Namadina *et al.*, (2024)
Biological and Environmental Sciences Journal for the Tropics 21(1) April, 2024
ISSN 0794 – 9057; eISSN 2645 - 3142
BEST JOURNAL 21(1): 1 - 11
Date received: 29/01/2024
Date accepted: 24/04/2024
https://dx.doi.org/10.4314/bestj.v21i1.1

Phytochemical, Antioxidant and Antibacterial Activities of *Vernonia amygdalina* Del. Leaf Extract

Maryam, S.I[1]., Abdulsalam, H[2]., Namadina, M.M[3]., Yunusa, A.Y[4]. and Shehu, S.A.[5]
[1]Department of Microbiology, Aliko Dangote University of Science and Technology, Wudil
[2]Department of Biological Sciences, Federal University of Technology, Minna, Niger State.
[3]Department of Plant Biology, Bayero University, Kano, Nigeria.
[4]Department of Biological Sciences, Federal University Wukari, Taraba State.
[5]Department of Biological Sciences, Bayero University, Kano, Nigeria.
Corresponding Author: mnmuhammad.bot@buk.edu.ng

ABSTRACT
Scientific standardization as well as possible therapeutic effects of some herbal preparations, alternatives against antibiotic resistant bacterial infections indicates a strong need for continuous effort to validate the use of plant materials as alternative therapy regimens with similar or higher antibiotic beneficial properties. The study was aimed to at evaluating the phytochemical, antioxidant, antibacterial activities of *Vernonia amygdalina* leaves. Phytochemical screening was achieved using standard qualitative phytochemical screening procedures. Antibacterial studies were carried out using agar well diffusing methods. The antioxidant activities of the methanol extract was determined by means of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Flavonoid, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrate and phenols were detected in methanolic extract. The methanolic extract showed higher radical scavenging ability than ascorbic acid at the highest concentration of 1000 µg/mL. The IC_{50} values of the methanol extract of *Vernonia amygdalina* leaves against DPPH free radical were 20.625 µg/ml. The Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the *Vernonia amygdalina* leaf extract recorded respective values of 15.625 mg/ml and 31.25 mg/ml against *Pseudomonas aeruginosa* and *Escherichia coli* and 7.8125 mg/ml and 15.625 mg/ml against *Staphylococcus aureus.* The FTIR analysis showed the presence of alcohol, phenol, carboxylic acid, aldehyde and ketone functional group. This study has proven the effectiveness and efficacy of *Vernonia amygdalina* against the tested clinical isolates.

Keywords*:* Antioxidant; Antibacterial; Phytochemical; *Vernonia amygdalina*

INTRODUCTION
A discussion of human life on earth would not be complete without a look at the role of plants, because plants have been an integral part of human society since the start of civilization (Shah *et al.*, 2013). Interestingly, many of today's drugs have been derived from plant sources. It is estimated that, plant materials are present in or have provided the models for 50% of western drugs (Shah *et al.*, 2013). Since ancient times, plants have been used to treat various ailments but due to lack of proper documentation, the knowledge was not properly transferred (Ajila and Olayede, 2012). However, recently various pharmacological researches have been reported for different types of medicinal plants (Omede *et al.*, 2018). The medicinal value of these plant species is due to the presence of bioactive chemical compounds such as flavonoids, alkaloids, saponins, phenolics amongst others (Omede *et al.*, 2018).

