PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (6) 1311-1318 June 2023

Assessment of Phytochemical Screening of *Vernonia amgydalina* Lin Leaves and its Antibacterial Potential *on Salmonella typhi* and *Staphylococcus aureus*

***[1]SAADU, H; [1]MUHAMMAD, D; [2]MUHAMMAD, AD**

**[1]Department of Biology, [2]Department of Business Education, School of Secondary Education Sciences, Federal College of Education, Katsina Katsina state, Nigeria*

**Corresponding Author Email: manfashi2006@yahoo.com; Tel: +2348024382028; +2348160397104*
Co-Authors Email djbida50@gmail.com; aysha4d@gmail.com

ABSTRACT: The phytochemical screening of *Vernonia amygdalina* Lin leaves and its antibacterial potential on *Salmonella typhi* and *Staphylococcus aureus* were investigated using standard methods. The Wattman No 1 sterilized paper discs (6mm in diameter) impregnated with *Vernonia amygdalina* ethanolic and aqueous extracts at the concentrations of 20 mg/ml, 10 mg/ml, 5 mg/ml and 2.5 mg/ml were tested on *Salmonella typhi* and *Staphylococcus aureus*. The zones of inhibition shown by the extracts at different concentrations against different strains, the turbidity of broth culture (susceptibility), Minimal Inhibitory Concentration (MIC) and bacterial growth Minimal Bactericidal Concentration (MBC) of the test organisms were studied. Susceptibility test revealed that VAEE have antimicrobial activities for *Salmonella typhi* and *Staphylococcus aureus* (clinical, reference and environmental strains). There was significantly higher difference with respect to the zones of inhibitions produced among the various extract concentrations against the test organisms (ANOVA $P<0.05$). The strains of *S. typhi* and *S. aureus* showed MIC and MBC at the extract concentrations of 10 mg/ml and 20 mg/ml respectively. The findings of this study suggested ethanol to be a good solvent for the extraction of the antibacterial substances in VA. Furthermore, some constituents; saponin tannin, flavonoid and alkaloid were shown to be presence in the leaves of VA, hence could be isolated and developed in to effective antimicrobial agents.

DOI: **https://dx.doi.org/10.4314/jasem.v27i6.36**

Cite this paper as: SAADU, H; MUHAMMAD, D; MUHAMMAD, A. D (2023). Assessment of Phytochemical Screening of *Vernonia amgydalina* Lin Leaves and its Antibacterial Potential *on Salmonella typhi* and *Staphylococcus aureus*. *J. Appl. Sci. Environ. Manage.* 27 (6) 1311-1318

Dates: Received: 07 June 2023; Revised: 18 June 2023; Accepted: 27 June 2023 Published: 30 June 2023

Keywords: *salmonella typhi; staphylococcus aureus;* Phytochemical Screening; *Vernonia amygdalina* Lin leaves

Plants were the main sources of materials to which the ancient man resorted to for preserving his health by treating diseases, where plant metabolites such as quinine, aspirin, morphine, benzein, saponin, caffeine among others were important drugs and which are still being used to date (Oliver, 1960; Sofowora, 2006). The claims of effective therapy for the treatment of diseases like pneumonia, ulcer, diarrhoea, dysentery, malaria fever, skin infections, boils, jaundice, asthma, cough, bronchitis, gyneacological diseases, diabetes by traditional herbalists, worldwide have prompted interest in specific investigation of such herbal medication (Abdulrahaman, 1990). Infact the herbal medication is cosmopolitan and has been practiced for several thousand years. The World Health Organization estimated that about 80% of the world population depends on the use of herbs for treatment of diseases (Obinawu, 1984; Eloff, 1998). The chemotherapy of *S. aureus*, Salmonellosis and other *Salmonella* related infections often encounter the problems of high cost of drugs, which may be hard to afford by the majority of the Third world populace who are mostly exposed and susceptible to diseases condition due to poor conditions of living. The side effect of the drugs used in treatments, In addition to antibiotics resistance by some strains of *Salmonella typhi, and S. aureus* is also a problem (Paul *et al.*, 1970; Cheesbrough, 2000). Garba (2005) reported that

