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**Research Article**

# Effect of Bitter Leaves Extract on Bacteria Isolated from Soils of Some Mechanic and non-Mechanic Sites in Makurdi Metropolis

**Grace Mwuese Gberikon[1*], Fredrick Ityolumun Iyoula[1], Smart Tersagh Ichor[1]**

[1]Department of Microbiology, Federal University of Agriculture, Makurdi Benue State, Nigeria

## Abstract

Effect of bitter leaves extract on bacteria isolated from soils of some mechanic and non- mechanic site in Makurdi metropolis was investigated. Forty (40) soil samples (20g each) were collected at the depth of 8-10cm from different mechanic shops in Makurdi metropolis (New garage, Kanshio and North bank) and soil from non-mechanic site. Fresh leaves of *Vernonia amygdalina* (bitter leaves) were purchased at Wadata market and identified at the Department of Plant Science, Federal University of Agriculture (FUAM). Serial dilution and pour plate technique was used for microbial analysis. Bacteria present were identified using cultural, morphological and biochemical techniques. Ethanol and methanol solvents were used to extract the bioactive constituents. Qualitative and quantitative phytochemical analyses were carried out on the bioactive components in the bitter leaves extracts. Susceptibility pattern of isolates were determined using Agar well diffusion assays. The genera *Pseudomonas, Staphylococcus, Proteus* and *Escherichia* were the predominant petroleum hydrocarbon degrading bacteria. Analysis from phytochemical screening showed that compounds such as alkaloids, tannins, saponins, flavanoids, terpenes, anthraquinones, reducing sugars, cardiac glycosides and phlobatannins were present. Alkaloids in the bitter leaves extract was more in quantity and quality as compared to other phytochemical components. Zones of inhibition of the plant extracts ranged between 20.00± 0.89mm - 32.78± 1.72mm for Methanol and 21.00± 2.37mm - 31.22± 2.05mm for Ethanol at 100mg/ml. The Minimum Inhibitory Concentration (MIC) of both solvents ranged between 23.83± 3.23m/l and 50.00± 0.00m/l.The MBC values ranged from 50.00± 0.00mg/l to 100.00± 0.00 mg/l. Bacteria from the control site which had no hydrocarbon products contamination were more susceptible than those from the mechanic sites. Results from findings have shown that *Vernonia amygdalina* (bitter leaves) has potentials to be used as an alternative source of antibacterial agents in the ever increasing menace of Multi-Drug Resistance.

**Keywords:** Mechanic sites; Non-Mechanic site; Bitter leaves; Extract, Antibacterial

## Introduction

Plants have developed the ability to synthesize some chemical compounds (metabolites). These metabolites have capacity to fight microorganisms and thus can be used for medicinal purposes. Bitter leaf (*Vernonia amygdalina*) is one of such effective plants that possess these features. It contains complex active components such as saponins, flavonoids, alkaloids and tannin that are of therapeutic importance (Burkill, 1985; Olamide and Agu, 2013). *Vernonia amygdalina* is a valuable medicinal plant that is widespread in East and West Africa (Burkill, 1985; Olamide and Agu, 2013). Nutritionally, the macerated

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***Corresponding author**
Grace Mwuese Gberikon,
Department of Microbiology, Federal University of Agriculture, Makurdi Benue State, Nigeria
Email: gracegberikon@yahoo.com

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leaves of the plant is used mainly in soup making in the tropics (Ijeh *et al.*,2008) and has been successfully used as a supplement in weaning foods (Eleyinmi *et al.*,2008). Several studies on bitter leaf extracts has been reported and shown to exert antibiotic action against drug resistant microorganisms (Burkill, 1985; Olamide and Agu, 2013). Hydrocarbon degraders are of different origins, despite their origin they have evolved traits over the years from their environments that make them resistant to antibiotics and this has constitute serious problems to public health. In an attempt to curb drug resistivity from microorganisms especially from bacterial origin, alternative ways has been utilize in the treatment of infections caused by bacteria. Extracts from some plants with proven bioactive components has been employed in alternative medicine to reduce incidence of antibiotic resistance.