Drug-resistant bacterial pathogens are a significant challenge in Africa, complicating the treatment of infectious diseases. The rise of multi-drug resistant organisms, particularly in nosocomial settings, further exacerbates this issue (Zainab, 2013). Infectious diseases remain a critical global health concern, contributing significantly to morbidity and mortality rates worldwide (WHO, 2012). While research and development efforts have historically provided new antibiotics to counter resistance, the rapid emergence of bacterial antibiotic resistance threatens to outpace these advancements, potentially leading to a global public health crisis. As such, there is a growing need for novel antimicrobials and innovative approaches to address this pressing issue (Zainab, 2013). Recognizing the limitations of conventional antibiotics, there is a shifting focus towards exploring natural substitutes. Plant-derived compounds have garnered attention due to their potential therapeutic benefits, including antioxidant properties. Omede *et al.* (2018) demonstrated the antioxidant activities of several plant species, highlighting their potential in maintaining health and potentially mitigating age-related degenerative disorders such as coronary heart disease, cancer, and neurodegenerative diseases. Despite their benefits, it is crucial to assess the safety of these natural antioxidants. The brine shrimp lethality assay is proposed as a valuable preliminary tool for evaluating the toxicity of plant extracts, offering a suggested pharmacological screening method for such extracts (Omede *et al.*, 2018).

The plant *Vernonia amygdalina* (Asteraceae) is a small evergreen shrub widespread in Africa. It has a common name ‘bitter leaf’ due to the fact that it is bitter to taste. However, the leaf is delicious in soup. The plant is three feet or more tall and grows plentifully in moist places. The leaf medicinal and nutritional properties cannot be underestimated (Omede *et al.,* 2018). Anti- bacteria, anti- malaria, anti-cancer and more recently antioxidant activities of some of the plant parts have been reported in literature (Omede *et al.,* 2018). The leaf is also effective in the treatment of scurvy, rheumatism, pile, indigestion, blood sugar control amongst others (Ajila and Oloyede, 2012). The aim of this study is to evaluate the phytochemical, antibacterial, antioxidant properties of *V. amygdalina* leaves.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Vernonia amygdalina leaves were collected from Botanical garden, Department of Plant Biology, Bayero University, Kano, Gwale Local Government Area, Kano State, Nigeria. The plant was identified and authenticated in the herbarium of the Plant Biology Department of Bayero University, Kano, Kano State, Nigeria and a voucher specimen number BUKHAN143 was deposited.

Preparation of Plant extracts

Fresh *Vernonia amygdalina* leaves were meticulously cleaned, air-dried, and finely ground into a coarse powder using a grinding machine. The powdered sample was carefully stored in airtight containers to maintain its integrity for further processing. Subsequently, 200 grams of the powdered leaves were immersed in 2 liters of methanol in a suitable container. The mixture was left to steep for 72 hours at room temperature (28 ±2ºC), with periodic agitation every hour to facilitate efficient extraction. After the steeping period, the extract was meticulously separated from the plant material. Firstly, it was passed through a muslin cloth to remove any coarse debris. Following this, the extract was filtered through a Whatman filter paper (No.1) to ensure clarity and purity of the final solution. The filtered extract was then carefully transferred into a clean evaporating dish.

The dish was placed on a water bath set at 50°C to facilitate the evaporation of the solvent. This process continued until all the methanol was completely evaporated, leaving behind the concentrated extract in the dish. Finally, the concentrated extract was carefully collected and stored in suitable containers under appropriate conditions for further analysis and experimentation.

Qualitative Phytochemical Screening of *V. amygdalina* Leaf extract

The plant extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the methods described by Evans (2009).

Tests for carbohydrates

Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicated the presence of carbohydrate.

Tests for Saponins

Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persists for 10-15 mins indicated presence of saponins.

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2 ml of 50% methanol in the presence of heated metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red color indicated the presence of flavonoids.

Test for Alkaloids

Wagner's Test: Few drops of Wagner's reagent were added into a portion of the extract, whitish precipitate indicated the presence of alkaloids.

Test for Steroids and Triterpenes

Liebermann-Burchard's test: Equal volumes of acetic acid anhydride were added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 ml) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicated the presence of triterpenes while blue or blue green indicates steroids.

Test for Cardiac Glycosides

Kella-killiani's test: A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides.

Test for Tannins

Ferric chloride test: 3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitates.

Test for Anthraquinones

Borntrager's test: Exactly 5ml of chloroform was added to the portion of the extract in a dry test tube and shaken for 5mins. This was filtered, and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicated the presence of free anthraquinones.