**Corresponding Author Email: manfashi2006@yahoo.com*

in recent years major typhoid epidemics caused by strains showing resistance to several antibiotics. The observation on traditional practices suggested that the plants materials have some medicinal values. These reasons prompted the need to conduct research and confirm the therapeutic potentials of the plant materials on *S. typhi*, and *S. aureus* as well as to determine the bioactive compounds of the aqueous and ethanolic extracts of *V. amygdalina*. The sensitivity of *S.typhi* and *S.aureus* to ethanolic and aqueous extracts of *V. amygdalina in vitro* was also investigated and minimal inhibitory concentration (MIC) and minimal Bactericidal Concentration (MBC) and the Minimum Bactericidal Concentration (MBC) of the extracts. Hence, the objective of this paper is to assess the phytochemical screening of *Vernonia amygdalina* Lin leaves and its antibacterial potential on *Salmonella typhi* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Sample Collection and Preparation of Extracts: The fresh leaves of *V. amygdalina* were collected around Samaru. Zaria, Kaduna State, Nigeria. It was identified and deposited at the herbarium of the department of Biological Science, Ahmadu Bello University Zaria, Kaduna State, Nigeria. The leaves were dried at room temperature in the laboratory and milled using pestle and mortar to powder form.

Solvent was used for the extraction of the *V. amygdalina* leaves: The powder of the leaf was extracted with absolute ethanol in ratio of 1:5 using a Soxhlet extractor for 36 hours. The residue was collected and stored in a dried and sterilized specimen bottle at 4^{o}C until required (Brain & Turner, 1975; Sofowora, 2006).

Phytochemical Screening of Vernonia. Amygdalina: Phytochemical analysis of the some of the compounds in the *V. amygdalina* ethanolic leaves extracts were carried out according to Cannell (1998) and Trease and Evans (1998) as described below:

Carbohydrates Test: Two tests, Molisch and Fehling, were carried out to determine the presence of carbohydrate in *V. amygdalina* leaves extracts.

a) *Molisch's Test*: Three (3) mls of each extract in a test tube two drops of Molisch's reagent was added followed by 1ml of concentrated Sulphuric acid (H_2SO_4) carefully down the side of the test tube.

b) *Fehling Test*: Five (2) mls of each extract 5ml of the mixture of Fehlings solution A and B were added and the mixtures boiled in a water bath for 5 minutes.

Flavanoid Test: Three tests, Shinoda test, 10% of Sodium Hydroxide (NaOH) & Diluted Hydrochloric acid (HCl) test and Concentrated H_2SO_4 (sulphuric acid) test were conducted for presence of flavanoid;

a) *Shinoda Test*: Few magnesium chips were added to 2ml portion of each extract and few drops of concentrated Hydrochloric acid (HCl) were added.

b) *10% of Sodium Hydroxide (NaOH) and Diluted Hydrochloric acid (HCl) Test*: To 2ml of each extract NaOH was added, followed by 4 drops diluted Hydrochloric acid (Hcl).

c) *Concentrated H_2SO_4 (Sulphuric acid) Test*: To 2ml of each extract 4 drops of Concentrated H_2SO_4. was added in the test tube.

Saponins Test: Two tests, frothing test and Haemolysis test, were carried out to determine the presence of saponin in *V. amygdalina* leaves extracts.

a) *Frothing Test*: To 2ml of each extract was added 1ml of methanol, followed by water about 4 times of its volume (8ml) and shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and observed over 30 minute's period of time. The foam appeared and remained between 15 to 35 minutes.

b) *Haemolysis Test*: To 2ml of each of the filtrate extract was added to 2ml of 1.8% aqueous Sodium Chloride (NaCl) in one tube and 2ml of distilled water was added to 2ml of 1.8% aqueous NaCl in another tube. Using syringe 5 drops of blood (sourced from Swiss albino rat) was added to each tube and their content was mixed gently (by inverting the tubes but not shaking).

Tannins test: Two tests, Lead Subacetate Solution and Ferric chloride ($FeCl_3$) solution, were used to determine the presence of tannins as follow;
a) *Lead Subacetate Solution Test*: To 2ml of each extract was mixed with 2ml methanol, followed by 8ml of water few drops of lead subacetate solution were also added.

b) *Ferric chloride ($FeCl_3$) solution Test*: To 2ml of each extract was mixed in 2ml ethanol and twice its volume was added with water followed by few drops of Ferric chloride ($FeCl_3$) solution.

