Original Research Article

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

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

Methodology: Crude methanolic leaf extraction of 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 (26mm at 1000mg/ml), the lowest against *Salmonella typhi* were 9mm at 1000mg/ml and 7mm at 200mg/ml, while the highest were 17mm at 400mg/ml and 14mm at 200mg/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 minimum inhibition concentration (MIC) and minimum Bactericidal (MBC) results, respectively shows *S. aureus* was found to be sensitive at only 100-200mg/ml, however *P. aeruginosa* was found to have the highest sensitivity with effect at all concentrations (25-200mg/ml) extract for the lowest (12.5).

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(remove it) contain(past form) pharmacologically active phytochemicals which could be responsible for the numerous medicinal properties exhibited by the plant leaf extract.

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

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].

Alkaloids, steroids, flavonoids, phenols, saponins, terpenes, cyanogenic glucoside, tannin, anthraquinone, phytate, oxalate, and lignans have been identified in *V. amygdalina* by phytochemical analysis [9,10].

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-leukemia, anti-inflammatory, analgesic, antipyretic, anti-oxidative, anti-helminthic, and antimicrobial [10-12].

Numerous studies indicate that its use is largely dose-dependent for successful results [13]. When taken as an antimalarial or to increase fertility [14], it may occasionally exhibit adverse consequences [15]. Therefore, the goal of this study is to evaluate the Phytochemicals and antimicrobial activities of *Vernonia amygdalina* (Better Leaf) leaf extracts.

MATERIALS AND METHODS

Study Area

The investigation was carried out at University of Maiduguri in Borno State, Nigeria. Between latitudes 10.2° N and 13.4° N and longitudes 9.8° E and 14.4 E, Maiduguri is at a latitude of 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 [16].

Plant Components

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

Methodology

Extraction of the Plant Material

1.5 litters of 95% methanol were added to the two-litter 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, the entire solution was concentrated on hot air ovum at 40 °C – 50 °C. Take the weight and transfer to an air tide container for analysis.

Phytochemical Screening of the Extract

Using the approach outlined by [17] [18], the crude aqueous 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 0.5g of the extract were mixed with 5.0 ml of 2 M aqueous hydrochloric acid. 1.0 ml of the filtrate will be treated separately with a few drops of Mayer's reagent; the appearance of buff-colored will be an indication for the presence of alkaloid; the appearance of orange red precipitate from Drangendoff's reagent; and the appearance of dark-brown precipitate from Wangner's reagent; will be an indication for the presence of alkaloid [19].

Test for Tannins

10ml of distilled water and precisely 0.5g of plant extracts was combined. A few drops of a 1% solution of ferric chloride will be added to the mixture after filtering, and 2.0 ml of the filtrate will be used for the test. The presence of tannin will be indicated by the presence of blue-black, green, or blue-green precipitate colors. The filtrate will be mixed with an equal volume of 10% lead ethanoate. The occurrence of tannins will be revealed by the production of white precipitate. Three drops of 10% HCL and one drop of methanol will be added to the filtrate of the extract when it is heated. The presence of tannins will be determined by a crimson precipitate [20-21].

i. Test for Phlobatannins

The extracts were heated in a little amount of distilled water before being filtered. With 1% aqueous HCI, the filtrate will be further heated. Phlobatannins' appearance [21].

ii. Test for Glycosides

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

2ml of acetic anhydride was added to the extract (0.5g). The mixture will cool in ice before being placed in a cone. Carefully added tetraoxosulphate (vi) acid will be used. If the color changes from violet to blue or bluish-green, a steroidal ring is present [22].

a. 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 2ml of chloroform to extract (0.5g). The presence of a steroidal ring is indicated by the appearance of a reddish-brown color or yellow in the interphase [22].

Test for Saponins

To ascertain the presence of saponins, the froth test and emulsion test as reported by [23] will be 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 noted.

(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 [24].

Test for free anthraquinones (Borntrager's test)

10ml of aqueous tetraoxosulphate (vi) acid was added to the extract (0.5g), agitated, and then transferred while it is still hot. Five milliliters of benzene will be mixed with the filtrate. After being separated, the benzene layer will be given half of its own volume of 10% ammonia solution. Indicators of mixed anthraquinones include pink, red, and violet coloring in the ammoniac (lower) phase [21].

Test for flavonoids

A. Test for Ferric Chloride

A tiny amount of the extract will be heated with water before being filtered. the filtrate, to 2ml.