Thin Layer Chromatography Profile

A TLC aluminum sheet measuring 20 x 20 cm coated with silica gel was utilized for this analysis, employing a one-way ascending technique. The sheets were subsequently cut into smaller sizes of 5 x 10 cm. The extract was dissolved in the initial extraction solvents, and spots were manually applied to the cut plates using capillary tubes.

The plates were then dried and developed in a solvent mixture of Hexane:Ethyl acetate (7:3) within a chromatographic tank. After development, the plates were sprayed with general detecting reagents (p-anisaldehyde/H_2SO_4, 10% H_2SO_4 in methanol) and viewed under UV light (365 nm). If applicable, the plates were heated at 110°C for 2 minutes. The number of spots, their colors, and the retardation factors (Rf values) for each spot were determined and recorded, while the chromatograms were scanned accordingly (Gennaro, 2000; Stahl, 2005).

Antioxidant activity

The antioxidant activity of *V. amygdalina* methanolic leaf extract was evaluated based on its radical scavenging ability using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), following a modified method adapted from Sani and Dailami (2015). Briefly, 200 µl of a 100 µM methanol solution of DPPH was mixed with 100 µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.8 µg/ml). The mixture was then allowed to react in the dark at room temperature for 30 minutes. Absorbance readings of the blank, test, and control samples were recorded at 517 nm using a spectrophotometer. The experiment was conducted in triplicate, and the scavenging activity was calculated using the formula below and expressed as a percentage of inhibition.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration corresponding to 50% inhibition (IC_{50}) was determined using probit analysis with SPSS 16.0 software. The IC_{50} values obtained were then compared with those of ascorbic acid, used as a standard antioxidant.

Microbiological analysis

The isolates underwent Gram staining to determine their Gram reaction and were cultured on various selective and differential media to observe their color, colony morphology, and fermentation abilities (Karzan *et al.*, 2017).

Isolation of Bacterial species

Specimens were cultured on sterile blood agar, chocolate agar, and MacConkey agar plates at 37°C for 24 hours in an incubator. Discrete colonies were selected based on their morphology and sub-cultured on blood agar and chocolate agar to obtain pure strains. Gram staining was performed on the isolated colonies, and based on their Gram reactions, they were inoculated onto different selective media such as mannitol salt agar, cetrimide agar, and eosin methylene blue agar. Various biochemical tests including catalase, coagulase, and oxidase tests were conducted. All isolates that grew on the selected agar media were then placed on nutrient agar and chocolate agar slants and stored in a refrigerator at 4°C for further use (Cheesbrough, 2010).

Identification and characterization

Identification and characterization of the bacteria were conducted using the Microgen Identification Kit (XYZ) following the manufacturer's specifications (API Biomerieux). Saline suspensions of the test organisms were added to the wells, and appropriate wells (1, 2, 3, and 9) were overlaid with sterile paraffin oil. After overnight incubation (18-24 hours) at 37°C, suitable reagents such as Nitrate A and B, Kovacs, Tryptophan deaminase (TDA), and Voges-Proskauer (VPI and II) were added to wells 8, 10, and 12 for additional tests, and color changes of the different tests were recorded. The results were converted into four to eight-digit codes depending on the organisms being tested and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Antibacterial Susceptibility Test

Preparation of Extract Concentration

Stock solutions of the plant extracts were prepared by adding 0.25 g of each crude plant extract to 1 ml of dimethyl sulfoxide (DMSO).

From each stock solution, concentrations of 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml were prepared using the two-fold serial dilution method (Srinivasan *et al.,* 2009).

Standardization of Bacterial Inoculum

Using an inoculum loop, overnight agar cultures were transferred into a test tube containing normal saline until the turbidity matched the 0.5 McFarland Standard as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

Susceptibility Test of Bacterial Isolates to Different Extract Concentrations

The antibacterial activity of *V. amygdalina* leaf crude extract against *S. aureus*, *E. coli*, and *P. aeruginosa* was evaluated using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized inoculum (in triplicate), and wells were filled with various concentrations of the crude extract. DMSO served as a negative control, while ciprofloxacin was used as a positive control. After incubation at 37°C for 24 hours, the diameter of inhibition zones was measured.