Steroid/Triterpenoids Test: Lieberman Buchard test was carried out to determine the presence of either steroid or triterpenoids in the extracts.

a) *Lieberman Buchard Test*: To 2ml of each extract in 2ml chloroform added equal volume of acetic anhydrate, followed by concentrated Sulphuric acid (H_2SO_4), down the side of the tube.

Anthraquinones Test: Borntrager's test was conducted out to determine the presence of anthraquinones in the extracts.
a) *Borntrager's Test*: To 2ml of each extract in 2ml chloroform added equal volume of diluted ammonium solution and chloroform solution were added, shaken vigorously and allowed to stand.

Alkaloid Test: Mayer's reagent test, Dragendoff's reagent test and Wagner reagent test were used to determine the presence of alkaloid in the extracts, as follow;

To 4ml extract each was dissolved in acidified alcohol (5% H_2SO_4 + 50% ethanol) and was filtered, the filtrate was basified with diluted ammonium solutions and the resulting solution was extracted with equal volume of chloroform (using separating funnel). The crude concentration of chloroform, (portion) solution was diluted with diluted H_2SO_4 using (separating funnel) to obtain the acid medium of the sample. Then each of the basic solutions were divided into three portions.

a) *Mayer's reagent Test*: To first portion of each of the sample solution obtained above, few drops of the mayers reagent was added.

b) *Dragendoff's reagent Test*: To second portion of each of the sample solution obtained few drops of Dragendoff's reagent were, added.
c) *Wagner reagent Test*: To third portion of each of the sample solution obtained few drops of Wagner reagent was added.

Oil and resins Test: The extract was applied on filter paper, establishment of transparent appearance was checked. When positive it a sign of the presence oil and resins in the samples.

Quinones Test: One 1mL of the extract in a test tube was allowed to react with 1mL concentrated *H_2SO_4 (Sulphuric acid)*. The appearance of red color indicated the presence quinones in the samples.

Anthocyanin/ Betacyanin Test: The extract in 1 mg was transferred to a test tube, then 2mL of 1 N sodium hydroxide (NaOH). The sample was boiled at 100^0C for about 10mins Anthocyanin presence was indicated by the formation of bluish-green color. However, Betacyanin indicated its presence when yellow color was formed in the samples.

Taste Identification: To 2ml of each extract in clean container was dissolve in 5ml distilled water and clean spoon was used to taste the solution, few drops were place on four tasting zones of the tongue; salty (front region), sour (sides regions), sweet (central region) and bitter (back region).

Collection of Strains: The clinical, environmental and reference strains of the test organisms *S. typhi, and S. aureus* were collected from Department of Microbiology Ahmadu Bello University (ABU), Zaria and Medical Microbiology, Teaching Hospital, ABU, Zaria. Each of the organism was grown on Nutrient Agar slant (Baker *et al.*, 2001).

Preparation Extract Concentration: The ethalonic extracts of *V. amygdalina* was prepared by two-fold serial dilution, giving extracts concentrations of 20mg/ml, 10mg/ml, 5mg/ml 2.5mg/ml and 1.25mg/ml. The aqueous extract of *V. amygdalina* using sterilized distilled water was prepared by the powdered leaves material were cold macerated in concentration 20mg/ml. and two-fold-serial dilution was carried out to give concentrations of 10mg/ml, 5mg/ml 2.5mg/ml and 1.25mg/ml. as described by (Fabry *et al.,* 1998; WHO, 2003).

Preparation of paper disc: The paper discs of 6mm in diameter were impregnated with the extracts at different concentrations of extracts by soaking the discs in each of the extract concentration. These gave concentrations of 2000µg/disc to 125µg/disc according to (Fabry *et al.,* 1998; WHO, 2003).

Susceptibility Test: The 0.5 McFarland Standard were used as a reference to adjust the turbidity of bacteria suspension, which causes turbidity in the solution that gave an inoculum size of; $1x10^8$ organism/ml. on the agar plates (CLSI, 2015a) Using flamed forceps the two to three impregnated discs were aseptically placed on the agar gently pressing down to ensure contact**.** After 24 hours incubation at 37^0C, each of the plate was examined and the zone diameter was measured in millimeters using transparent meter rule on the under surface of the Petri-dishes according to (Bauer *et al.* (1966; Ching, 2006; CLSI. 2015b).

Minimum Inhibitory Concentration (MIC) Test: MIC was conducted to find out the least concentration at which the extracts, can inhibit the growth of the test organisms. Turbidity cause by the bacterial growth was examined and the lowest concentration of each extract that showed no visible growth was recorded as

the MIC. According to the method described by (Hewitt *et al.,* 1969; Reimer *et al.,* 1981).