## Materials and Methods

### *Sample Collection and Sample Size*

Forty soil samples, (twenty (20g) each) were collected from different mechanic sites located in three parts of Makurdi Metropolis namely: New garage mechanic site, mechanic village Kanshio and mechanic site North bank. Soil samples were also collected from non – mechanic site and used as control. Twenty (20) g of each Soil sample was collected at the depth of 8-10 cm by using a clean and dry sterile spatula in clean polythene bags, labelled and transported in ice box for preservation to prevent changes in microbial load for basic isolation at the advanced biological science laboratory, University of Agriculture Makurdi (Sudhir *et al.*, 2014)**.**

### *Media Used for Isolation*

Nutrient agar (NA), Mueller Hinton agar, Peptone water and Normal Saline were employed

### *Isolation and Enumeration of Hydrocarbon Utilizing Bacteria*

The vapour phase transfer method was adopted for enumeration of hydrocarbon utilizing bacteria. Serial dilution of the soil samples was achieved by shaking 1.0 g of the soil samples in 9 ml of distilled water in test tubes and dilution was done up to $10^5$. An aliquot (1 ml) of dilutions was plated out in triplicates on a sterile Nutrient Agar (oxoid) as described by Olukunle (2013). Sterile Whitman No. 1 filter paper saturated with crude oil was placed on the inside cover of each of the Petri dishes and incubated with the agar side up. The filter supplied the microorganisms with hydrocarbon by vapour phase transfer to the inocula. The plates were placed in the incubator (Gallenkamp Duostat Incubator size 2 England) at 37 $^0$C for 2 days after which colonies were counted to determine titre (viable counts per unit) of soil samples (Ichor *et al.*, 2014). This was repeated to all 40 samples collected.

### *Purification of Bacteria Isolates*

The streak method was used to subculture the bacteria isolates that grew on the Nutrient Agar. An inoculating loop was sterilized using hot flame, allowed to cool before it was used to take part of the grown bacterial colonies from the cultured agar and streaked on the surface of a fresh Nutrient Agar. The pure strains obtained were preserved on nutrient agar slants at 4 $^o$C for further studies as described by Olukunle (2013).

### *Characterization of Bacteria Isolates*

Individual bacteria colonies were identified by cultural, morphological and biochemical techniques using the taxonomy scheme of Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). The cultural characterization of bacteria colonies isolated was done by observing the colonies for color, shape, edge, elevation and surface appearance displayed on Nutrient Agar. Biochemical test for bacteria identification included gram staining, spore staining, catalase test, oxidase test, Indole test, coagulate test, urease test, methyl red and Citrate test.

### *Collection and Identification of Bitter Leaf (Vernonia amygdalina)*

Fresh leaves of *Vernonia amygdalina* (bitter leaf) free from disease were purchased in a clean polythene bag at Wadata market, Makurdi, Benue state, Nigeria and was immediately transported to the herbarium of Department of Plant Science and Biotechnology, University of Agriculture, Makurdi for identification. The leaves were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, the leave materials were air dried on sterile blotter under shade and then ground into fine powder using laboratory Pestle and Mortar. Powdered samples were stored in tightly closed reagent bottles for subsequent extraction and bioassay as described by Ghamba1 *et al*. (2014).

### *Preparation, Extraction and Concentration of Leaf Extracts*

Exactly 50g of the bitter leaves powder was soaked in 100 ml of 70 %w/v Methanol and 70 %w/v Ethanol for three days. At the end of the extraction, the extracts were filtered using Whatman No.1filter paper. The filtrate was concentrated in vacuum at 30 $^o$C. After complete evaporation, the extract was weighed and preserved aseptically at 4 $^o$C until when required for use (Newton *et al.*, 2002).

### *Sterility Proofing of Extract*

The extracts were tested for sterility after filtration by introducing 2 ml of the sterile extract into 10 ml of sterile Mueller Hilton broth. This was incubated at 37 °C for 24 hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period (Oluyele and Oladunmoye, 2017).

***Qualitative Determination of Phytochemicals***

The phytochemical components of the powdered plant leaves were analysed according to the method described by Ghambal *et al*. (2014). The leaves were screened for tannins, alkaloids, flavonoids, anthraquinones, saponins, terpenoid, phlobatanins, deoxy-sugar and cardiac glycosides.

