



Antimicrobial Activity and Phytochemical Screening of Methanolic Leaf Extract of *Vernonia amygdalina*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Crude methanolic leaf extract of *Vernonia amygdalina* was evaluated to determine its bioactive constituents, the antimicrobial properties, measure the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against some selected bacterial organisms.

Place and Duration of Study: The investigation was carried out at University of Maiduguri in Borno State, Nigeria. The herb obtained from Lake Chad Research Institute were identified and validated

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by the department of Forestry and wild life, Mohammed Lawan College of Agriculture (MOLCA) in Maiduguri, Borno State.

Methodology: Crude methanolic leaf extraction of the plant, qualitative phytochemical screening, antimicrobial sensitivity against some disease-causing organisms, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried out.

Results: Phytochemical screening of *V. amygdalina* leaves extract revealed the presence of tannins, saponins, terpenoids, flavonoid, carbohydrates and cardiac glycosides. The antimicrobial sensitivity shows *P. aeruginosa*, had the highest sensitivity with effect at all concentrations (26 mm at 1000 mg/ml), the lowest against *Salmonella typhi* were 9 mm at 1000 mg/ml and 7 mm at 200 mg/ml, while the highest were 17 mm at 400 mg/ml and 14 mm at 200 mg/ml. When tested at all concentrations (200-1000 mg/ml), *S. aureus*, *S. pyogenes*, *B. subtilis*, *Corynebacteria species*, and *K. pneumonia* showed greater sensitivity than *Salmonella typhi* but less sensitivity to *P. aeruginosa*. Based on the outcomes of the MIC and MBC results, *S. aureus* was found to be sensitive to the extract at 100 and 200 mg/ml, while *P. aeruginosa* was found to have the highest sensitivity to the extract at all concentrations (25-200 mg/ml) with the exception of 12.5 mg/ml of the extract.

Conclusion: The study highlighted the antimicrobial effects of *V. amygdalina* leave extracts on some pathogens thereby verifying the traditional healer's claim. Also, it was concluded that the extract of *V. amygdalina* contained pharmacologically active phytochemicals which could be responsible for the numerous medicinal properties exhibited by the plant leaf extract.

Keywords: *Vernonia amygdalina*; methanol; antimicrobial; phytochemicals; minimum inhibitory concentration; minimum bactericidal concentration.

1. INTRODUCTION

Plants have been utilized to cure and manage a variety of ailments due to the presence of phytochemicals. Worldwide, there is a steady rise in the usage of medicinal herbs. The advent of resistant diseases and the advancement of scientific understanding of herbal remedies as significant treatment alternatives have led to an increase in the quest for therapeutic molecules originating from plant species [1]. Despite the significant advances in modern medicine, phytotherapy is still widely utilized today since medicinal plants have been used to prevent and treat a variety of health issues for many years [2-4]. Depending on a number of variables like the weather, ecology, the time and age of collection, and other considerations, the various phytochemicals generated by plants can vary both qualitatively and quantitatively [4-5].

Vernonia amygdalina is well known for its wide range of uses in traditional medicine [4]. A member of the family Asteraceae, *Vernonia amygdalina* is also known as bitter leaf in English, ewuro in Yoruba, Shiwaka in Hausa, and olubo in Igbo [6].

Bitter leaf is a 2–5 m tall shrub plant that thrives in a variety of ecological zones throughout Africa. It produces enormous amounts of forage and is drought tolerant. The leaf is green and has a distinct odour and harsh taste [7]. Non-nutritional

elements such alkaloids, saponins, tannin, terphenoids, glycosides, and steroids are to blame for the bitter test [6,8].

Vernonia amygdalina is a perennial shrub. It is a vegetable that is frequently utilized in tropical African cuisine and folk medicine. Traditional users claim that this plant has palpable health advantages. Numerous vitamins, minerals, and phytochemicals are present in it, including iodine, alkaloids, anthraquinones, edotides, sesquiterpene lactones, and steroid glycosides. The health advantages of the plant are linked to these bioactive substances [9].

Vernonia amygdalina has favourable effects on health, which are invariably attributed to its nutritional and phytochemical richness. Many of its mechanisms of action have not been fully understood in humans, despite the presence of compounds with anticancer effects, antioxidant properties, antimalarial properties, anti-inflammatory properties, antibacterial characteristics, and hypolipidemic benefits [9].