Minimal Bacterial Concentration (MBC) Test: The minimum bactericidal concentration (MBC) test is use to assess the ability of an antimicrobial agent to kill a bacterial isolate NCCLS. (1999). Thus, MBC tests are performed to determine the bactericidal activity of ethanolic and aqueous extracts of V*ernonia amygdaina* as potential antimicrobial agent against a bacterial isolate.

This was determined by sub-culturing from the bottles in the MIC series after 24 hours of incubations with extract at different concentrations. They were streaked on Mueller-Hinton Agar plates and incubated at 37^0C for 24 hours, bacterial growth was examined and the lowest concentration of each extract that showed no bacterial growth was recorded as the MBC.

RESULTS AND DISCUSSION

Physical characteristics of both the ethanolic and aqueous extracts of V*ernonia amygdaina*, were shown to be darkish brown, crystalline solid with sweetish bitter taste with frothing property and pungent odour as shown Table 1.

Table 1: Physical characteristics of V. *amygdaina* ethanolic and aqueous extracts

Characteristics	Ethanolic leaves extract	Aqueous leaves extract
Color	Darkish brown	Brown
Taste	Sweetish-bitter	Sweetish-bitter
Odour	Pungent	Pungent
Appearance	Solid	Granules
Froth	Positive	Positive

The results of the phytochemical screening showed the presence of carbohydrate, Flavanoid, Saponins, Tannins, Steroid/Triterpenoids, Alkaloid, Quinones. However, Anthocyanin/Betacyanin, Oil and resin and Anthraqunoses were comfirmed to be absence in the sample.

The presence of many secondary metabolites in the V*ernonia amygdaina* ethanolic leaves extract table 2 could further confirms the therapeutic potential of V. *amygdaina* as ascribed by (Oyewole and Oladele, 2017).

Alkaloid for instance have been reported to exhibited effective anti-inflammatory, anti-diarrheal, anti-diabetic and anti-cancer activities (Monika, Anil, Aakanksha and Priyanka, 2012). Tannins have therapeutic effects against haemorrhage, diarrhea and microbial infections (Oyewole et al., 2021). The compounds presence in the sample possesses wide range of therapeutic values supported the ethnomedicinal usage of V. *amygdaina* in the treatment of some diseases such typhoid fever in northern Nigeria.

Table 2: Summary of phytochemical components of V*ernonia amygdaina* ethanolic leaves extract

SNO	Constituent and test	Reactions
1	**Carbohydrate**	
	a) Molisch's Test	+
	b) Fehlingh Test	+
2	**Flavanoid**	
	a) Shinoda Test	+
	b) 10%NaOH&Dil. HCl	+
	c) Conc. H_2SO_4	+
3	**Saponins**	
	a) Frothing Test	+
	b) Haemolysis test	+
4	**Tannins**	
	a) Lead subacetate Sol.	+
	b) $FeCl_3$ Sol.	+
5	**Steroid/Triterpenoids**	
	a) Liebarman Burchard Test	+
6	**Anthraqunoses**	
	a) Borntrager method	-
7	**Alkaloid**	
	a) Mayer's reagent	+
	b) Dragendoff's Test	+
	c) Wagner Test	+
8	**Oil and resin**	
	a) Filter paper Test	-
9	**Quinones**	
	a) Quinones Test	+
10	**Anthocyanin/ Betacyanin**	
	Anthocyanin Test	+

Key *+ Presence in appreciable amount + Presence - Absence*

The mean diameter zone of inhibition produced by the discs of VAEE at 0% to 40% concentrations in respect of the clinical, environmental and reference strains of *S. typhi* and *S. aureus* are shown in Plate 1 and 2 and Figure 1. *S. typhi* clinical strains, recorded the highest susceptibility at 10% and 20% concentrations with 29 mm and 24.67 mm in diameter.

Similarly, *S. typhi* environmental strain, recorded, 24 mm and 13 mm in diameter, while *S. aureus* clinical strain recorded 22.50 mm and 11.83 in diameter, followed by *S. aureus* reference strains with 18.08 mm and 10.67 mm in diameter at the same concentrations.

However, *S. aureus* environmental strain was the least with 19.67 mm and 9.17 mm in diameter zone of inhibitions, but all the strains showed resistance at 0% to 5% concentrations.