Determination of Minimum Inhibitory Concentration (MIC)

The tube dilution method was used to determine the MIC. Plant extracts were serially diluted, and test tubes containing varying concentrations were inoculated with standardized suspensions of test organisms. Tubes were incubated at 37°C for 24 hours, and growth was observed. Sub-culturing onto nutrient agar confirmed bacterial growth inhibition.

Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by sub-culturing broth culture from tubes used for MIC determination onto fresh nutrient agar plates. The lowest concentration without growth after incubation was considered the MBC (Adesokan *et al.,* 2008).

Fourier Transformed Infrared Spectroscopy Analysis (FTIR)

Air-dried sample of methanol extract of *V. amygdalina* was analyzed for identification of characteristic functional groups using Fourier Transform Infrared (FT-IR) spectrophotometer (Shimadzu 8400) at the Multi-User Laboratory, Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. A small quantity (0.1g) of extract sample and 0.025g of dry potassium bromide (KBr) were homogenized using mortar and pestle. A portion of the homogenized mixture was placed on the disc and pressed using a mini hand press to form a KBr thin film and the disc was placed in the FT-IR spectrophotometer in which spectra was measured by accumulating 64 scans at 4 cm-1 resolution in the spectral range of 4000 to 400 cm-1. Percentage transmittance was plotted against wavelengths. The FT-IR spectra were used to identify the functional groups of active metabolites based on the peak values in the infra red region.

RESULTS

Flavonoid, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrate, and phenols were detected while glycoside and anthraquinones were absent in the *V. amygdalina* extract (Table 1). Thin layer chromatography profile of methanol extract of *V. amygdalina* leaves revealed five (5) spots with *p-Anisaldehyde* spray and the Rf values (Table 2).

Table 1. Qualitative Phytochemical screening of methanol extract of *V. amygdalina* leaves

Metabolites	Inferences
Alkaloid	+
Flavonoid	+
Saponins	+
Cardiac glycoside	-
Tannins	+
Steroid	+
Triterpenes	+
Phenol	+
Anthraquinones	-
Carbohydrate	+

Table 2. TLC results of Methanolic extract of *Vernonia amygdalina* leaves

Extract	Solvent system	Number of Spots	Distance of spots(cm)	RF-Value
Methanol extract	HE:EA (7:3)	5	6.8	0.6,0.7,0.8,0.91,0.95

HE (Hexane), EA (Ethyl acetate)

Free radical scavenging ability of the methanolic extract of *V. amygdalina* leaves was evaluated using DPPH radical. Ascorbic acid was used as positive control. It was determined that methanolic extract of *V. amygdalina* leaves possessed higher radical scavenging ability of 95.30 % at the highest concentration of 1000 µg/mL and was compared with standard where it showed 92.0 % activity (Table 3).

Table 3. Antioxidant activities of Methanolic extract of *V. amygdalina* leaves

Analyte	Concentration (µg/mL) / % Inhibition							
	1000	500	250	125	62.5	31.25	15.6	7.8
Methanol extract	95.3	94.0	91.5	79.5	59.7	50.8	45.2	42.9
Ascorbic acid	92.0	93.3	93.0	93.4	93.2	92.1	92.3	90.8

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend methanol extract < Ascorbic acid. The IC_{50} values of the methanol extract of *V. amygdalina* leaves against DPPH free radical were 20.625 µg/ml.