Alkaloids, steroids, flavonoids, phenols, saponins, terpenes, cyanogenic glucoside, tannin, anthraquinone, phytate, oxalate, and lignans have been identified in *V. amygdalina* by phytochemical analysis [10,11]. These phytochemicals may be the cause of its physiological effects, which include those that are hypolipidemic, anti-diabetic, anti-microbial,

anti-allergic, antibacterial, antimalarial, anti-fungal, anti-cancer, anti-leukaemia, anti-inflammatory, analgesic, antipyretic, antioxidative, anthelmintic, and antimicrobial [11-13].

V. amygdalina extracts in aqueous, ethanol, and methanol suppress bacteria and fungi linked to food deterioration and pathogenicity. Extracts of *V. amygdalina* leaves have been reported to be effective against a variety of microorganisms, including *Candida albicans*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Periodontal bacteria are prevented from proliferating when chewing on sticks made of *Vernonia amygdalina* wood. There is evidence that its leaves can inhibit the growth of a number of dangerous bacteria and viruses [14-16]. Alkaloids, flavonoids, and saponins are what give *V. amygdalina* its antibacterial characteristics. Vernodalol, vernodalin, and vernolide are some of the specific chemicals linked to this plant's antibacterial properties (sesquiterpene lactones) [16]. Sesquiterpene lactones have been linked to antibacterial, anti-protozoal, and anti-tumor effects [17-19].

The leaves of *Vernonia amygdalina* are primarily consumed by humans. Washing is frequently required to lessen the harsh taste that water-soluble saponins generate. Sesquiterpene lactones (such as vernodalin, vernolepin, and vernomygdin) and steroid glucosides are what give *V. amygdalina* its bitter flavor (vernioniosides). Usually, a fresh leaf is washed before being added to food while it is being prepared. African breadfruit, yam porridge, and other foods including soups (such as Ogbono, Egusi, Okro, and the well-known bitter leaf soup) can all be made using *V. amygdalina*. The leaves are consumed as a vegetable and aid with digestion. The leaves have been used in place of hops in the beer-brewing sector [20]. The stems are eaten by domestic animals. They are also used by humans as a chewing stick. The leaves may be used as a hop substitute and an antioxidant [21].

Traditional medicine has utilized *Vernonia amygdalina* extensively to manage and treat a variety of illnesses. It is employed in some situations to control and treat conditions like diabetes, malaria, and gastrointestinal problems. Additionally, a local infusion of the leaves or roots is used to treat microbiological infections, schistosomiasis, cough, hepatitis, and other

disorders that are spread through sexual activity [22,23]. The roots and leaves are used in traditional medicine to cure fever, hiccups, and renal issues while, [24]; the wood, from the root, can be used to clean the tooth [25]. According to reports [26], *V. amygdalina*'s fresh leaves work wonders as a purgative. In some parts of Africa, it is used to make cough medicine [27].

One of *Vernonia amygdalina*'s most potent substances, saponins are complex molecules whose glycans are triterpenoids or steroidal [28,29]. Saponins are starch glycosides that have three hydroxyl groups connected to complicated sugar components [28]. It is an amorphous white or light brown powder that initially tastes pleasant before turning bitter and even acidic. The gastrointestinal tract is thought to be too irritated by saponins for them to be employed internally [28,30]. *Vernonia amygdalina*'s steroidal saponin levels appear to be higher than those of other plants [28,31]. The leaves of *Vernonia amygdalina* also contain flavones (flavonoids), which are antioxidants with therapeutic benefits against carcinogens and aging [28,32].

Clinical research is needed to support the benefits of this plant product on health. More animal models' experiments are required to prove the potency of this plant product. Based on findings from compositional analysis and animal bioassays, this plant product is believed to have health advantages. There is a need to establish these claims in humans. There are a lot of claims on this plant-based on ethnomedicine and traditional uses [9].

Numerous studies indicate that its use is largely dose-dependent for successful results [33]. When taken as an antimalarial or to increase fertility [34], it may occasionally exhibit adverse consequences [35]. Therefore, the goal of this study is to evaluate the phytochemicals and antimicrobial activities of *Vernonia amygdalina* (Bitter Leaf) methanolic leaf extracts.