***Quantitative Phytochemical Analysis***

The phytochemicals which are present in the ethanolic and methanolic extracts of bitter leaf (*V. amygdalina*) was quantified by standard procedures using high performance liquid chromatography (HPLC)

***Assessment of Antibacterial Activity***

***Standardization of the bacterial cell suspension***

Five colonies of each test organism from mechanic and non-mechanic site were picked into sterile test-tube containing sterile nutrient broth and incubated at 37 $^0$C for 24 hours. The turbidity produced by these organisms was adjusted and used to match the turbidity (opacity) of 1.5 x $10^8$ cfu/ ml McFarland 0.5 standard calibration as described by Anibijuwon *et al*, (2012).

***Agar well diffusion method (zone of inhibition)***

The methanolic and ethanolic aqueous extracts were tested for antibacterial activity by Agar well diffusion. Exactly 1ml of Inocula was measured from the peptone water in bijou bottles and introduced on to the surface of sterile plates. Mueller Hinton agar (MHA) was sterilized in flasks, cooled to 45 – 50 $^0$C and then pour plated into sterilized Petri dishes with inocula, the plates were gently rock and allowed to solidify but covered to prevent atmospheric contamination. A Cock borer of 6 mm in diameter was sterilized by flaming and used to create four ditches at the centre of the plate. The holes so created were then filled with the plant extracts and standard antibiotic (ciprofloxacin) as control drug. The plates were allowed to stand for one hour for pre-diffusion of the extracts and control. Incubation was done at 37 $^0$C for 24 hours. The diameters of the zones of inhibition were measured in millimetres (Ibekwe *et al.*, 2001).

***Determination of minimum inhibitory concentration (MIC) of the extracts on test organisms***

The initial concentration of the plant extract (100 mg / ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extract (stock solution) into 5 ml of sterile peptone water to obtain 75 mg/ ml concentration. The above process was repeated several times to obtain the dilutions 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml. each concentration was inoculated with 0.1 ml of the standardised bacterial cell suspension and incubated at 37$^0$ C for 24 hours (Ibekwe *et al.*, 2001). The growth of the inoculum in the broth was indicated by no/low turbidity or cloudiness of the broth while growth was indicated by high turbidity and the lowest concentration of the extract at which there was no/least growth as indicated by clear broth is taken as the minimum inhibitory concentration (MIC). Negative controls were set up as follows; Peptone water only, Peptone water and plant extract, and finally positive control containing peptone and a test organism (Ibekwe *et al.*, 2001).

***Determination of minimum bactericidal concentration (MBC)***

The MBC was determined by taking a loopful from each tube that showed no growth during MIC determination and streaked onto Mueller Hilton agar plates and incubated at 37° C for 24 hours. The least concentration at which no growth was observed was noted as the MBC (Ibekwe *et al.*, 2001).

***Statistical Analysis***

The data was analyzed using one-way ANOVA with assistance of SPSS version 20 and means were compared using Duncan multiple range test (DMRT). The results were expressed in terms of mean ± standard deviation. All data presented are mean values of triplicate measurement (n=3) obtained from the separate runs.

## Results and Discussion

**Table 1:** Qualitative Phytochemical Screening of Bitter Leaves (*Vernonia amygdalina)* Extracts

| Phytochemicals | Ethanol | Methanol |
|---|---|---|
| Alkaloids | +++ | +++ |
| Anthraquinones | ++ | ++ |
| Cardiac glycosides | ++ | ++ |
| Flavonoids | ++ | ++ |
| Phlobatannins | + | + |
| Reducing sugars | ++ | ++ |
| Saponin | ++ | ++ |
| Tannins | ++ | + + |
| Terpenes | ++ | ++ |

Key: + = Presence of constituent.
+ + = high presence of constituent.
+ + + = Very high presence of constituent