Table 4: Antioxidant Activities of the Methanolic extract of *V. amygdalina* leaves

Sample	IC_{50} (µg/mL)
Methanol	20.625
Ascorbic acid	0.01

Antibacterial activities of methanol extract of *V. amygdalina* leaves showed inhibition on all the tested clinical isolates of *S. aureus*, *E. coli* and *P. aeruginosa* at 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml (Table 5). The MIC and MBC of the *V. amygdalina* leaf extract recorded respective values of 15.625 mg/ml and 31.25 mg/ml against *P. aeruginosa* and *E. coli* and 7.8125 mg/ml and 15.625 mg/ml against *S. aureus.*

Table 5: Antibacterial activity of methanol extract of *V. amygdalina* leaves

Clinical isolates	Concentration (mg/ml)/Diameter zone of inhibition (mm)						MIC	MBC
	250	125	62.5	31.25	CPR	DMSO		
S. aureus	14	13	11	09	38	06	7.8125	15.625
E. coli	14	12	11	08	37	06	15.625	31.25
Pseudomonas	15	14	12	10	40	06	15.625	31.25

Table 6. Peak Positions and Probable Inter-atomic bond of Isolated Compounds from *V. amygdalina* methanolic leaf extract by FTIR

IR-value	Functional group	Secondary metabolite
3324.80	O-H Stretching vibration of alcohol and phenols	Steroid, terpenoids, saponins, phenols, carbohydrates
2921.21	C-H (Stretching), C=O Stretching of Carboxylic acid and ketone	Flavonoids, terpenoids, steroid, saponins
1629.72	C=C Stretching, N-H bending of primary amines	Alkaloid, steroid, terpenoids, saponins, fatty acid
1443.69	C-N stretching of aromatic amines, C-H bending	Alkaloids
1033.42	C-H stretching of aromatic hydrocarbon, C-O Stretching	Terpenoid, steroid , saponins, carbohydrate

DISCUSSION

Flavonoid, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrate, and phenols were detected in methanolic extract of *V. amygdalina* leaves. Glycoside was absent in the extract and this is in agreement with the work of Alhassan *et al*. (2014) and Abubakar *et al*. (2015). But Sharall *et al*. (2013) reported that some members of Asteraceae family have anthraquinone and cardiac glycosides.

These primary and secondary metabolites in plants have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Atinga *et al.,* 2021). Flavonoids have been shown to provide antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity. Flavonoid also has immense antioxidant and anti-inflammatory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Atinga *et al.,* 2021). Tannins have been used in the treatment of wounds especially those emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision. The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Atinga *et al.,* 2021) that will aid in chemo taxonomical classification system and further phylogenetic studies in Asteraceae family.

The phytochemical screening of *V. amygdalina* leaf extract recorded the presence of compounds carbohydrates, flavonoids, saponins and alkaloids present in the extract and responsible for antimicrobial activities as reported in similar studies (Hamid *et al*., 2011). A study conducted by Sharall *et al*. 2013) reported the presence of fatty acids and terpenes responsible for bactericidal activity. Hamid *et al.* (2011) also reported that phenolics, flavonoids, saponins and phorbol esters as antimicrobial compounds in *V. amygdalina.*

The reduction of DPPH radical is one of the popular and simpler ways to measure antioxidant activities of medicinal plants. The potential of plant extracts to inhibit DPPH radical is strongly linked to their ability to donate electrons to the radical (Daniel and Dluya, 2016). Normally, DPPH radical is stable in various solvents including methanol, ethanol and water. Therefore, the radical is usually prepared in a solution of either ethanol or methanol (Fukumoto and Mazza, 2000). In the present study, the DPPH radical was prepared in ethanol. The *in-vitro* DPPH scavenging assay was preferred in this study because it is rapid, easy, reliable and less expensive since it does not require specialized device and methods.

The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Raja *et al*., 2010). When these molecules are formed, the absorbance decreases and the DPPH solution decolourises from violet colour to pale yellow. The degree of discolouration is an indication that the plant extract has the potential to scavenge free radicals as a result of its ability of hydrogen donation. More yellowish colour of DPPH is an indicator stronger antioxidant activity of the extracts (Sowunmi and Afolayan, 2015). The results obtained in this study showed dose dependent DPPH scavenging activities of the two fractions of methanol extract. It was however, noted that the methanolic leaf extract of *V. amygdalina* had higher DPPH scavenging abilities than ascorbic acid at 500 and 1000 µg/mL. This could be due to the crude nature of extract as compared to the refined standard drug. This result does not corresponded with the observations of Igbinosa *et al*. (2011), who found that *Vernonia amygdalina* had lower DPPH activities than ascorbic acid (standard) at 500 and 1000 µg/mL . The results of methanol extract of *V. amygdalina* agrees with a study by Kambli *et al.* (2014), who found that the DPPH scavenging activity of *F. racemosa* was considerable but not higher than that of the standard drug. The good antioxidant property of *V. amygdalina* leaf extract against DPPH, corroborate well with findings of Degollado *et al*. (2014), who noted that *F. odorata* had a good antioxidant activity against DPPH radicals.

