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In vitro antibacterial activities of *Vemonia amygdalina* extract on microoganisms isolated from *Clarias gariepinus*

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Abstract
This aim of this study is to determine the antimicrobial potential of *Vemonia amygdalina* leaf extract on common microorganisms that affect *Clarias gariepinus*. Aqueous and methanol extraction procedures were adopted using sohxelet extractor. Qualitative phytochemical screening was carriedout using the method described by Ogunmefun and co-researchers. Different techniques were use for quantitative determination of phytochemical constituents of leaf extract of *V. amygdalina*. Simple random sampling technique was employ in sampling. Seventy- two swab samples was collected from different fishes from the six different ponds with an average of twelve samples per pond representing 10% of the total population. The samples were screen for pathogenic microorganisms. Antimicrobial screening of the aqueous and methanolic extracts was carriedout using the agar well diffusion method. The study shows the presence of phenol, tannins, saponin, alkaloids and flavinoid in both methanol and aqueous extraction, with saponin (5.85±5.90mg/g) and tannins (9.51± 9.47mg/g) higher than others for methanol extract. For aqueoes extraction, tannnin (8.30± 8.27mg/g) were more in dominance than other phytochemicals. Five microoganisms were isolated from the fishes and these are; *Streptococcus* spp, *Staphylococcus* spp, *Escherichia coli*, *Pseudomonas aureginosa* and *Salmonella* spp. Antimicrobial activity of methanol leaf extract of *V. amygdalina* at 5mg/ml and 10mg/ml showed zones of inhibition of 8mm and 14mm for *Esherichia coli*. *Staphylococcus aureus* at 10mg/ml had 10mm inhibition zone. *Escherichia coli* presented zone of inhibition of 10mm when 10mg/ml aqueous extract of *V. amygdalina* was use, while *Staphylococcus* spp exhibited 8mm zone of inhibition with 5mg/ml of the extract. Extracts of *V. amygdalina* could be use for the treatment of *E. coli* and *Staphylococcus* spp since both organisms were sensitive to the extracts. *Pseudomonas aureginosa* and *Salmonella* spp showed zones of inhibition of 7mm and 8mm at 10mg/ml. Minimum inhibitory concentration of *V. amygdalina* leaf extracts for aqueous and methanol extract of *P. aureginosa* and *Salmonella* spp is 10mg/ml.

Keywords: Microoganisms, inhibition zones, invitro-antimicrobial activities, *V. amygdalina*, methanol and aqueous extracts

Introduction
In Africa, phytomedicine has been in existence for hundreds of years ago ever before the colonial administration and is still in use today with about 80% of the population depending on herbal medicine for its primary health care delivery (Elujoba *et al*, 2005; Okigbo and Mmeka, 2006) [9, 28].

Infectious disease accounts for one halve of all deaths in the tropical countries irrespective of efforts made in controlling the incidence of epidemic (Okigbo and Ajalie, 2005) [33].

Medicinal plants and traditional preparation with antimicrobial activities have been use extensively in the West African regions. These plants of medicinal important have proven to be very effective even where treatments with antibiotics failed (Oshim *et al.*, 2016) [30].

Vemonia amygdalina commonly called bitter leaf (English), Shuwaka (Hausa), Ewuro (Yoruba), Oriwo (Edo), and Olugbu (Igbo) is a perennial shrub belonging to the family Asteraceae (Ghamba *et al.*, 2014; Gashe and Zeleke, 2017) [12, 11]. *Vemonia amygdalina* is a shrub that can grow to 10 m tall with petiole leaf of about 6 mm in diameter, elliptic in shape and grow throughout tropical Africa. In various parts of West Africa including Nigeria, it is locally use as vegetable in soups (Etim *et al.*, 2012; Habtamu and Melaku, 2018) [10, 15]. All parts of the plant are pharmacologically useful. Both the roots and leaves has been used in phytomedicine to treat fever, hiccups, kidney disease and stomach discomfort, among others (Anibijuwon *et al.*, 2012; Hamowia *et al.*, 2004) [3, 16].

The aqueous and methanolic extracts of this plant was known to have great antimicrobial effect against different microorganisms responsible for various human infections (Arekemase *et al.*, 2013) [4].