Susceptibility was recorded when clear zone of inhibitions was produced by the extracts, this implies the presence of antibacterial compounds in the leaves of *V. amygdalina*. The frothing feature of the leaves indicated the presence of saponin.

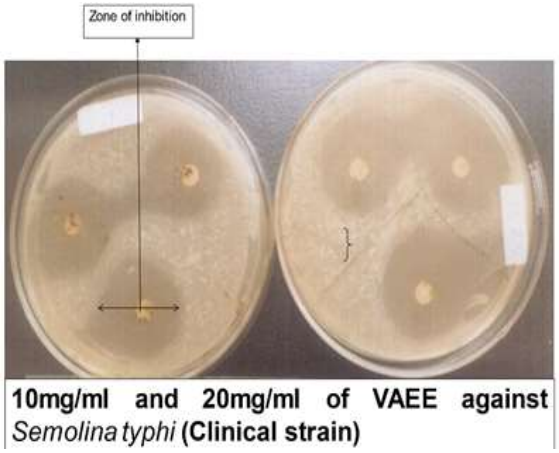

Plate 1: The zones of inhibitions of 20mg/ml and 10mg/ml *Vernonia amygdalina* ethanolic extract (VAEE) against *Salmonella typhi* (clinical strain)

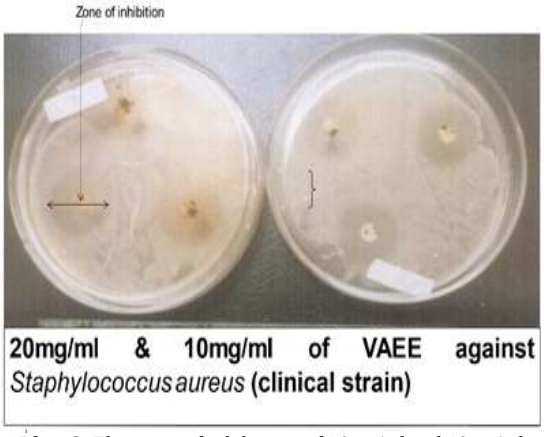

Plate 2: The zones of inhibitions of 20mg/ml and 10mg/ml *Vernonia amygdalina* ethanolic extract (VAEE) against *S aureus* (clinical strain)

This characteristic of the extract is in line with Merck index (1983) description of *sa*ponin. The saponins and alkaloids are suggested to be the bioactive compounds which showed activity of the extracts against the test organisms. The presence of bioactive compounds has been linked to the antibacterial activity such as inhibition of growth (El-Mahmood and Ameh, 2007). The compounds offer some protection to the plant against microbial infections (Farnsworth, 1982; Monika, Anil, Aakanksha and Priyanka, 2012). The presence of some of the phytochemical components like saponins and alkaloid compounds have been attributed to the activity of the crude drug observed (De and James, 2002; Oyewole and Oladele, 2017). Moreover, many plants contain non- toxic glycosides that can get hydrolyzed to release phenolic compounds that are toxic to microbial pathogens (Aboaba *et al*., 2001). Therefore, the compounds detected in these extracts may be responsible for the antibacterial activity and may have a causal role in protecting the plants from microbial attack *in vivo*. Phytochemical substances at least in part, if not all should be valuable in the multi-chemical defense against microbial attack (Kubo *et al*., 1995). The mean diameter zone of inhibition produced by the discs of VAEE against the three strains (clinical, environmental and reference strains) of *S. typhi* and *S. aureus* are shown in and **Fig 2.** The highest susceptibility at 20mg/ml was shown by clinical strains of *Salmonella typhi* and *Staphylococcus aureus, which* recorded 23.64 mm in diameter. Similarly environmental strains, recorded 21.17 mm in diameter, while the reference strains recorded 16.27 mm in diameter. These were followed susceptibility at 10mg/ml where zones of inhibition shown were 17.29 mm, 11.23 mm and 10.56 mm in the strains of clinical, environmental and reference respectively. However, all the strains were not susceptibility, at 5mg/ml and below concentrations.

