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Antibacterial Activities of Vernonia Amygdalina Extracts against Salmonella Typhi

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Abstract: **Original Research**

***Background*:** Plant extracts, used alone or in combination, were used as a traditional and local means of treatment for both infectious and non-infectious disorders long before the revolutionary discovery of medications that could block bacterial activity. This is aimed at determining the potentials of Vernonia amygdalina leaf extracts, its phytochemicals, and antibacterial activities against pure isolate of Salmonella typhi obtained from cultured plate media. ***Methods*:** An experimental study was carried out in the Microbiology Laboratory at the Bingham University Karu, Nasarawa State. Salmonella Typhi Samples were isolated and collected from selective cultured media (SSA) in the laboratory for microbiological analysis. Specified concentrations of *Vernonia amygdalina* extract was obtained from the powdered leaf by subjecting it to aqueous and ethanol extraction. The extracts were used to determine their antimicrobial activities against the Salmonella Typhi isolate. ***Results*:** The test results revealed the presence of various phytochemicals, such as flavonoids, alkaloids, saponins, and tannins in the extract. The Aqueous extract demonstrated activity against S. typhi, with growth inhibition zones measuring from 6.0±0.1mm at 62.5mg/ml to 18.0±1.2mm at 500mg/ml. In comparison, the Ethanolic extract showed growth inhibition zones ranging from 4.0±0.1mm at 62.5mg/ml to 22.0±1.5mm at 500mg/ml against S. typhi, while Amoxicillin, serving as the control antibiotic, inhibited growth with zones ranging from 2.0±0.2mm at 31.25mg/ml to 30.0±1.0mm at 500mg/ml against S. typhi. ***Conclusion*:** It was discovered that the plant extracts killed the organism with MBCs of 250 mg/ml for the ethanolic extract and 500 mg/ml for the aqueous extract, and that they inhibited the development of the test organism with a MIC of 250 mg/ml for both aqueous and ethanolic extracts. According to the concentration employed, this suggests that the extracts may be bacteriostatic or bactericidal. The findings showed that crude extracts of Vernonia amygdalina were just as effective as traditional antibiotics.

Keywords: Salmonella Typhi, Extracts, Vernonia Amygdalina, Ethanol, Aqueous.

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INTRODUCTION

Sir Alexander Fleming's 1928 discovery of penicillin marked the beginning of the modern age of antibiotics [1]. Plant extracts, used alone or in combination, were used as a local treatment for both infectious and non-infectious disorders long before the revolutionary discovery of medications that could block bacterial activity. Traditionalists did this without knowing which of the phytochemicals contained in these plants were active against such diseases [2].

It has been demonstrated that a variety of plants are necessary for human survival not only as food sources but also as sources of

industrial raw materials and medicinal compounds. It has been demonstrated that medicinal plants' organs contain chemicals beneficial for medicine production and physical therapy [3]. The plant genus Vernonia bears William Vernon's name, an English botanist who discovered and catalogued this genus in Maryland during the late 1700s. The uses of plants in the genus Vernonia are numerous and include, among other things, food and medicine [4].

Vernonia amygdalina, commonly known as Bitter leaf is a well-known medicinal plant belonging to a group of dicotyledonous plants otherwise called African leaves found in the family *Asteraceae* [5]. Vernonia amygdalina has been utilized for generations as a food and medicine, and its benefits are still relevant today. Due to its effectiveness in managing and treating a wide range of medical issues, the plant has several applications in traditional medicine. Many parasitic diseases, including helminthiasis and amoebic dysentery, hiccups, typhoid, yellow fever, stomach ache, convulsions, boils, burns, diabetes, jaundice, inflammatory diseases, candidiasis, pile, cancer, viral diseases, bacterial infection, gastro-intestinal (GIT) disorders, liver diseases, kidney problems, and nausea, can be effectively treated with it. Additional conditions include menstruation discomfort, diarrhea, hepatitis, eczema, anaemia, hypertension, cough, febrile convulsion, urinary tract inflammation, wound dressing, and other STDs [6].

Smashed Vernonia amygdalina leaves are commonly applied to wounds and cuts as first aid in rural areas of Eastern and Western Nigeria, where access to proper healthcare facilities is limited. This practice has demonstrated remarkable efficacy in preventing bacterial infection over time.