The reported biologically active phytoconstituents from *V. amygdalina* are alkaloids, flavonoids, terpenes, saponins, coumarins, xanthones, phenolic acids, lignans, steroids, anthraquinones (Tona *et al.*, 2004) [35]. Despite the vast traditional used of *V. amygdalina*, it is still considered among the under exploited plants of economic significance. *Clarias gariepinus* not only play an important role in the demand of food for humans (Patel, *et al*., 2018) [31], but is known to habour some microorganisms. Pathogens that can cause fish diseases comprise: viral infections, bacterial infections, fungal infections, protozoan infections and water mould infection (Govind *et al*., 2012) [37]. Fishes are expose to different environmental pollutants, including drugs and chemicals. The most common microoganisms of fish particularly in freshwater aquaria, include; *Edwardsilla* spp, *Enterobacter* spp, *Salmonella* spp, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp *and Escherichia coli*. These microoganisms causes diseases that manifest clinically on fishes (Walakari *et al.*, 2014) and are as follows: columnaris, gill disease, ick (ich), dropsy, tail and fin-rot among others. Antibiotics are use frequently to control fish diseases caused by bacteria, but there is an increasing risk of developing antibiotic resistant strains. The complaints by many farmers within the area under study of high cost of drugs and poor performance of drugs used, suggests that the antibiotics used for common bacteria treatment are no longer effective. The aim of this study is to find an alternative drug (herbal extract) to the antibiotics being used that will be effective and affordable for treatment of bacteria diseases in fish.

Materials and Methods

Collection and Identification of Plant Material: The fresh leaves of *Vemonia amygdalina* plant used in this study were purchase from Isinweke daily market in Ihitte-Uboma Local Government Area in Imo State, South Eastern Nigeria. The leaves were package in a dry polythene bag and transported to the laboratory of Michael Okpara University of Agriculture, Umudike. The plant materials were identify and authenticated at the Department of Botany by Dr. Nkaa Francis of the same Institute. Sample collection lasted two months from April to May of 2021.

Processing of the Pant Material: The fresh leaves of *Vemonia amygdalina* was thoroughly clean and dried at room temperature for three weeks at the Veterinary Microbiology Laboratory bench in the College of Veterinary Medicine of Michael Okpara University of Agriculture, Umudike. The dried plant material was then pulverised to fine powder using an electric blender and stored in sterile container until the time of extraction and analysis.

Extraction procedure: Solvent was use for the preparation of the extract, namely distilled deionized water and methanol 99.9% concentration.

The Aqueous Extract: The method as described by Newton (2002) was use. Two hundred and fifty grams (250g) of the milled powdered leaves of *V. amygdalina* was introduce into a beaker and 200 ml of distilled deionized water was added gradually and stirred intermitently and vigorously with a glass rod every 20mins for 48 hours. The combination settled after 3 h using the infusion method. The extracts were filtered using Whatman no.1 filter paper.

The methanol extract: Some fraction (250g) of the plant was weighed, later wrapped in Whatman no.1 filter paper, and placed in the holding chamber of the soxhlet extractor. Then, 500 ml of the 99.9% methanol was use as solvent for the extraction of the leaves using the reflux method for a period of 48 h. The extracts were then concentrated by evaporating to dryness using rotary evaporator at a temperature 4 ºC. A dark- green coloured mass for *V. amygdalina* obtained was stored in airtight bottle at 4 ºC in a refrigerator until ready for use. The stored extract (yield) 20mg/ml was reconstituted using dimethyl sulfoxide and distilled water in a 2fold serial dilution to obtain extracts of several concentrations 10, 5, 2.5 and 1.25 mg/ml and stored at 4 ºC prior to determination of the minimum inhibitory concentration.

The Qualitative Phytochemical Screening of leaf extract of *V. amygdalina*: The phytochemical screening was carriedout using the method described (Ogunmefun *et al*., 2017) [26].

Test for phenols and tannins: Using Standard Method reported by Trease and Evans (1989) [36], Two millilitres (2ml) of 2% solution of Ferrous chloride (FeCl) was mixed with the crude extract. Black or blue green colour indicated the presence of tannins and phenols.

Tests for flavonoids: Using the method reported by Trease and Evans in 1989 [36]. Pieces of magnesium ribbon and concentrated hydrogen chloride (HCl) was mixed with the crude plant extract, after few minutes, the appearance of a pink colour scarlet indicated the presence of flavonoids.

Test for saponins: Test was carriedout using the method described (Ogunmefun *et al*., 2017) [26]. Five millilitres of distilled water was added onto crude plant extract in a test tube and it was shaken vigorously. The foam formation indicated the presence of saponins.