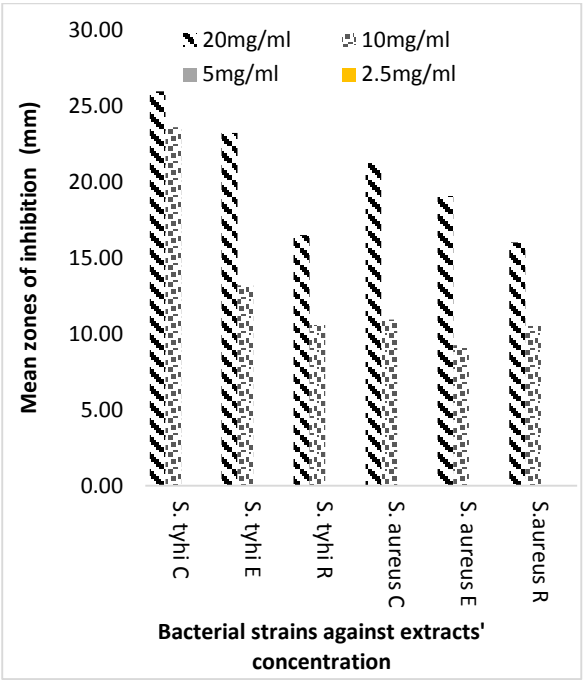

Fig 1: Mean zones of inhibition produced by the *Vernonia amygdalina* ethanolic extract (VAEE) against *Salmonella typhi* and *Staphylococcus aureus*

All the strains in test bacteria were resistant to the *Vernonia amygdalina* aqueous extract (VAAE) across all the concentrations. As there is no mean diameter zone of inhibition produced by the discs impregnated in VAAE with respect of the six strains; clinical, reference and environmental strains of *Salmonella typhi* and *Staphylococcus aureus*. The test organisms (*S. typhi*, and *S. aureus*) were susceptible to the extracts.

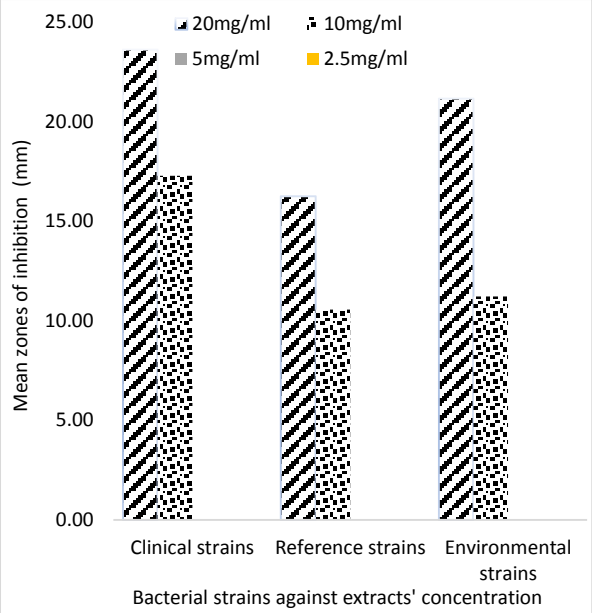

Fig 2: Comparison of the mean zones of inhibition produced by the *Vernonia amygdalina* ethanolic extract (VAEE) against clinical, reference and environmental strains of *Salmonella typhi* and *Staphylococcus aureus*

The MIC values indicated *in vitro* antibacterial activity of the *V. amygdalina* ethanolic extract. The susceptibility increases with the increase in the extract concentrations of 10mg/ml – 20mg/ml. This agrees with Robert e*t al.,* (1974) who reported that, the inactivation of a susceptible bacterial population is dependent on the relative concentration of the two reactants, the bacteria and the chemical. The Minimum bactericidal concentrations (MBC) values obtained for the VAEE at 20mg/ml against *S. typhi* (clinical strains) showed no bacterial growth. However, other strains of *S. typhi* (environmental and reference) have shown bacterial growth, hence MBC across the extract concentrations. Similarly, *S. aureus* (clinical, environmental and reference strains) also showed no MBC across the extract concentrations. Similarly, there was a statistically significant difference in susceptibility among the various *Vernonia amygdalina* extracts' concentrations and the test organisms. (P > 0.05) Table 3. The clinical strains of *S. typhi* showing highest susceptibility.