The public's health is seriously threatened by the rising incidence of drug misuse and improper usage of antibiotics. Research has demonstrated that one of the main causes of antimicrobial resistance worldwide is drug addiction [7]. Abuse of local medications is typically caused by an incorrect dosage.

The study is aimed at investigating the inhibitory activities of Vernonia amygdalina a plant extract, on the bacterial pathogen Salmonella typhi.

MATERIALS AND METHODS:

Study Area:

This is an experimental study carried out in the Microbiology Laboratory at the Bingham University Karu, Nasarawa State. Salmonella species Samples were isolated using PCR/ NGS methods and collected from selective cultured media (SSA) in the laboratory for microbiological analysis.

Vernonia amygdalina:

Fresh leaves of Vernonia amygdalina were collected near the Old Male Hostel, Bingham University Karu in July 2024. It was identified and authenticated at the Department of Biological Sciences by a Botanist in the Department.

Confirmatory Tests:

To confirm that the isolate collected were indeed the right one, the organism was subjected to biochemical tests and sub-cultured on selective media - Salmonella Shigella Agar (SSA).

Preparation of Extract:

The leaves of *Vernonia amygdalina* were separated from the plant stalk, cleaned and air-dried under the shade for a few days, crushed into coarse particles using mortar and pestle, then collected in a clean airtight container.

Extraction Procedure:

Specified concentrations of *Vernonia amygdalina* extract was obtained from the powdered leaf by subjecting it to aqueous and ethanol extraction.

Aqueous Extraction:

In a stoppered bottle, 400ml of distilled water (1:4 w/v) was added to 100g of powdered leaf. For 72 hours, the mixture was let to stand at room temperature while being constantly stirred. In order to liberate the soluble phytochemicals, the plant's cell wall was broken down and softened. The resulting combination was filtered using Whatman Filter Pater No. 1 to strain and

clarify it after 72 hours, and it was then dried off using an evaporating dish placed over a water bath. Before being used, the aqueous extract was kept at 4°C in an appropriate sterile container [8].

Ethanol Extraction:

In a stoppered bottle, 100g of the powdered leaf was cold macerated for 72 hours with 400ml of 70% ethanol. Whatman Filter Pater No. 1 was used to filter the resulting slurry, which was then dried off using an evaporating dish placed over a water bath. Before being used, the water bath was kept in an appropriate, sterile container with a relatively consistent temperature [9].

Preparation and Standardization of Inoculum:

The process of standardizing bacterial inoculate involved using a loop to select four to five identical colonies from the culture plate, moving them into nutritional broth (tryptic soy broth), stirring for a short while, and then incubating them for roughly three hours at 37°C [10, 11]. The turbidity generated was corrected to match 0.5 Barium Sulfate (BaSO4) or 0.5 McFarland turbidity standard (108 cfu/ml), which was then adjusted to 105 cfu/ml.

Antimicrobial Susceptibility Test of V. amygdalina Extracts on Isolates:

Agar diffusion method was used to test if *Salmonella typhi* is sensitive to both aqueous and ethanolic extracts of *Vernonia amygdalina*. Mueller Hinton Agar (MHA) was used following procedures outlined by Anibijuwon [10]. Mueller Hinton Agar was prepared, poured into fifteen plates and allowed to solidify. The agar plates were inoculated by pouring the standardized inoculum into each one. A sterile cotton swab was used to spread the inoculate evenly on the surface of the agar and the excess drained off. The plates were left on the bench for 1hour so that the inoculate could properly diffuse into the agar. A sterile cork borer was used to make ditches on the plates. Varying concentrations of the aqueous and ethanolic extracts, as well as the control antibiotic – amoxicillin (i.e. 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml) were made using two-fold serial dilution and 0.5ml of each concentration was dropped in separate plates and appropriately labelled. The plates were left on the bench for few minutes for the extract to diffuse into the agar and later incubated at 37°C for 24hours. After incubation the zone of inhibition which represents antibacterial activity was measured using a metre rule by taking the diameter of each ditch. The zone of clearance around each ditch was also measured by taking measurement from the edge of the plate to the point where the growth of the organism started. The test was conducted in triplicate.