**Table 2:** Quantitative Phytochemical Screening of Bitter Leaves (*Vernonia amydalina)* Extract in Respect to Medium of Extraction (Mg/G)

| Phytochemical Contents | Ethanol | Methanol | Means± SD |
|---|---|---|---|
| Tannins | 1.37± 0.014[b] | 1.53± 0.014[b] | 1.45± 0.93 |
| Alkaloids | 6.51± 0.007[g] | 7.03± 0.007[g] | 6.77± 0.30 |
| Saponins | 4.32± 0.14[f] | 4.23± 0.014[f] | 4.27± 0.05 |
| Phlobatanins | 0.92± 0.014[a] | 1.12± 0.014 [a] | 1.02± 0.12 |
| Flavonoids | 2.87± 0.014[d] | 3.02± 0.014[e] | 2.94± 0.09 |
| Terpenes | 2.46± 0.014[c] | 2.34± 0.014[c] | 2.40± 0.07 |
| Anthraquinones | 1.95± 0.014b[b] | 2.05± 0.000[c] | 2.00± 0.06 |
| R. Sugars | 4.23± 0.014[f] | 3.65± 0.014[e] | 3.94± 0.33 |
| C. glycosides | 4.61± 0.007[f] | 3.82± 0.000[f] | 4.22± 0.46 |
| | 3.25±1.75 | 3.20± 1.73 | 3.22± 1.73 |

Extraction Medium F (1, 26) = 0.45 P= 0.510
Phytochemicals F (8, 26) = 247.28 P< 0.001
Means with same alphabets are not significantly different.
Means with different alphabets are significantly differe

**Table 3:** Antibacterial Activity of Ethanolic and Methanolic Extracts of Bitter Leaves (*Vernonia amygdalina*) and Ciprofloxacin on Bacteria Isolates from Mechanic and non- Mechanic Sites

| | Zones of Inhibition (mm) | | | |
|---|---|---|---|---|
| **Bacteria Isolates** | **Ethanol** | **Methanol** | **Ciprofloxacin** | Mean + SD |
| *Acinetobacter* spp | *28.33*± 2.88[a] | 26.83± 3.19[a] | 40.00± 8.60[c] | 31.72± 7.99 |
| *B. cereus* | 29.33± 4.27 [a] | 28.44± 4.22[a] | 44.11± 7.17[c] | 33.96± 8.97 |
| *B. subtilis* | 27.67± 3.16[a] | 27.56± 4.00[a] | 44.89± 5.39[c] | 33.37± 9.27 |
| *E. coli* | *28.33*± 2.81[a] | 30.00± 5.34[b] | 45.08± 6.93[c] | 34.47± 9.22 |
| *Enterococccus* spp | 27.56± 2.56[a] | 26.00± 3.28[a] | 42.78± 4.84[c] | 32.11± 8.49 |
| *K. pneumonia* | 22.00± 3.22[a] | 20.33± 1.51[a] | 40.67± 4.76[c] | 27.67±10.02 |
| *K. oxytoca* | 21.00± 2.37[a] | 20.00± 0.89[a] | 42.83± 5.04[c] | 27.94± 11.26 |
| *Micrococcus* spp | 24.33± 3.28[a] | 23.56± 3.28[a] | 39.22± 8.15[c] | 29.04± 9.00 |
| *P. Vulgaris* | 30.67± 3.00[b] | 28.89± 3.82[a] | 44.67± 2.92[c] | 34.74± 7.85 |
| *P. mirabilis* | 31.22± 2.05[b] | 32.78± 1.72[b] | 45.89± 1.76[c] | 36.63± 6.94 |
| *P. aeruginosa* | 28.50±3.43[a] | 28.08± 3.15[a] | 43.00± 4.11[c] | 33.19± 7.85 |
| *P. fluorescens* | 29.00± 3.46[a] | 28.78± 4.94[a] | 41.89± 8.71[c] | 33.22± 8.58 |
| *P. stutzeri* | 25.83± 3.92[a] | 27.33± 4.76[a] | 42.50± 6.25[c] | 31.89± 9.09 |
| *S. aureus* | 25.83± 3.92[a] | 27.33± 4.76[a] | 42.50± 6.25[c] | 31.89± 9.09 |
| *Salmonella* spp | 29.83± 3.07[a] | 28.42± 3.06[a] | 45.92± 3.61[c] | 34.72± 8.65 |
| | 27.81±3.98[a] | 27.33± 4.64[a] | 43.40± 5.85[b] | 32.85± 8.92 |