2. MATERIALS AND METHODS

2.1 Study Area

The investigation was carried out at Department of Veterinary Medicine, University of Maiduguri in Maiduguri, Borno State, Nigeria. Maiduguri is located between latitudes 11.8° N and 13.2° N and longitudes 9.8° E and 14.4 E, it lies at latitude 354 m above sea level. The dry (October–May) and wet (June–September)

seasons are the most common. The yearly rainfall is between 9 and 198 mm, the temperature goes from 13°C to 41°C, and the relative humidity fluctuates between 19% and 78% but stays around 45% during the wet season. Seven to nine hours a day are spent in the sun [36].

2.2 Plant Components

The Lake Chad Research Institute provided the *V. amygdalina* leaves, which the Forestry Department of the Mohammed Lawan College of Agriculture (MOLCA) in Borno State identified and validated the plant. The leaves were cleaned with fresh water before being dried under a shed at room temperature. The dried leaves were grounded into a fine powder using a wooden pestle and mortar and stored in a clean plastic container for further processing.

2.3 Methodology

2.3.1 Extraction of the plant material

Exactly 1.5 liters of 95% methanol were added to the two-liter round bottom flask in which the powdered material had been previously placed. The combination was refluxed for approximately two hours, after which the solution was withdrawn for filter paper-based debris removal. After evaporation a (green) substance was obtained and subsequently concentrated on hot air ovum at 40°C – 50°C.

2.3.2 Phytochemical screening of the extract

Using the approach outlined by [37,38], the crude methanolic extract of *V. amygdalina* was subjected to qualitative chemical screening for the identification of the various classes of chemical constituent.

i. Test for alkaloids

On a steam bath, precisely 0.5 g of the extract were mixed with 5.0 ml of 2 M aqueous hydrochloric acid. 1.0 ml of the filtrate were treated separately with a few drops of Mayer's reagent; the appearance of buff-colored was an indication for the presence of alkaloid. Also, the appearance of orange red precipitate from Drangendoff's reagent; and the appearance of dark-brown precipitate from Wangner's reagent; was an indication for the presence of alkaloid [39].

2.3.3 Test for tannins

Exactly 10 ml of distilled water and precisely 0.5 g of plant extracts was combined. A few drops of a 1% solution of ferric chloride was added to the mixture after filtering, and 2.0 ml of the filtrate was used for the test. The presence of tannin was indicated by the presence of blue-black, green, or blue-green precipitate colors. The filtrate was then mixed with an equal volume of 10% lead ethanoate. If white precipitate appears, it indicates the presence of tannins. The addition of three drops of 10% HCL and one drop of methanol to the filtrate of the extract after heating, the presence of tannins was determined by the presence of crimson precipitate [40-41].

i. Test for Phlobatannins

The extract was heated with little amount of distilled water before filtration. The filtrate was further boiled with 1% aqueous HCL. The presence of phlobatannins was revealed by the formation of red precipitate [41].

ii. Test for Glycosides

a. Liebermann-Bur Chard's Test. (Test for Steroid Nucleus).

2 ml of acetic anhydride was added to the extract (0.5 g). The mixture was cool in ice before being placed in a cone. Tetraoxosulphate (vi) acid was carefully added. The presence of a steroidal ring was suggested by the appearance of a reddish-brown colour or yellow in the interphase [42].

b. Salkowski's Test. (Test for steroidal Nucleus)

Tetraoxosulphate (VI) acid was carefully added by the side of the test tube to generate a lower layer after the addition of 2 ml of chloroform to extract (0.5 g). The presence of a steroidal ring is indicated by the appearance of a reddish-brown color or yellow in the interphase [42].

2.3.4 Test for saponins

To ascertain the presence of saponins, the froth test and emulsion test as reported by [43] was employed. In a 100 ml beaker, distilled water (20 ml) was mixed with a little amount of each plant extract, boiled, and the filtrate utilized for the test:

(a) The froth test was performed using 5 ml of filtrate diluted with 20 ml of water, forcefully shaken, and left to stand for 30 minutes. The outcome was recorded.