Test for alkaloids: Test was carriedout using the method described by Harborne (1998) [17]. Test substance (plant extracts in powder) was mix with few drops of 2N HCL. An aqueous layer formed was decanted and one or two drops of Mayer's reagent added. Formation of white turbidity or precipitate indicates the presence of alkaloids.

Quantitative Determination of Phytochemical Constituents of leaf extract of *V. amygdalina.*

Determination of Flavonoid: Flavonoid determination was by the method reported by Ejikeme *et al*. (2014) [8]. Exactly 50 cm^3 of 80% aqueous methanol was added to 2.50 g of sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of methanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each wood sample. Each wood sample filtrates was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated as:

% Flavonoid = (weight of Flavonoid)/(weight of sample) ×100

Determination of Tannins: Analytical method for quantitative determination of tannin was according to Amadi *et al*. (2004) and this is by dissolving 50 g of sodium tungstate (Na2WO4) in 37 cm^3 of distilled water, Folin-Denis reagent was obtain. To the reagent prepared above, 10 g of phosphomolybdic acid (H3PMo12O40) and 25 cm^3 of orthophosphoric acid (H3PO4) was added. Two-hour reflux of the mixture was carriedout, cooled, and diluted to 500 cm^3 with distilled water. One gram of each wood powder (sample) in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 cm^3 volumetric flask. Addition of 5.0 cm^3 Folin-Denis reagent and 10 cm^3 of saturated Na_2CO_3 solution into 50 cm^3 of distilled water and 10 cm^3 of diluted extract (aliquot volume) was carried out after being pipetted into a 100 cm^3 conical flask for colour development. The solution was then, allowed to stand for 30 minutes in a water bath at a temperature of 25 ^{0}C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer, optical density was measured at 700nm, and then compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 cm^3 mark (1 mg/cm^3) were used to obtain tannic standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis reagent (5 cm^3) and saturated Na_2CO_3 (10 cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was allow to stand for 30 minutes in a water bath at 25^{0}C. Optical density was ascertain at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration graph was plotted.
The following formula was use in the calculation:

Tannic acid (mg/100g) = (C × extract volume ×100)/(Aliquot volume ×weight of sample)

Where C is concentration of tannic acid read off the graph.

Determination of Saponin: Saponin quantitative determination was carriedout using the method reported by Obadoni and Ochuko, (2002) [25]. Exactly 100 cm^3 of 20% aqueous methanol was added to 5 grams of each wood powder sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55 ^{0}C. The residue of the mixture was re-extracted with another 100 cm^3 of 20% aqueous methanol, after filtration, it was heated for 4 hours at a constant temperature of 55 ^{0}C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90 ^{0}C, 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel, and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice and 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated in percentage:

% Saponin = (weight of saponin)/(weight of sample) ×100

Determination of Alkaloids: Quantitative determination of alkaloid was according to the methodology by Harborne, (1973) [18]. Exactly 200 cm^3 of 10% acetic acid in methanol was added to each wood powder sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop-wise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance Model B-218, the residue was dry in an oven and the percentage of alkaloid expressed mathematically as:

% Alkaloid = (weight of alkaloid)/(weight of sample) ×100

Determination of Phenols: Defatting of 2 g wood powder sample was carriedout for 2 hours in 100 cm^3 of ether using a soxhlet apparatus. The defatted sample (0.50 g) was boiled for 15 minutes with 50 cm^3 of ether for the extraction of the phenolic components. Exactly 10 cm^3 of distilled water, 2 cm^3 of 0.1 N ammonium hydroxide solutions, and 5 cm^3 of concentrated amyl alcohol were also added to 5 cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm, 0.20 g of tannic acid was dissolved in distilled water and diluted to 200 mL mark (1 mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution was pipetted into five different test tubes to which 2 cm^3 of NH3OH, 5 cm^3 of amyl alcohol, and 10 cm^3 of water were added. The solution was made up to 100 cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 505 nm keay *et al*. (1964) [20].

Preparation of Crude Plant Extract: A 2 g amount of extract was weighed out, using a Mettler balance, and then dissolved in 5 ml of dimethyl sulphoxide (methanol extract) or 5 ml of water (water extracts). Subsequently, each solution was serially diluted to obtain 10.0, 5.0, 2.5 and 1.25 mg/ml concentrations.