Antibacterial Effects of V. amygdalina at Different Concentrations:

The antibacterial activity of *Vernonia amygdalina* at the five concentration levels above (i.e. 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml) were assessed. This was done in order to evaluate the activity and efficacy of different concentrations of *V. amygdalina* against *S. typhi*.

Determination of Minimum Inhibitory Concentration (MIC):

Broth dilution method was used to determine the MIC of both aqueous and ethanolic extracts. Varying concentrations of the extracts which include 500mg/ml, 250mg/ml, 125mg/ml, 62.50mg/ml and 31.25mg/ml were made using two-fold serial dilution method. Peptone water was prepared and 5ml was added to ten sterile test tubes, the test tubes were appropriately labelled and 0.1ml of the test organism was inoculated into the various tubes. Finally, 1ml of each concentration of the extract was added. The tubes were incubated aerobically for 24hours at 37°C. Positive control was equally set up by using peptone water and test organisms without the extract. The tube with the lowest concentration of extract which did not show any visible growth after the period of incubation was taken as the MIC [10].

Determination of Minimum Bactericidal Concentration (MBC):

From the test tubes used in the determination of MIC, the tubes that showed no visible growth were sub-cultured on Mueller Hinton Agar (MHA), with the concentration preceding the MIC also plated as a positive

control. The plates on which no growth was observed were selected and the lowest concentration was adopted as the MBC.

Phytochemical Screening of the Extracts:

The plant extract was analysed in order to detect the presence of constituent phytochemicals such as flavonoids, alkaloids, tannins, saponins and steroids.

Test for Flavonoids (Alkaline Reagent Test):

2-3 drops of sodium hydroxide (NaOH) were added to 2ml of the plant extract. Initially it turned to a deep yellow colour but it gradually became colourless by adding few drops of dilute HCL, showing that flavonoids were present [12].

Test for Saponins (Frothing Test):

3ml prepared solution of the extract was mixed with 5ml distilled water in a test tube. The tube was stoppered with the thumb and shaken vigorously for about two minutes. It was allowed to stand for few minutes and observed for honey-comb froth, which is an indication of the presence of saponins [13].

Test for Alkaloids (Wagner's Test):

Wagner's reagent was prepared by dissolving 2g Potassium iodide and 1.2g iodine in 5ml distilled water and the solution was further diluted to 100ml with distilled water. 1ml of the reagent was added to 2ml of the extract; a reddish-brown precipitate indicates the presence of alkaloids [14].

Test for Tannins (Braymer's Test):

2ml of 2% Ferric Chloride ($FeCl_3$) solution mixed with crude plant extract. Black or blue-green colouration confirms the presence of tannins [15].

Test for Steroids (Liebermann-Burchard Test):

100mg of the extract was shaken with chloroform in a test tube; few drops of acetic anhydride were added to the test tube and boiled in a water bath and rapidly cooled. 2ml of concentrated H_2SO_4 was added to the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids [14].

Statistical Analysis:

One-way Analysis of Variance (ANOVA) was used as a statistical method to examine the differences in the effects of Vernonia amygdalina aqueous and ethanolic extracts against the test organism at various doses.

Using Pearson Correlation as a statistical tool, the relationship between the different concentrations of Vernonia amygdalina and the corresponding zones of inhibition on the test organism was also examined. Values were deemed statistically significant only if they were $p < 0.001$.

RESULTS

Physical Properties of Vernonia amygdalina Extracts

After being crushed and pounded, the dry sample weighed 200g. For both aqueous and ethanolic extraction, 100g of the powdered material and 400ml of solvent were utilized. The aqueous and ethanolic extracts weighed 3g and 5g, respectively, following filtration and evaporation. Both extracts were sticky and dark green in colour.

Phytochemical Constituents of Vernonia amygdalina Extracts

Table 1 displays the findings of Vernonia amygdalina crude extracts' phytochemical screening. The results showed that phytochemicals like alkaloids, flavonoids, tannins, and saponins were present. The levels of these phytochemicals varied between the ethanolic and aqueous extracts of Vernonia amygdalina. No steroids were used.