(b) A tiny amount of filtrate was mixed with 2.5 ml of fehling's solutions A and B in an equal volume. A brick-red precipitate's appearance was a sign that glycosides were present [44].

2.3.5 Test for free anthraquinones (Borntrager's test)

Exactly 0.5 g of the plant extract was shaken with 10 ml benzene. After filtering the mixture, the filtrate was mixed with 5 ml of a 10% ammonia solution. The combined product was shaken. Anthraquinones when present will appear as pink, red, or violet color at the lower phase [41].

2.3.6 Test for flavonoids

A. Test for Ferric Chloride

A tiny amount of the extract was heated with water before being filtered. A few drops of 10% ferric chloride were added to 2 ml of the filtrate. The presence of a phenolic hydroxyl group was indicated by a green, blue, or violet coloration.

B. The Shinoda Test

The extract was heated, filtered, and 0.5 g of it was distilled in ethanol. After adding a few drops of strong hydrochloric acid to the filtrate, three pieces of magnesium chips were added [45].

C. Lead Acetate Test

Exactly 5 ml of distilled water were used to dissolve up to 0.5 g of the extract. 3.0 ml of 10% lead acetate solution was added. The presence of flavonoids was revealed by the formation of buff-colored precipitate [39].

D. Sodium Hydroxide

A tiny amount of the extract was dissolved in water and filtered. To give the filtrate a yellow coloration, 2 ml of a 10% sodium hydroxide aqueous solution was added. When strong hydrochloric acid was added, the color changed from yellow to colorless, indicating the presence of flavonoids [41].

2.3.7 Test for cardenolides

The extract was dissolved in ethanol in a little amount. It was then followed by the addition of concentrated tetraoxosulphate (VI) acid and 1 ml of acetic acid anhydride. The sample's color changed from pink to violet, indicating the presence of terpenoid [42].

i. Keller-Killian's test

0.5 g of the extract was dissolved in 2 ml of glacial acetic acid with a drop of ferric chloride solution. A brown ring that formed during the interphase indicated the existence of cardenolide's distinctive digitoxose sugar. A violet ring appeared just below the brown ring while the acetic acid layer; a greenish ring appeared just below the brown ring and gradually spread throughout this layer [41].

2.3.8 Test for carbohydrates; general test (Molisch's test)

To the extract that had been dissolved in distilled water, a few drops of Molisch's reagent were added. Following this, 1 ml of concentrated tetraoxosulphate (VI) acid was added by the side of the test tube, forming an acid layer below the aqueous layer. After letting the mixture sit for two minutes, it was diluted with 5 ml of purified water. The development of a reddish to dull violet color at the boundary between the two layers was seen as a positive test [41].

2.3.9 Test for monosaccharide (Barfoed's test)

In distilled water, the extract (0.5 g) was dissolved before being filtered. In a test tube, 1 ml of the filtrate was combined with 1 ml of Barfoed's reagent before being heated over a water bath for two minutes. A crimson cuprous oxide precipitate was regarded as a positive test result [39].

2.3.10 Test for free reducing sugars (Fehling's test)

The extract (0.2 g) was dissolved in distilled water and filtered. Few drops of Fehling's solution were added to the filtrate and then heated on a water bath for 2 minutes. Appearance of a reddish-brown precipitate of cuprous oxide indicates the presence of combined reducing sugars [41].

2.3.11 Test for combined reducing sugar's

The extract (0.2 g) was boiled with 5 ml of diluted hydrochloric acid to hydrolysed it, and the resultant filtrate was then neutralized with sodium hydroxide solution. It was then cooked on a water bath for two minutes with a few drops of Fehling's solution added. Because mixed sugars were present, a reddish-brown cuprous oxide precipitate formed [41].

2.3.12 Test for ketones (Salivanoff's test)

A little amount of the extract was mixed with a few resorcinol crystals and 2 ml of strong hydrochloric acid, and the mixture was then allowed to boil for five minutes. The presence of ketones was indicated by a red coloration [44].

2.3.13 Test for soluble starch

With 1 ml of 5% potassium hydroxide that had been cooled and acidified with tetraoxosulphate (VI) acid, a small amount of the extract was added. If the colouring was yellowish, then soluble starch was present [44].