Media preparation: All media used were prepared according to the manufacturer's directives. The media includes; nutrient, MacConkey, Salmonella-shigella, deoxyocholate, blood and eosin methylene blue agar. Media preparations were as described by Lammert *et al*. (2007) [21]. A given quantity in grams of the media was measured and suspended in distilled water, then, sterilized by autoclaving at standard temperature and pressure within a given time. The media was allowed to cool at a known temperature before poured into petri-dishes.

Gram-staining technique: The technique as described by Gram (1888) was employ. Make a smear and fix it by

passing the slide rapidly over a flame. Cover the slide with, crystal violet and allow it to act for 30 seconds. Pour off and wash freshly with distilled water. Cover with fresh iodine solution and allow to react for 30 seconds. Pour off the iodine solution and wash freely with distilled water. Cover with acetone and allow it to act for about 30 seconds (until the stain stops carrying out).Wash thoroughly with water. Counter- stain with safranin for 30 seconds and Wash with distilled water, blot and dry. View under the microscope with oil immersion lens.

Catalase test: Test was carriedout as described by Karen (2010). Pour 2-3ml of fresh hydrogen peroxide solution into a test tube. Using a sterile wooden or glass rod, remove a good piece of growth of the test organism and immerse it in the 3% hydrogen peroxide solution. Look for immediate bubbling, which indicates the production of oxygen. Active bubbling produced along the side of a glass rod is a positive result.

Oxidase test: The test was as described by Patricia and Luara (2010). The indirect paper strip procedure in which a few drops of the reagent were added to a filter paper strip. A loop full of suspected colony was smeared into the reagents zone of the filter paper. Bacterial colonies having cytochrome oxidase activity developed a deep purple color at the inoculation site within 10 seconds. As a precautionary measure, stainless steel or nichrome inoculating loops or wires were not use for this test because surface oxidation products formed by metals when flamed for sterilizing may result in false positive reactions. Report as 'oxidase positive' if a blue-purple color is produced. Otherwise, report as 'oxidase negative'.

Collection and isolation of sample organism: Samples were taken from under the gill (by lifting it up) of the fish using sterile swab sticks, inoculated into nutrient agar plates and incubated at 37 ^{0}C for 48hrs. Colonies of the growth organisms were subcultured into different media like, eosin methylene blue (EMB), *Salmonella-Shigella* agar (SSA), blood agar and MacConkey agar and incubated at 37^{0}C for 48hrs. The presence of green metallic sheen was confirmatory of *Escherichia coli*, presence of in-complete zones of haemolysis confirms *Staphylococcus aureus* in blood agar. while complete zones of haemolysis on blood agar was confirmatory of *Streptococcus* spp. Discrete raise colonies with black centres in Salmonella shigella agar is suggestive of *Salmonella* spp, while clear blue colonies on MacConkey agar is suggestive of *Pseudomonas aeruginosa*.
Preparation of Test Organisms and Tests for Potency of Bacteria Pathogen: The organisms isolated were stored in stock culture in the Department of Veterinary Microbiology, College of Veterinary Medicine of the University. Organisms obtained were as follows: *Escherichia coli*, Staphylococcus aureus, Streptococcus spp, Pseudomonas aeruginosa and Salmonella spp.
The bacteria organisms were sub-cultured in nutrient agar. Overnight cultures were prepared by inoculating approximately 2ml nutrient broth with colonies of the appropriate organism taken from the agar slant. Broths were incubated overnight at 37 ^{0}C. Inoculants were prepared by diluting overnight cultures in saline to approximately 10^8 CFU /ml for each of the organisms.
Experimental Animal: *Clarias gariepinus* were obtained from 6 different farms in Ikwuano Local Government Area and kept in 6 different artificial ponds.
Determination of antimicrobial activity of the extract**:** The antimicrobial screening of the aqueous and methanolic extracts was carriedout using the aqar well diffusion method as described by Lino and Deogracious (2006) [22]. Nutrient agar was poured into sterile Petri dishes and allowed to solidify. About 1.0 ml of the test culture was dropped on the appropriate solidify agar and spread over the surface of the medium using sterile swab stick. Wells of approximately 6 mm in diameter were made in the agar medium using sterile cork borer. Each well was filled with 0.2 ml of the appropriate concentration of the extract. The dishes were allowed to stand for 40 min at room temperature to allow for proper diffusion of the extract to occur. Control experiments were set up with 0.2 ml of 99% methanol and 0.2 ml distilled deionized water in separate wells. The plates were incubated at 37 ^{0}C for 24 h. All tests were performed in 5 replicate and antimicrobial activity was expressed as the mean diameter of the clear zone (mm) produced by the plant extract. Zones of clearance round each well means inhibition and the diameter of such zones was measure in millimeter (mm).
Determination of Minimum Inhibitory Concentration (MIC)**:** The MIC of the extracts for each susceptible test organism were determined by a method described by Okeke *et al*. (2001) [27] and Okoli *et al*. (2002) [29]. The minimum inhibitory concentration (MIC) in mg/ml was determined by comparing the different concentration of the extracts that have different zones of inhibition and then selecting the lowest concentration of each extract (Agatemor, 2009) [2].