Extracts and Control F (2, 361) = 555.39, P < 0.001.
Bacteria Isolates F (14, 361) = 7.98, P < 0.001.
Means with same alphabets are not significantly different.
Means with different alphabets are significantly different

**Table 4:** Minimum Inhibitory Concentrations of Bacteria Isolates in Respect to Location of the Study Sites in Makurdi

| Bacteria Isolates | North Bank | Kanshio | New Garage | Control | Mean+SD |
|---|---|---|---|---|---|
| *Acinetobacter* spp | 37.50± 13.69$^{b}$ | 0.00± 0.00$^{a}$ | 37.50± 0.00$^{b}$ | 0.00± 0.00$^{a}$ | 37.50± 13.06 |
| *B. cereus* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00 |
| *B. subtilis* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00 |
| *E. coli* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 27.17± 3.97$^{a}$ | 50.00± 0.00$^{c}$ | 44.29±10.27 |
| *Enterococcus* spp | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00 |
| *K. pneumonia* | 25.00± 0.00$^{a}$ | 22.67± 4.41$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 23.83± 3.22 |
| *K. oxytoca* | 37.50± 0.00$^{b}$ | 0.00± 0.00$^{a}$ | 37.50± 0.00$^{b}$ | 0.00± 0.00$^{a}$ | 37.50± 13.06 |
| *Micrococcus* spp | 29.17± 10.21$^{a}$ | 29.17± 10.21$^{a}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00$^{c}$ | 36.11± 12.78 |
| *P. vulgaris* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00 |
| *P. mirabilis* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00 |
| *P. aeruginosa* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00 |
| *P. fluorescens* | 50.00± 0.00$^{c}$ | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00$^{c}$ | 50.00± 0.00 |
| *P. stutzeri* | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00 |
| *S. aureus* | 37.50± 13.69$^{b}$ | 37.50± 13.69$^{b}$ | 37.50± 13.69$^{b}$ | 37.50± 13.69$^{b}$ | 37.50±12.77 |
| *Salmonella* spp | 50.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00 |
| | 44.44± 10.45$^{a}$ | 43.93± 11.18$^{a}$ | 43.30± 10.99$^{a}$ | 48.44± 6.12$^{b}$ | 44.84±10.19 |

Bacteria Isolates F (14, 234) = 17.86, P< 0.001.
Study Site F (3, 234) = 3.87, P = 0.01.
Means with same alphabets are not significantly different
Means with different alphabets are significantly different

**Table 5:** Minimum Bactericidal Concentrations of Bitter Leaves (*Vernonia amygdalina)* Extract on Bacteria Isolates in Respect to Locations of the Study Sites in Makurdi

| Bacteria Isolates | North Bank | Kanshio | New Garage | Control | Mean +SD |
|---|---|---|---|---|---|
| *Acinetobacter* spp | 58.33± 20.41$^{a}$ | 0.00± 0.00$^{a}$ | 58.33± 20.41$^{a}$ | 0.00± 0.00$^{a}$ | 58.33± 19.46 |
| *B. cereus* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00 |
| *B. subtilis* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00 |
| *E. coli* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00 |
| *Enterococccus* spp | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00 |
| *K. pneumonia* | 50.00± 0.00$^{a}$ | 50.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 50.00± 0.00 |
| *K. oxytoca* | 75.00± 27.39$^{b}$ | 0.00± 0.00$^{a}$ | 75.00± 27.39$^{b}$ | 0.00± 0.00$^{a}$ | 75.00± 26.11 |
| *Micrococcus* spp | 58.33± 20.41$^{a}$ | 50.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 58.33± 20.41$^{a}$ | 55.56± 16.17 |
| *P. vulgaris* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00 |
| *P. mirabilis* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00 |
| *P. aeruginosa* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00 |
| *P. fluorescens* | 100.00± 0.00$^{c}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00$^{c}$ | 100.00± 0.00 |
| *P. stutzeri* | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00$^{c}$ | 0.00± 0.00$^{a}$ | 100.00± 0.00 |
| *S. aureus* | 75.00± 37.39$^{b}$ | 75.00± 27.39$^{b}$ | 75.00± 27.39$^{b}$ | 75.00± 0.00$^{b}$ | 75.00± 25.54 |
| | 85.71± 22.74 | 85.65± 22.74 | 88.30± 21.14 | 91.49± 18.12 | 87.50± 21.70 |