B. The Shinoda Test

The extract (0.5g) were warmed and filtered after being resolved in ethanol. Then, a few drops of com will be poured to the filtrate, followed by the pieces of magnesium chips. HCI Flavonoids will be present if the coloration is pink, orange, or red to purple [25].

C. Lead Acetate Test

5ml of distilled water were used to dissolve up to 0.5g 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 [19].

D. Sodium Hydroxide

A tiny amount of the extract was dissolved in water and filtered. To give the filtrate a yellow coloration, 2ml 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 [21].

Test for Cardenolites

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 [22].

i. Keller-killian's test

In 2ml of glacial acetic acid that also contained a drop of ferric chloride solution, the extract (0.5g) was dissolved. The next step was adding 1ml of concentrated tetraoxosulphate (VI) acid. A brown ring that formed during the interphase indicated the presence of the cardenolide-specific digitoxose sugar. During the acetic acid layer, a violet ring developed just below the brown ring; a greenish ring appeared just below the brown ring and slowly spread throughout this layer [21].

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 5ml 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 [21].

Test for Monosaccharide (Barfoed's test)

In distilled water, the extract (0.5g) was dissolved before being filtered. In a test tube, 1ml of the filtrate was combined with 1ml 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 [19].

Test For free Reducing Sugars (Fehling's test)

The extract (0.2g) was filtered after being dissolved in distilled water. The presence of reducing sugars was detected by formatin, which appeared as a red cuprous oxide precipitate after heating the filtrate with 5 ml each of fehling's solutions A and B. [21].

Test for Combined Reducing sugar's

The extract (0.2g) was boiled with 5ml of diluted hydrochloric acid to hydrolyze 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 [21].

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 assumed to be indicated by a red coloration (24).

Test for Soluble Starch

With 1ml of 5% potassium hydroxide that had been cooled and acidified with tetraoxosulphate (VI) acid, a small amount of the extract was added. It was determined that the coloring was yellowish and that soluble starch was present [24].

Antimicrobial Activity Assessment

Microorganism Source

Staphylococcus aureus, Escherichia coli, klebsiella pneumonia, Pseudomonas aeruginosa and Salmonella typhi the only fungus utilized Candida albicans were used as the test organisms in this investigation. They were acquired from the university of Maiduguri Teaching Hospital's department of medical microbiology.

Anti-Microbial Test

The clinical and laboratory standards institute CLSI, [26] method for conducting the anti-microbial susceptibility test was used, with only minor modifications made by [27]. 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 1.5000 for gram-positive bacteria and gram-negative bacteria, respectively [27]. 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. Amoxicave (30 mg), ceflunat (30 ug), levoxine (5 ug), loxacine (5 ug), and reflotab were the standard anti-microbials utilized in the test, which was done in triplicate (5 ug)

Minimum Inhibitory Concentration (MIC)

The MIC is defined as the concentration at which there is no discernible turbidity in the test tubes. Following [28] earlier description, [27] 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 0-195mg/ml to 50mg/ml using nutrient broth, and were then inoculated with 0.2ml solution of the test organism. This yielded the stocked extract concentration of 100mg/mg. After 24 hours of 37°C incubation, the tube was checked for turbidity at the lowest concentration where there was none.

Minimum Bactericidal Concentration (MBC)

MBC is the minimal bactericidal concentration was determined from Broth dilution test resulting from the MBC tubes as describe previously [27-28]. By inoculating the content of each test tube on a nutrient agar plate. The plate was then incubated at 37℃ for 24hrs. The lowest concentration of the extract that showed no growth was noted and recorded as a minimum bactericidal concentration.

RESULTS

Qualitative phytochemical analysis

The resulting extract was evaporated to dryness in a hot air oven and then subjected to phytochemical analysis, as indicated in table 4, after being made from the methanolic leaf extract of dried powdered Acacia nilotica. Table 1 lists the different chemicals found in the crude extract.

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 (26mm at 1000mg/ml), followed by *Salmonella typhi* (9mm at 1000mg/ml) and (7mm at 800mg/ml), *Corynebacteria* species (11mm at 600mg/ml), and *Staphylococcus* (1mm at 400mg/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 [27, 29]. 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 Table 3 shows that the extract has significant action against candida and E. coli. This study contradicted the conclusion of [27].

P. aeruginosa was found to have the highest sensitivity to the extract with effects at all concentrations (200-2100mg/ml), followed by *K. pneumonia, S. pyogenes, B. subtilis, and S. aureus, with Salmonella typhi* being recorded with the lowest sensitivity at only 800-1000mg/l. 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 Cardenolites	
	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, cardenolites, saponnins, flavonoids, and carbohydrates but the absence of alkaloid, phlobtannins, and anthraquinone.

