

Treatment of *Providencia rettgeri* has been difficult due to its antibiotic resistance. Certain strains of *Providencia rettgeri* have been shown to be resistant to Ceftriaxone and Cefepime (Zhou *et al.*, 2014). Also, Aibinu *et al.* (2011) reported a case of Quinolone resistance in Nigeria. The bacterium is also resistant to antimicrobials such as Ciprofloxacin, Cephalosporins, and Ampicillin based on the studies of Magiorakos *et al.* (2012) and Shin *et al.* (2018). Furthermore, resistant genes can be found on the same plasmid in bacteria and can be easily transported to the environment according to Wiedenbeck and Cohan (2011) and Oyetibo *et al.* (2010).

Conclusion

Given the impact of hydrocarbon contamination on the ecosystem and the necessity to clean up contaminated places, bioremediation solutions can be considered. This research looked into the ability of some microorganisms to breakdown petroleum hydrocarbons. Furthermore, all of the isolates showed promise in terms of bioremediation abilities, indicating that they might be used as either indigenous or exogenous microorganisms to aid in the environmentally friendly cleanup of petroleum hydrocarbons and derivatives. In addition, the plants studied in this research had a significant number of bioactive chemicals that can help prevent pathogen invasion and strengthen the immune system. Furthermore, since the microorganisms were discovered to be antibiotic-resistant, antibiotics can be used to combat a variety of diseases connected with them.

Competing Interest

The authors declare no competing interests

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