2.3.14 Antimicrobial activity assessment

Microorganism source

Staphylococcus aureus, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Corynebacterium species* and *Salmonella typhi* the only fungus utilized *Candida albicans* were used as the test organisms in this investigation. These isolates were acquired from the Department of Medical Microbiology, University of Maiduguri Teaching Hospital.

2.3.15 Anti-microbial test

The clinical and laboratory standards institute CLSI, [46] method for conducting the anti-microbial susceptibility test was used, with only minor modifications made by [47]. The test was performed using a stock concentration of 100 mg/ml that was created by dissolving 1 gram of crude extract into 10 ml of sterile distilled water using dilution ratios of 1: 100 and 1:500 for gram-positive bacteria and gram-negative bacteria, respectively [47]. The surface of sterile petri dishes containing sterile solid nutritional agar was aseptically inoculated with around 0.5 ml of the diluted cultures. After incubating at 37°C for 24 hours, discs impregnated with the crude extract at a concentration of 5 mg were aseptically mounted on agar. The inhibitory zone was then measured in mm using a transparent meter rule. Amoxiclav (30 mg), ceflunat (30 ug), levofloxacin (5 ug), loxacin (5 ug), and riflatab were the standard anti-microbials utilized in the test, which was done in triplicate (5 ug)

2.3.16 Minimum inhibitory concentration (MIC)

The MIC is defined as the concentration at which there is no discernible turbidity in the test tubes. Following [48] earlier description, [47] used some magnification to obtain a concentration. For the bacteria that displayed reasonable sensitivity to the test extract, the MIC was established. The microorganism used in this test was created using broth dilution procedures. The working concentrations were made using two-fold serial dilution techniques, which varied from 0-195 mg/ml to 50 mg/ml using nutrient broth, and were then inoculated with 0.2 ml solution of the test organism. This yielded the stocked extract concentration of 100 mg/mg. After 24 hours of 37°C incubation, the tube was checked for turbidity at the lowest concentration where there was none.

2.3.17 Minimum bactericidal concentration (MBC)

MBC is the minimal bactericidal concentration determined from Broth dilution test resulting from the MBC tubes as describe previously [47-48]. By inoculating the content of each test tube on a nutrient agar plate, the plate was then incubated at 37°C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as a minimum bactericidal concentration.

3. RESULTS

3.1 Qualitative Phytochemical Analysis

The mixture was refluxed for two the results from the phytochemical analysis, are indicated in Table 1. Alkaloids were absent as well as free and combined anthraquinone. Cardiac glycoside, terpenoid, saponin, tannins, flavonoids were present in the extract. Others includes presence of reducing sugar, cardenolides and soluble starch (Table 1).

3.2 Antibacterial Sensitivity Study

Table 2 lists the outcomes of the *in vitro* antimicrobial screening. The results showed that the extract significantly inhibited the test organism, with *Pseudomonas aeruginosa* showing the highest activity across concentration levels (26 mm at 1000 mg/ml), followed by *Salmonella typhi* (9 mm at 1000 mg/ml) and

(7mm at 800 mg/ml), *Corynebacterium species* (11 mm at 600 mg/ml), and *Staphylococcus* (1 mm at 400 mg/ml). Although not statistically comparable to that provided by the extract, the zone of inhibition created by the majority of antibiotic discs against some of the organisms was determined to be substantial in respect to those activities produced by the organisms under examination. However, inhibitory zones with widths less than 10 mm were regarded as active. This observation is consistent with research from [47,49]. According to the results of the minimum inhibition concentration (MIC) tests shown in Tables 3 and 4, it was found that the extract had the broadest activity against gram positive organisms (*Staphylococcus aureus*, *Staphylococcus pyogenes*, *Bacillus subtilis*, *Klebsiella pneumonia*, and *Pseudomonas*

aeruginosa) at the concentrations of 200 mg/ml, 100 mg/ml, and 50 mg/ml, while lowest activity was recorded against the organisms at 25 mg/ml and 12.5 mg/ml. The extract exhibit considerable activity against *Candida albicans* and *E. Coli* in Table 2.