The IC_{50} values of the leaf extract of *V. amygdalina* against DPPH free radical were 20.625 µg/ml. The moderate IC_{50} values obtained from this study, showed that the extract had moderate antioxidant activities against DPPH radicals. This argument is in line with studies by Mbaebie *et al*. (2012), who worked on stem bark extract of *S. latifolia* against DPPH radical, and obtained an IC_{50} value of 0.126mg/ml thereby strongly recommending the plant to be used as an antioxidant supplement. The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend extract < Ascorbic acid. It is interesting to note that the lower the IC_{50} value, the higher the scavenging activity of the plant extract (Sowunmi and Afolayan, 2015). Flavonoids and phenols naturally exhibit strong scavenging abilities for free radicals due to their hydroxyl groups (Mohamed *et al.,* 2010) which are attached to their aromatic ring structures and help to quench the radicals either by donating their electrons and thus neutralizing them or via the electron delocalization over all three ring system achieved by ortho-dihydroxyl of the B-ring and 4-oxo group of the ring C of the flavonoid, which actively reduce radicals like DPPH and Fe^{3+} to Fe^{2+} ions. Phenols are common in diet and are divided into two classes; the benzoic derivatives like gallic acid and cinnamic derivatives like caffeic acids.

Gallic acid is the best known polyphenol working efficiently in polar medium, the compound has greater ability of scavenging hydroxyl free radicals through deprotonation. Gallic acid is a prolific scavenger, with a greater ability to deactivate variety of ROS and RNS mainly via electron movement through the cellular physiological pH (Mareno *et al*., 2014). The mechanism of antioxidant action of phenolic compounds is through inactivation of lipid free radicals or by preventing the decomposition of hydroperoxides into free radicals (Kibiti and Afolayan, 2015).

The result disagrees with most studies that showed activity of *V. amygdalina* on Gram negative organisms, which may be attributed to the fact that cells of Gram negative bacteria are less viable thus less affected by the extract inhibitory activity when compared to those of Gram positive bacteria. Gram negative bacteria also possess efflux system that extrude antibacterial agents out of the cell (Tenovar, 2006). Arekemase *et al*. (2011) recorded high activity of the plant extract on *P. aeruginosa*, *E. coli* and *S. aureus* at 250 mg/ml. Kalimathu *et al.* (2010) in *Pseudomonas aeruginosa* and *Staphylococcus aureus* with leaf extract of *Vernonia amygdalina.* This study recorded moderate antibacterial activity on Gram positive *S. aureus* and on gram negative *E. coli* and *Pseudomonas aeruginosa* which might be attributed to their difference in cell wall composition. The antibacterial effects of *V. amygdalina* had been previously studied and reported that the extract displayed potent antibacterial activity against *S. aureus, P. aeruginosa* and *E. coli* and host of other bacteria, giving inhibitory concentration as low as 7.8125 mg/ml which confirms the potency of this plant in treating human infections (Arekemase *et al.,* 2011).

The main functional groups of organic compounds detected by FTIR in methanolic extract of *V. amygdalina* were 3324.80 cm^{-1} and 2921.21 cm^{-1} which indicated the presences of OH^- (alcohol and phenol) and COOH (carboxylic acid).Oxalic acid, hexadecanoic acid, 9-octadecanoic acids (oleic acids) and acetic acids could be the major compounds detected in the leaf extract.