Results

A 99.9% methanol and distilled deionized water was use for the extraction. More yields were achieved with aqueous extract of *V. amygdalina* 25g than methanol extracts which yielded 8.0g. Saponin and tannin was present in reasonable amount in qualitative analysis of all the constituents found in *Vemonia amygdalina* using methanol extraction method. Other phytochemical constituents are alkaloid, phenol and flavenoid. Aqueous extraction method had saponin in larger amount qualitatively, than all other constituents. In quantitative analysis using methanol method, saponin and tannin with (5.85±5.90mg/g) and (9.51±9.47mg/g) had the highest qualitative presentation, while alkaloid had the least of (2.22±2.18mg/g). Comparative presentation evaluated show that tannin possess the largest amount (8.30±8.27mg/g) quantitavely and alkaloid the least (1.23±1.19mg/g) using aqueous method. The microorganisms obtained from the samples are *Staphylococcus* spp, *Escherichia coli*, *Streptococcus* spp, *Pseudomonas aeruginosa and Salmonella* spp. At concentration of 5mg/ml, *Escherichia coli* had the highest inhibition zone of 8mm while, *Pseudomonas aeruginosa* and *Salmonella* spp had the least of 0mmmg/ml. *Escherichia coli* and *Staphylococcus* spp at 10mg/ml had a higher zone of inhibition of 14mm and 10mm, while *Pseudomonas aeruginosa* and *Salmonella* spp had the least inhibition zones of 7mm and 8mm respectively. *Escherichia coli* and *Staphylococcus* spp had an inhibition zone of 10mm and 8mm at 10mg/ml concentration, 5mm and 3mm at 5mg/ml concentration while, *Pseudomonas aeruginosa* had the least of 5mm at 10mg/ml contation. Minimum inhibitory concentration of *Vemonia amygdalina* leaf extract

for methanol and aqueous method was 10mg/ml for *P. aureginosa* and *Salmonella* spp. For other microorganisms, 5mg/ml was the minimum inhibitory concentration using methanol and 7mg/ml for aqueous method. *Escherichia coli* is most sensitive to the extract than other microoganisms.

Table 1: The Qualitative Analysis of *Vemonia amygdalina* on Methanol Extraction.

Samples	Alkaloid	Saponin	Tanin	Phenol	Flavonoid
Vemonia amygdalina	+	++	++	+	+

Key: + = present, ++ = highly present

Table 2: The qualitative analysis of *Vernonia amygdalina* on aqueous extraction

Samples	Alkaloid	Saponin	Tanin	Phenol	Flavonoid
Vemonia amygdalina	+	++	+	+	+

Key: + = present, ++ = highly present

Table 3: The methanolic quantitative analysis of alkaloid, saponin, tannin, phenol and flavonoid in *Vemonia amygdalina.*

Samples	Alkaloid (mg/100g)	Saponin (mg/100g)	Tanin (mg/100g)	Phenol (mg/100g)	Flavonoid (mg/100g)
Vernonia amygdalina	2.22±2.18	5.85±5.90	9.51±9.47	3.32±3.28	4.72±2.80

Table 4: The aqueous quantitative analysis of alkaloid, saponin, tannin, phenol and flavonoid in *Vemonia amygdalina.*

Samples	Alkaloid (mg/100g)	Saponin (mg/100g)	Tanin (mg/100g)	Phenol (mg/100g)	Flavonoid (mg/100g)
Vemonia amygdalina	1.23±1.19	4.80±4.50	8.30±8.27	2.32±2.28	3.75±1.85

Table 5: Antimicrobial activity of methanol leaf extract of *Vemonia amygdalina* with zones of inhibition measured in (mm) at different concentration.