Bacteria Isolates F (13, 207) = 37.27, P < 0.015.
Study Site F (1, 207) = 0.73, P= 0.98.
Means with same alphabets are not significantly different
Means with different alphabets are significantly different

Bacteria strains isolated from mechanic and non-mechanic sites were aerobic and belong to the genera *Acinetobacter, Bacillus, Escherischia, Enterococcus, Klebsiella, Micrococcus, Proteus, Pseudomonas, Staphylococcus* and *Salmonella* which confirm the work carried out by Kafizadeh *et al.*,2011).

The Phytochemical Screening of the various Bitter leaves (Vernonia amygdalina) extracts determined in this study showed the presence of the following secondary metabolites; Alkaloids, Tannins, Saponins, Flavanoids, Terpenes, Anthraquinones, Reducing sugars, Cardiac glycosides and phlobatanins. Similarly, Ghamba1 *et al.* (2014) on *Vernonia amygdalina* (bitter leaves) aqueous and ethanol leaves extracts detected the presence of flavonoids, alkaloids, anthraquinones, saponins, Tannins, cardiac glycosides, steroids, Terpenoids and Cardenolide. In addition, this study contradicts the result of Anibijuwon *et al.* (2012) who detected components of the plant to the exclusion of components such as alkaloids, flavonoids and phlobotannins using ethanol for extraction from the same plant.

The observed quantitative distribution of the various phytochemicals in the extracts could be due to their relative solubility in the various solvents used for extraction. Alkaloids content was highest and Phlobatanins was the lowest in both ethanol and methanol. Alkaloids are very important in medicine and constitute most of the valuable drugs (Edeoga *et al.*, 2005).

The significant difference observed in antibacterial activity of the leaves extracts of *V. amygdalina* when compared to the standard antibiotic (Ciprofloxacin) could be due to the fact that organic extracts are in crude form compared to synthetic antibiotics which have high degree of purity (Muhammad *et al.*, 2014). While the results of the susceptibility testing of hydrocarbon degraders to ethanolic and methanolic extracts of the leaves of *Vernonia amygdalina* demonstrated that the plant possess strong activity against the isolated hydrocarbon degraders. *Proteus mirabilis, Staphylococcus aureus, Escherichia coli Bacillus cereus* had the highest zones of inhibition whereas *Acinetobacter spp, Micrococcus spp, Kleibsiella oxytoca and Klebsiella pneumonia* were less sensitive at 100 mg/ml.

The observed difference between the study sites could be attributed to the effect of the hydrocarbon present from the polluted site which corroborates the findings of Mathe *et al.* (2012) that soil contaminated with hydrocarbon leads to increased microbial resistance.

In the absence of selection pressure, such as hydrocarbon contamination, the indigenous microbial population is characterized by lower biodegradation potential and tolerance toward antibiotics (Mathe *et al.*, 2012). Furthermore, the Measurement of minimum inhibitory concentration (MIC) and the corresponding bactericidal concentration (MBC) for the extracts showed that ethanol extract was more effective in inhibition and extermination of the growth of hydrocarbon degraders at lower concentration than the methanol extract. This supports the previous findings of Ogbuile *et al. (*2007) who claimed that ethanol and soxhlet extractions are the best method to give optimum antibacterial effect of this plant.

## Conclusion

The study showed that the predominant petroleum hydrocarbon degrading bacteria in Makurdi metropolis were from the genera *Pseudomonas, Staphylococcus, Proteus* and *Escherichia.* It was established from results of this study that there were presence of secondary metabolites in the leaves of *V. amygdalina.* The leaves extracts of *V. amygdalina* exerted antimicrobial effects on both Gram positive and Gram negative bacteria spp which is bacteriostatic and bactericidal. Bitter leaves extract have potentials as an alternative source of antimicrobial agents in the ever increasing menace of Multi-Drug Resistance.

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