CONCLUSION

In conclusion, the phytochemical screening of the methanol extract of *V. amygdalina* revealed the presences of flavonoid, alkaloids, tannins, saponins, steroids, triterpenes, carbohydrate, and phenols. The results obtained in this study clearly demonstrated the effectiveness of methanolic leaf extract of *V. amygdalina* on *S. aureus, P. aeruginosa* and *E. coli*. The DPPH radical scavenging ability of the extract showed moderate antioxidant activities. It also revealed the probable functional groups of chemical compounds in the crude extracts by FTIR analysis which are responsible for the antibacterial activities. Based on the result of the analysis therefore, the work can be considered as part of an effort to validate the use of *V. amygdalina* in traditional medicine as well as source of future discovery of antibacterial drugs.

REFERENCES

Adesokan, A.A., Akanji, M.A., Yakubu, M.T., Soladoye, A.O. and Lawal, O.K. (2008). Effect of administration of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark on brewer‟s yeast induced pyresis in rats. *African Journal of Biochemistry Research*, 165**:** 164-169.

Alhassan, M. A., Ibrahim, M., and Musa, I. A. (2014). Phytochemical Screening and Antimicrobial Evaluation of Stem Bark Extract of *Pseudocedrela kotschyi* (Herms). *British Journal of Pharmaceutical Research,* 4(16): 1937-1944.

Arekemase, M.O., Kayode, R.M.O. and Ajiboye, A.E. (2011). Antimicrobial activity and phytochemical analysis of *Jatropha curcas* plant against some selected microorganisms. *International Journal of Biology*, **3**(3):52-59.

Atinga, V., Musa, T.L., Ibrahim, H.M., Ayeni, E.A., Namadina, M.M., Jajere, U.A. and Ali, U.Y. (2021). Standardization of stem bark of Pseudocedrela kotschyi (Meliaceae). *Journal of Pharmacognosy and Phytochemistry*, 10(6): 19-23

Cheesbrough, M. (2010). Biochemical tests to identify bacteria: District Laboratory Practice in Tropical countries. Part 2, update edition Cambridge University Press. pp 62-105.

Daniel, D. and Dluya, T. (2016). In vitro Biochemical assessments of methanol stem bark extracts of *Ficus sycomorus* Plant. *Jordan Journal of Biological Sciences*, 9 (1). 63 – 68.

Degollado, J., Yolo, T. and Santiago, A. (2014). Hypoglycemic effect and in vitro antioxidant activity of the dichloromethane fraction from the leaves of *Ficus odorata* (B.) *International Journal of Research and Development in Pharmacy and Life Sciences* 3 (5) 1163-1173.

Evans, W. C. (2009). *Trease and Evans pharmacognosy"*, 16th edition, W. B. Saunders Ltd., London, 10 – 11.

Fukumoto, R. and Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of agricultural and food chemistry*, 48(8), 3597-3604.

Hamid, A.A., Aiyelaagbe, O.O. and Usman, L.A. (2011). Essential oils: Its medicinal and pharmacological uses. *International Journal of Current Research*, **3**(2):086-098.

Igbinosa, O., Igbinosa, H., Chigor, N., Uzunuigbe, E., Oyedemi, O., Odjadjare, E. and Igbinosa, O. (2011). Polyphenolic contents and antioxidant potential of stem bark extracts from *Jatropha curcas* (Linn). *International Journal of Molecular Sciences*, 12(5), 2958-2971.

Kalimuthu, K., Vijayakumar, S. and Senthilkumar, R. (2010). Antimicrobial activity of Biodesiel Plant *Jatropha curcas L. International Journal ofPharmaceutical Sciemces*, **12**(1):49-54.

Kambli, J., Ashwini, C. and Rohini, K. (2014). Phytochemical screening, and evaluation of antibacterial, antioxidant and cytotoxic activity of *Ficus racemosa* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6 (4); 6-14.

Karzan, M. K., Faeza, B. O. and Shahida, N. Y. (2017). Isolation and Identification of Urinary Tract Infectious Bacteria and Exploring their Anti-drug Potential against Some Common Antibiotics. *J Microb. Biochem. Technol*. **9**(6): 285-289.