Table 2. The Antimicrobial Activities of *Vernonia amygdalina* leaf Extra on micro-organism Inhibition zone

S/N	ORGANISMS	1000	800	600	400	200
1.	*Staphylococcus aureus*	17	15	13	11	9
2.	*Streptococcus pyogenes*	20	18	15	13	11
3.	*Bacillus subtilis*	18	16	14	12	10
4.	*Corynebacterium spp.*	18	13	11	9	7
5.	*Escherichia coli*	0	0	0	0	0
6.	*Salmonella typhi*	9	7	0	0	0
7.	*Pseudomonas aeruginosa*	26	23	20	17	14
8.	*Klebsiella pneumonia*	28	18	15	13	11
9.	*Candida albicans*	0	0	0	0	0

NB.0=Resistance

Table 3 The Antimicrobial activities of *Vernonia amygdalia* leaf extract showing minimum Inhibitory Concentration of (mg/ml)

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

Key

NB. (- = Negative and + = positive)

The table above displays the antimicrobial activity of plant (*Vernonia amygdalina* leaf) extract on various organisms tested on, with the majority of results demonstrating sensitivity to the extract. *P. aeruginosa* was found to have the highest sensitivity, with effects at all concentrations (25-200mg/ml) save for the lowest (12.5), whereas *S. aureus* Only 100–200mg/ml was shown to be the sensitivity range for. *S. pyogenes. B. subtilis K. Pneumonia* exhibits moderate sensitivity to the extract, between 50 and 200 mg/ml.

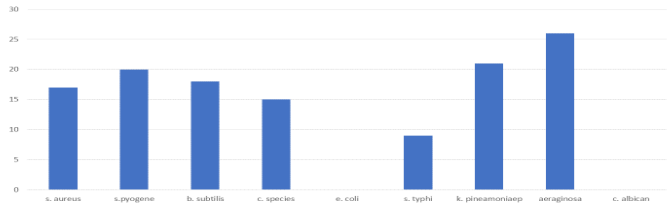

Fig. 1 Sensitivity test results against some microorganisms

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.	*Staphylococcus aureus*	-	-	+	+	-
2.	*Streptococcus pyogenes*	-	-	-	+	-
3.	*Bacillus subtilis*	-	-	+	+	+
7.	*Pseudomonas aeruginosa*	-	-	-	+	+
8.	*Klebsiella pneumonia*	-	-	-	+	+

Key

NB. (- = Negative and + = positive)

The minimum inhibitory concentration of the plant's (*Vernonia amygdalina* leaf) extract on the various organisms tested is shown in Table 4. The majority of these organisms exhibit sensitivity to the extract, with *P. aeruginosa* having the highest sensitivity and showing effects at all concentrations (25-200mg/ml) except for the lowest (12.5) and *S. aureus* having the lowest sensitivity. Only 100–200 mg/ml of aureus was demonstrated to be sensitive. *S. Pyogenes, B. subtilis, K. Pneumonia* exhibits moderate sensitivity to the extract, between 50 and 200 mg/ml.

DISCUSSION

The sensitivity test against some microorganisms was demonstrated in the invitro antimicrobial screening shown in table 5. *P. aeruginosa*, which had the highest sensitivity with effect at all concentrations (26mm at 1000mg/ml), was documented to have significant levels of inhibition from the extract among the test organisms. 20 mm at 600 ml/g and 23 mm at 800 ml/g, the lowest against *Salmonella typhi* were 9mm at 1000mg/ml and 7mm at 200mg/ml, while the highest were 17mm at 400mg/ml and 14mm at 200mg/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*. 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 [29]. Based on the outcomes of the minimum inhibition concentration (MIC) and minimum microbial concentration (MMC) results, respectively, shown in Tables 6 and 7. *S. aureus* was found to be sensitive at only 100-200mg/ml, however *P. aeruginosa* was found to have the highest sensitivity with effect at all concentrations (25-200mg/ml) extract for the lowest (12.5). The moderate sensitivity of *S. pyogenes, B. subtilis, and K. pneumonia* ranges from 50 to 200 mg. Phytochemical screening of *V.*

amygdalina leaves extract revealed the presence of the following constituent's tannins, saponins, terpenoids, flavonoid, carbohydrates and cardiac glycoside.

CONCLUSION

This 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* 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.

RECOMMENDATION

Following the study's findings, it is advised that additional extensive research be encouraged to learn more about the plant's therapeutic characteristics. This research can involve qualitative and quantitative analysis as well as a look at the mineral makeup of the entire plant.

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