Table 3: Summary of ANOVA for six strains of bacteria against the four concentration of *V. amygdalina* extract

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
S *typhi* C	4	89.17	22.2925	122.6415
S *typhi* E	4	71.33	17.8325	103.8656
S *typhi* R	4	60.16	15.04	73.43047
S *aureusC*	4	73	18.25	79.7326
S *aureus* E	4	56.17	14.0425	57.73316
S *aureus* R	4	61.33	15.3325	80.96316
0.4	6	158.32	*26.38667	7.644267
0.2	6	130.17	*21.695	20.69171
0.1	6	86.67	14.445	33.97527
0.05	6	36	6	0

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Test organisms	182.1242	5	36.42485	4.221311	0.013522	2.901295
Extracts' concentration	1425.667	3	475.2224	**55.07399	2.49E-08	3.287383
Error	129.432	15	8.6288			
Total	1737.224	23				

*Where d f = Degree of freedom, Ss = Sum of square, Ms = Mean of sum of square * Significant difference (p> 0.05).*

The MBC was observed when the lowest concentration of the extracts that resulted in no growth of bacteria after subculture onto Mueller Hinton agar (Alan *et al.*, 1977). The result of this research is in agreement with the result obtained by Aladesanmi, *et al* (2007) who explained that the leaf extract of *Markhamia tomentosa* showed good anti-staphylococca activity as well as anti-activities against *Pseudomonas aeruginosa* and *Bacillus subtilis*. The VA leaves extract showed a wide range of medicinal uses as it had a curative effect on experimental Schistosomiasis in mice (Ogboli *et al.,* 2000).

Conclusion: The susceptibility and MIC at 10mg/ml concentrations and below, which signified antibacterial activity of the *V. amygdalina* in respect to *S. typhi* and *S. aureus* in clinical, environmental and reference strains. However, MBC values at 20mg/ml was shown only on the clinical strain of *S. typhi*. Thus, confirmed the claims of some local herbalists that the

leaves of *V. amygdalina* to be efficacious in the management diseases related to *S. typhi* and *S. aureus*.

Acknowledgement: The authors would like to thank the Tertiary Education Trust Fund (Tetfund) Abuja Nigeria, for funding this research work. Additional thanks go to Malam Adamu Department of Pharmacognosy, Ahmadu Bello University, Zaria where the extraction and phytochemical screening of the leaves was carried out.

REFERENCES

Abdulrahaman, EM; Dimlong, LD (1990). The practice of Traditional Medicine in Gynaecological diseases, *Clin. Pharmacy and herbal Med.* 6: 12-16.

Aboaba, OO; Etuwape, BM (2001). Antibacterial property of some Nigeria species, *Biol. Res. J.* 13:183-188.

Aladesanmi, J; Iwalewa, EO; Adebajo, AC; Akinkunmi, EO; Taiwo, BJ; Olorunmola, FO; Lamikanra, A (2007). Antimicrobial and Antioxidant Activity of some Nigerian Medicinal plants, *African J. Traditional, Complimentry and Alternative Medicines*, Vol.4 (2):173-184.

Alan, A; Pollock, MD; Stephen, A; Barger, MD; Alma, S; Richmond, MD; Michael, S; Simberkoff, MD; James, J; Rachal, Jr, MD (1977). Amikacin Therapy for serious Gram-Negative Infection, *J. of the American Med. Assoc.*, 237 (6): 562-564.

Baker, FJ; Silverton, RE; Palister, CJ (2001). *Baker and Silvertons Introduction to Medical Laboratory Technology.* (7th edition). Bounty press ltc. Ibadan., Pp 311, 312-315.

Bauer, AW; Kirby, WMM; Sherris, JC; Turck, M (1966). Technical section: Antibiotic susceptibility Testing by a Standardized Single disk method, *The American J. of Clin. Pathology.* 45: (4)403.

Brain, KR; Turner TD (1975). *The practical evaluation of phytopharmaceuticals.* Wright-Scientechnica, Bristol, Pp. 101-109.

Cheesbrough, M (2000). *District laboratory practice in tropical countries* (part 2). Co-published by Syndreate of the University of Cambridge, Cape town, Pp178-187.

Ching, C (2006). *Disk diffusion susceptibility Testing* (Kirby-Bauer Method) Animal Disease Diagnostic Laboratory (ADDL), West Lfayette September, 20, 2006.

CLSI. (2015a). Performance Standards for Antimicrobial Susceptibility Testing, 25th informational supplement. Approved supplement M100-S25. CLSI, Wayne, PA.

CLSI. (2015b). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 10th ed. Approved standard M7-A10. CLSI, Wayne, PA.