Test organism	Zone of inhibition (mm)				
	Methanol	1.25mg/ml	2.5mg/ml	5mg/ml	10mg/ml
S. aureus	0	0	3	4.0	10.0
Streptococcus spp	0	0	4	4.8	8.5
E. coli	0	0	5	8.0	14.0
P. aeruginosa	0	0	0	0	7.0
Salmonella spp	0	0	0	0	8.0

Table 6: Antimicrobial activity of aqueous leaf extract of *Vemonia amygdalina* with zones of inhibition measured in (mm) at different concentration.

Test organism	Zone of inhibition (mm)				
	Distilled water	1.25 mg/ml	2.5 mg/ml	5.0 mg/ml	10 mg/ml
Staphylococcus aureus	0	0	0	3.0	8.0
Streptococcus spp	0	0	0	4.6	6.5
Escherichia. Coli	0	0	0	5.0	10.0
P. aeruginosa	0	0	0	0	5.0
Salmonella spp	0	0	0	0	7.0

Table 7: Minimum inhibitory concentration of *Vemonia amygdalina* leaf extract

Test organism	Aqueous extract (mg/ml)	Methanol extract (mg/ml)
S. aureus	7	5
Streptococcus spp	7	5
E. coli	7	5
P. aeruginosa	10	10
Salmonella spp	10	10

Discussion

A higher yield of *Vemonia amygalina* was obtain from aqueous extract than methanol extract and this may be responsible for the general use of aqueous extract by the populace in various preparations including for food consumption. Similarly, the cost effectiveness of the aqueous method is highly desirable, when compared to the methanol extract. Quantitative analysis of *Vemonia amygdalina* using methanol extract had alkaloid (2.22±2.18mg/g) as the lowest phytochemical while Saponin (5.85±5.90mg/g) and tannin (9.51±9.47mg/g) were the highest phytochemical. The high presence of saponin present pharmacological influence or activities and this is in agreement with the findings of Oakenfull and Scott (1998) [24]. Oakenfull and Scott in their study reported that saponins possess antibiotic and antiviral properties. The study shows that tannin was present in high amount and this was also supported by Buzzini *et al*. (2008) [5]. Buzzini and his co-workers, in their report stated that tannins possess some antifungal, antimicrobial and antiviral properties that could be use for the treatment of inflammation, gonorrhoea, burns and diarrhea. The presence of alkaloids in this work and their synthetic derivatives are use as basic medicinal agents for their bacterial effects and this is in consistent with the

findings of Stray (1998) [33]. Flavonoids are plant nutrients that when consumed in the form of vegetables are non-toxic as well as potentially beneficial to the human body; up till now, more than 200 different flavonoids have been isolated from vegetables such as *Vemonia amygdalina* as reported by Taiz and Ziegler (2006) [34]. The susceptibility of the test microorganisms relates to the inhibition zones size in millimetres via agar well diffusion assay.

The phytochemical screening conducted on the leaf extract of *Vemonia amygdalina* revealed the presence of phytochemical constituents such as alkaloids, saponins, tannins, phenols and flavonoids which has been shown to possess some pharmacological activities and this agrees with the research done by Trease and Evans (1989) [36]. The antimicrobial effect of *Vemonia amygdalina* leaf extract is due to the bioactive compounds such as the phytochemical constituent present in the leaf as reported by Doughri (2007) [7]. The level of inhibition of the pathogen by the test extracts shows the extracts to be appreciably potent. Generally, the activity of plant extracts against disease causing microorganisms and their use in traditional remedies is consider to be a function of the phytochemicals in the plants and this is in agreement with Sofawara (1993) [32]. The methanol extract showed more activity than the aqueous extracts on all isolates, and this is in agreement with the work by Agatemor (2009) [2]. The findings in this work showed that the methanolic extract possesses more antimicrobial activity than aqueous extract. *Escherichia coli* was showed the greatest sensitivity, while *Pseudomonas aeruginosa* showed the least of all the isolates and thia is in agreement (Adetutu *et al*. 2011) [1]. The finding in this work is contrary to a previous report Chekole *et al*. (2016) [6] that plant extracts display stronger antimicrobial effect on the gram positives bacterial strains than on their gram- negative counterpart.

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