Kibiti, M. and Afolayan, J. (2015).Preliminary Phytochemical Screening and Biological Activities of Bulbine abyssinica Used in the Folk Medicine in the Eastern Cape Province, South Africa. Journal of Evidence-Based Complementary and Alternative Medicine, 2015.

Mareno, T., Galano, A. and Russo, N. (2014). Radical scavenging ability of gallic acid toward OH and OOH radicals. Reaction mechanism and rate constants from the density functional theory. *The Journal of Physical Chemistry B*, 118(35), 10380-10389.

Mbaebie, O., Edeoga, O. and Afolayan, J. (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*, 2(2), 118-124.

National Committee for Clinical Laboratory Standards (2008).Performance Standards for Antimicrobial Susceptability Testing; Ninth Informational Supplement. NCCLS document M100-S9. National Committee for Clinical Laboratory Standards, Wayne, PA.

National Committee for Clinical Laboratory Standards NCCLS (2000).Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standards-fifth edition. NCCLS document M7-A5. NCCLS. 2000: Wayne, PA, USA.

Njoku, P.C. and Akumufula, M.I. (2007). Phytochemical and Nutrient evaluation of *spondias mombi* Leave. *Pakistani Journal of Nutrition*, 6(6):613-615.

Omede, A., Suleiman, M.S., Atanu, F.O., Momoh, S., Friday, E.T., Sheneni, V.D., Jegede, E.R. (2018). *Evaluation of antioxidant and cytotoxic properties of Vernonia amygdalina, International Journal of Cell Science & Molecular Biology*, **4(** 4): 80-86.

Raja, K. R.R., Arumugam, R. and nantharaman, A. (2010*). In vitro* antioxidant activities of ethanol extract from *Enhalus acoroides* (L. F.) Royle, *Asian Pacific Journal of Tropical Medicine*. **3** (11): 898–901.

Sharall, V., Verma, M., Sharma, S. and Malik, A. (2013).Determination of Phytocomponents of *Jatropha curcas* root by GC-MS Analysis and their Termcidal activity.

Shah, S., Siraj-ud-Din, Rehmanullah (2013). Pharmacognostic Standardization and FTIR analysis of various parts of *Sageretia thea*. *In. Journal of Bioscience*. 3(3): 108-114.

Sowunmi, L. I. nd Afolayan, A. J. (2015). Phytochemical constituents and antioxidant properties of acetone extract of cleome gynandra (L.) growing in the Eastern Cape, *South Africa. African Journal of Traditional, Complementary and Alternative Medicines*, 12(3): 1-8

Sowunmi, L. I., and Afolayan, A. J. (2015). Phytochemical constituents and antioxidant properties of acetone extract of cleome gynandra (L.) growing in the Eastern Cape, *South Africa. African Journal of Traditional, Complementary and Alternative Medicines*, 12(3): 1-8

Srinivasan, L., Sasaki, Y., Calado, D.P., Zhang, B., Paik, J.H., DePinho, R.A., Kutok, J.L., Kearney, J.F., Otipoby, K.L. and Rajewsky, K. (2009). PI3 kinase signals BCR-dependent mature B cell survival. *Cell*, **139**:573-586.

Sylvester, N.M. (2016). Phytochemical and Antivenom activity of Albizia chivelieri stem bark on *Naja nigricollis* Broadley Envenomated Mice. M.sc Research Dissertation (Unpublished).Department of Pharmacognosy and Drug Development Library. Ahmadu Bello University, Zaria. Pp: 47-48.

Tenovar, F.C. (2006). Mechanisms of antibacterial resistance in bacterial. *American Journal of Medicine,* **34**:3-10.

Zainab, Y.I. (2013). Analysis of The Crude extracts of *Jatropha curcas* For Phytochemicals and their Antimicrobial activities against Clinical Bacterial Isolates. M.Sc. Dissertation Submitted to the Department of Microbiology, Ahmadu Bello University, Zaria. 1-10.