De, N; James, NA (2002). Antibacterial spectrum of extracts of *Ocimum gratisssium* L (Basil) and *Xylopia aetiopica, A. Rich De Niger J. of Basic and Applied Sci*. 11:165-175.

El Astal, ZY; Ashour, AERA; Kerrit, AAM (2005). Antimicrobial Activity of some Medicinal plants extracts in Palestine, *Pakistan J. of Med.*, 21 (2) 187-193.

El-Mahmood, AM; Ameh, JM (2007). In vitro antibacterial activity of Parkia biglobosa (Jacq.) root bark extract against some microorganisms associated with urinary tract infections, *African J. of Biotechnology*. 6 (11):1272-1275.

Eloff, JN (1998). Which extracts for screening and Isolation of antimicrobial Components from plants, *J. Ethnopharmacology.* 66: 1-8.

Fabry, W; Okumo, PO; Ansory, R (1998). Antibacterial activity of East African Medicinal Plants, *J. of Ethno pharmacology.* 60: 78-84.

Farnsworth, AC (1982). *The role of ethnopharmacology drug development from plants*. John Wiley and Sons, England (Ciba Symposium 154). Pp2-10.

Fidelia, UF (2004). *Plant Resource of Tropical Africa* 2 PROTA foundation/Backheys Publisher/CTA Netherlands, Pp. 543-546.

Garba, SA (2005). *Vaccines: The solution to the prevention of disease.* Inaugural lecturer series 6, 12th May 2005, Federal University of Technology, Minna. Pp. 17-30.

Hewitt, JH; Coe, AW; Parker MT (1969). The detection of Methicillin Resistance in *Staphylococcus aureus, J. of Medical Microbiol.* Vol. 2: 444-445.

Kubo, A; Lunde, C; Kubo, I (1995). Antimicrobial activity of the 'Olive oil' flavour compound, *J. of Agric. Food Chem*. 43: 1629-1633.

Merck Index (1983). *An Encyclinicalodia of Chemicals, drugs and biologicals*. 10th Ed. Rahway,10204 Merck and Co. Inc. P.8218.

Monika, J; Anil, B; Aakanksha, B; Priyanka, P (2012). Characterization and in vitro antiurolithiatic activity of cerpegin alkaloid from Ceropegia bulbosavar. Lushi root. *Int. J Drug Dev Res*. 4: 154-160.

NCCLS. (1999). Methods for Determining Bactericidal Activity of Antimicrobial Agents. Approved guideline. NCCLS document M26-A. NCCLS, Wayne, PA.

Ogboli, AU; Nock, IH; Abdurahman, EM; Ibrahim, NDG (2000). Medical Application of Vernonia amygdalina Del leaf extracts in the treatment of Schistosomiasis in mice, *Nigeria J. of Natural products and Med*. 4:73-75.

Oliver. B (1960). *Medicinal Plants in Nigeria*, Nigeria College of Art, Science and Technology. Ibadan Pp 4-8.

Oyewole, OI; Boyede, DO; Mutiat, OB; Oyedotun, MO; Alabi, KE; Oladele, OO; Oyewole, IO (2021). Chemical profiling, phytochemical constituents and in vitro antioxidant activities of ethanolic leaf extract of Talinum triangulare. Current Research in Chemistry. 13:26-37.

Oyewole, OI; Oladele, JO (2017). Changes in activities of tissues enzymes in rats administrated Ficus exasperate leaf extract. *Int. J. of Biochem Sci*. 11:378-386

Paul, D; Hoeprich, E; Jack, B; Fritz, HK (1970). Susceptibility of "Methicillin" Resistant *Staphylococcus aureus* to 12 Antimicrobial agents, California *American Society for Microbiology, Antimicrobial agents and Chemotheraphy*. (1969)104-110.

Reimer LG, Stratton CW, Reller LB. (1981). Minimum inhibitory and bactericidal concentrations of 44 antimicrobial agents against three standard control strains in broth with and without human serum. *Antimicrob Agents Chemother* 19:1050–1055.

Robert, C; Duguid, JP; Marmion, BP; Swain, RHA (1974). *Medical Microbio.* Longman group limited, London, Great-Britain, Pp 71-78

Sofowora, A (2006). *Medical plants and Traditional Medicine in Africa*. Spectrum book, Pp.3-10. www.spectrumbooksonline.com

WHO (2003). *Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the developing World*. Geneva, Pp. 207-209.