P. aeruginosa was found to have the highest sensitivity to the extract with effects at all concentrations (200-1000 mg/ml), followed by *K. pneumonia*, *S. pyogenes*, *B. subtilis*, and *S. aureus*, with *Salmonella typhi* being recorded with the lowest sensitivity at only 800-1000 mg/ml. Table 2 shows zone of growth inhibition (mm) at various concentrations of the plant's (*Vernonia amygdalina*) *E. coli* and *Candida albicans* were found to be resistant over the 200–1000 mg/ml range of extract concentration.

Table 1. Phytochemical test results

S/N	TEST(S)	Values
1.	Test for Alkaloid	
	I. Dragendroff's reagent test	-
	II. Mayer's reagent test	-
2.	Test for cardiac glycoside	
	I. Salkowski's test	+
	II. Liebermann burchard's test	+
3.	Test for free Anthraquinone	-
4.	Test for combined Anthraquinone	-
5.	Test for Terpenoid	+
6.	Test for Cardenolides	
	I. Keller-killian's test	+
7.	Test for Saponins	
	I. Frothing test	+
8.	Test for Tannins	
	I. Ferric Chloride test	+
	II. Lead Acetate test	+
9.	Test for Flavonoids	
	I. Shinoda's test	+
	II. Ferric Chloride test	+
	III. Lead Acetate test	+
	IV. Sodium Hydroxide test	-
10.	Test for soluble Starch	+
11.	Test for phlobatannins	-
12.	Test for Carbohydrates	
	I. Molish's test	-
	II. Test for monosaccharide (barfoed's test)	+
	III. Test for free reducing sugars (fehling's test)	+
	IV. Test combined reducing sugar	+
	V. Test for ketoses	-

Key: + indicates presence, - indicates absence

According to Table 1 above, the phytochemical analysis of *V. amygdalina* revealed the presence of cardiac glycoside, terpenoid, tannin, cardenolides, saponins, flavonoids, and carbohydrates but the absence of alkaloid, phlobatannins, and anthraquinone.

Table 3 displays the minimum inhibitory concentration (MIC) of the leaves of *Vernonia amygdalina* extract on various organisms tested on, with the majority of results demonstrating sensitivity to the extract. *P. aeruginosa* was found to have the highest MIC, with effects at concentrations (25-200 mg/ml), and lowest activity at 12.5 mg/ml, whereas *S. aureus* demonstrate activity at 100–200 mg/ml concentration, *S. pyogenes*. *B. subtilis* *K.*

Pneumonia exhibits moderate activity to the extract, between 50 and 200 mg/ml concentration.

Table 4 displays the minimum bactericidal concentration (MBC) of the leaves of *Vernonia amygdalina* extract on various bacterial isolates. The results indicates that most of the isolates were sensitive to the extract. *P. aeruginosa* was found to have the highest MBC, with effects at concentrations (50-200 mg/ml), whereas *S. aureus* demonstrate activity at 100–200 mg/ml concentration, *S. pyogenes*. *B. subtilis* *K.* *Pneumonia* exhibits moderate activity to the extract, between 50 and 200 mg/ml concentration.

Table 2. The antimicrobial activities of *Vernonia amygdalina* leaf extra on micro-organism inhibition zone

S/N		1000	800	600	400	200
1.	<i>Staphylococcus aureus</i>	17	15	13	11	9
2.	<i>Streptococcus pyogenes</i>	20	18	15	13	11
3.	<i>Bacillus subtilis</i>	18	16	14	12	10
4.	<i>Corynebacterium spp.</i>	18	13	11	9	7
5.	<i>Escherichia coli</i>	0	0	0	0	0
6.	<i>Salmonella typhi</i>	9	7	0	0	0
7.	<i>Pseudomonas aeruginosa</i>	26	23	20	17	14
8.	<i>Klebsiella pneumonia</i>	18	18	15	13	11
9.	<i>Candida albicans</i>	0	0	0	0	0

NB.0=Resistance

Table 3. The antimicrobial activities of *Vernonia amygdalina* leaf extract showing minimum Inhibitory Concentration of (mg/ml)

S/N	Organisms	200	100	50	25	12.5
1.	<i>Staphylococcus aureus</i>	-	-	+	+	+
2.	<i>Streptococcus pyogenes</i>	-	-	-	+	+
3.	<i>Bacillus subtilis</i>	-	-	-	+	+
4.	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-
5.	<i>Klebsiella pneumonia</i>	-	-	-	+	+

Key: MIC; indicates the lowest concentration of the plant extract that will inhibit the visible growth of a microorganism.

NB. (- = Negative and + = positive)

Table 4. The Antimicrobial activities of *Vernonia amygdalina* leaf extract showing minimum Bactericidal Concentration of (mg/ml)

S/N	Organisms	200	100	50	25	12.5
1.	<i>Staphylococcus aureus</i>	-	-	+	+	+
2.	<i>Streptococcus pyogenes</i>	-	-	-	+	+
3.	<i>Bacillus subtilis</i>	-	-	+	+	+
7.	<i>Pseudomonas aeruginosa</i>	-	-	-	+	+
8.	<i>Klebsiella pneumonia</i>	-	-	-	+	+

Key: MBC; indicates lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media

NB. (- = Negative and + = positive)

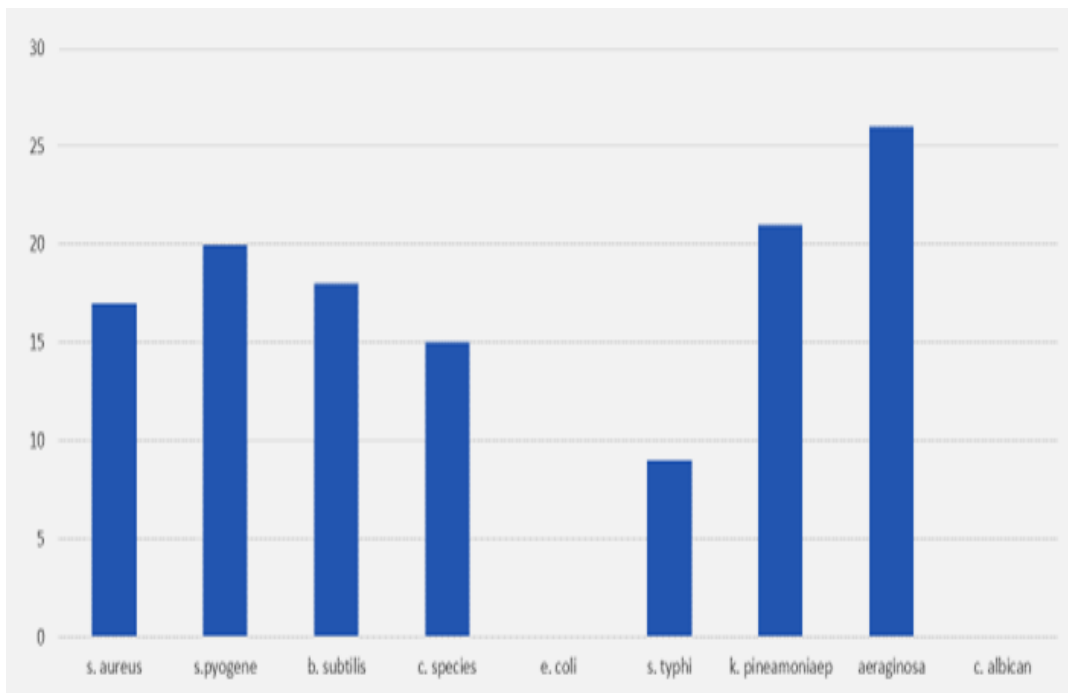


Fig. 1. demonstrated the effect of varying concentration of the plant leaf extract in a dose dependent manner. *Pseudomonas aeruginosa* shows the highest peak of activity (26 mm at 1000mg/ml), and activity declines as the concentration decreases. *Escherichia coli* and *Candida albicans* demonstrated no activity at all concentration of the extract (indicating resistance)

4. DISCUSSION

The results obtained from the phytochemical analysis of methanolic leaf extract of *Vernonia amygdalina* are in accordance with the findings obtained by other researchers [49-54]. The absence of alkaloids and anthraquinones contradicts the findings of Usunomena and Ngozi [55], that reported the presence of these bioactive metabolites. The variation in observation could be due to differences in solvent used for the study, geographical location, processing and extraction methods. The presence of phytochemical components may be responsible for the observed antimicrobial activity of the leaf extract. These findings conform to the report of Ojmelukwe and Amaechi [9]. When the extract was tested using comparative test methods by impregnated disc gel diffusion protocol against the test organisms, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Corynebacterium species* and *Salmonella typhi*, a specific pattern regarding the effect of the extract on inhibitory zone radii was discernible,

except for *Escherichia coli* and *Candida albicans*. When the extract was deployed in a range of concentrations from 200 mg/ml to 1000 mg/ml. it was discovered that 1000 mg/ml, 800 mg/ml and 600 mg/ml demonstrated highest inhibition radii in the increasing order of concentration against the test organisms, thus indicating dose dependent effect and antimicrobial potency. This finding agrees with Ibrahim and colleagues [56]. Lower concentrations of the extract are 400 mg/ml and below produced lower zone of inhibition radii significantly less than extract at 1000 mg/ml and 800 mg/ml. At lower concentration, these observations tend to imply that the extract antimicrobial activity increased as the concentration increased in a dose dependent manner. *Candida albicans* and *Escherichia coli*, on the other hand, demonstrated zero activity, this agrees with the study by Oshim and colleagues [57], that reported the resistance of *Candida albicans* to the extract at all concentrations. This implies that the two organisms are resistance to the extract at all concentrations. The sensitivity test against the microorganisms was demonstrated in the *in vitro* as shown in Table 2.

Pseudomonas aeruginosa, showed the highest sensitivity (26 mm at 1000 mg/ml) with effect at all concentrations including 20 mm at 600 mg/ml and 23 mm at 800 mg/ml. *Salmonella typhi* demonstrated the lowest levels of inhibition of the extract among the test organisms with inhibition radii, 9 mm at 1000 mg/ml and 7 mm at 800 mg/ml, when tested at all concentrations (200-1000 mg/ml). Other test isolates like *S. aureus*, *S. pyogenes*, *B. subtilis*, *Corynebacterium species*, and *K. pneumonia* showed greater sensitivity than *Salmonella typhi* but less sensitivity to *P. aeruginosa*. The antibiotic disc's zone of inhibition against the tested organism was determined to be notable in respect to the study's sensitivity but not statistically in comparison to the extract's zone of inhibition. However, zones of inhibition with a diameter of 10 mm were thought to be active. This observation is consistent with research from Ngatu and colleagues [13]; Dumas and colleagues [58] which showed that the extracts of *V. amygdalina* exhibited inhibitory activity on all tested bacteria including *Staphylococcus aureus*, *Salmonella enterica* and *Klebsiella pneumoniae* [59]. Habtamu and Melaku [60], demonstrated the chloroform extract of *V. amygdalina* showed strong activity against *S. aureus* with an inhibition zone of 21 mm. This is in contrary to the findings in the current study which demonstrated higher activity of the extract to *Pseudomonas aeruginosa*, followed by other organisms. Chukwuemeka and colleagues [61], showed that the extract inhibited *S. aureus*, *Bacillus subtilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* activities in mice, which agrees to this study. Based on the outcomes of the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) results, respectively, as shown in Tables 3 and 4, *S. aureus* was found to be sensitive at only 100-200 mg/ml However, *P. aeruginosa* was found to have the highest sensitivity with effect at all concentrations (25-200 mg/ml) except for the lowest (12.5 mg/ml).

5. CONCLUSION

This study highlighted the antimicrobial effects of *V. amygdalina* methanolic leaves extracts on some bacterial isolates to confirm their folkloric use. The extract was observed to contain pharmacologically active phytochemicals which could be responsible for the numerous medicinal properties exhibited by the plant leaf extract and has a lot of potential as an antibacterial agent for the treatment of infection-causing pathogens.

Further work should be carried out in order to isolate the active compounds for further antimicrobial, pharmacological and clinical testing.

6. RECOMMENDATION

The leaf extract of *Vernonia amygdalina* demonstrated antimicrobial activity which makes it an excellent functional component to be utilized in the treatment of various infectious diseases. Further studies and additional extensive research are encouraged to discover the mode of action, plant's therapeutic characteristics, quantitative analysis, the mineral makeup of the entire plant and the specific phytochemical component responsible for demonstrated antibacterial activity. Additional human clinical studies are required to discover effective and safe dosages for the treatment of various diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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