Antibacterial Activities of Vernonia amygdalina Extracts

Table 2 displays Vernonia amygdalina's antibacterial properties against Salmonella typhi. Carefully assessed and documented were the inhibitory zones of the different extract and control concentrations. The extracts' proportional antibacterial activity determines the S. typhi growth inhibition zones.

The findings demonstrated that Salmonella typhi was inhibited by aqueous extract at concentrations of 500.00 mg/ml, 250.00 mg/ml, 125.00 mg/ml, 62.50 mg/ml, and

31.25 mg/ml, respectively, with diameters of 18 mm, 12 mm, 10 mm, 6 mm, and 0 mm. Inhibition diameters for the ethanolic extract were 22 mm, 12 mm, 8 mm, 4 mm, and 0 mm for concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml, respectively. Growth inhibition diameters for the control plates were 30 mm, 15 mm, 10 mm, 8 mm, and 2 mm for amoxicillin concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, and 31.25 mg/ml respectively.

A graphic representation of the zones of growth inhibition created by various concentration levels of both aqueous and ethanolic extracts of *Vernonia amygdalina* is shown on Figure 1.

Clear zones of 500mg/ml and 250mg/ml of the aqueous extract are shown on table 2 below.

Dose-Response Effects of V. amygdalina on Salmonella typhi

The zones of inhibition for the test organism S. typhi and the concentration of Vernonia amygdalina were significantly correlated ($p < 0.001$) according to the dose-response model, as seen in Figure 2. The aqueous extract, ethanolic extract, and control had the following correlation coefficient values: r2 = 0.85, r2 = 0.97, and r2 = 0.98, respectively. Accordingly, the findings showed that differences in the concentration of the plant extracts accounted for 85%, 97%, and 98% of the variation in the widths of the inhibitory zones against Salmonella typhi produced by the aqueous extract, ethanolic extract, and control, individually, as shown in figure 2 below.

Minimum Inhibitory Concentration of Vernonia amygdalina

The minimum inhibitory concentration (MIC) of *Vernonia amygdalina* against *Salmonella typhi* is shown in Table 3. The results obtained showed that the minimal inhibitory concentration of both the aqueous and ethanolic extracts of *V. amygdalina* against *S. typhi* is 250mg/ml.

Minimum Bactericidal Concentration of Vernonia amygdalina

The minimum bactericidal concentration (MBC) of *Vernonia amygdalina* against *Salmonella typhi* is shown in Table 4 below. The results obtained from plating the MIC of both extracts and the concentrations preceding and succeeding them shows that the MBC of the aqueous extract is 500mg/ml while that of the ethanolic extract remained 250mg/ml (same as the MIC) as shown in table 4 below.

Table 1: Phytochemical constituents of Vernonia amygdalina

Phytochemicals	**Observation**	
	Aqueous Extract	Ethanolic Extract
Saponins	++	++
Tannins	+	+
Flavonoids	-	+
Steroids	-	-
Alkaloids	+	+

Key: (+) = Positive; (++) = Strongly Positive; (-) = Negative

Table 2: Zones of inhibition of Vernonia amygdalina extracts against *S. typhi*

Type of Extract	**Extract concentration (mg/ml)**				
	500	250	125	62.5	31.25
Aqueous extract	18.0±1.2	12.0±0.5	10.0±0.2	6.0±0.1	0.0
Ethanolic extract	22.0±1.5	12.0±0.2	8.0±0.5	4.0±0.1	0.0
Amoxicillin	30.0±1.0	15.0±0.5	10.0±0.0	8.0±0.5	2.0±0.2

Zones of inhibition are in millimeter (mm)

Table 3: Minimum inhibitory concentration of *V. amygdalina* extracts against *S. typhi*

Type of Extract	Extract concentration (mg/ml)				
	500	250	125	62.5	31.25
Aqueous extract	-	-	+	+	+
Ethanolic extract	-	-	+	+	+

Key: (+) = Growth, (-) = No Growth.

Table 4: Minimum bactericidal concentration of *V. amygdalina* extracts against *S. typhi*

Type of Extract	Extract concentration (mg/ml)		
	500	250	125
Aqueous extract	-	+	+
Ethanolic extract	-	-	+

Key: (+) = Growth, (-) = No Growth

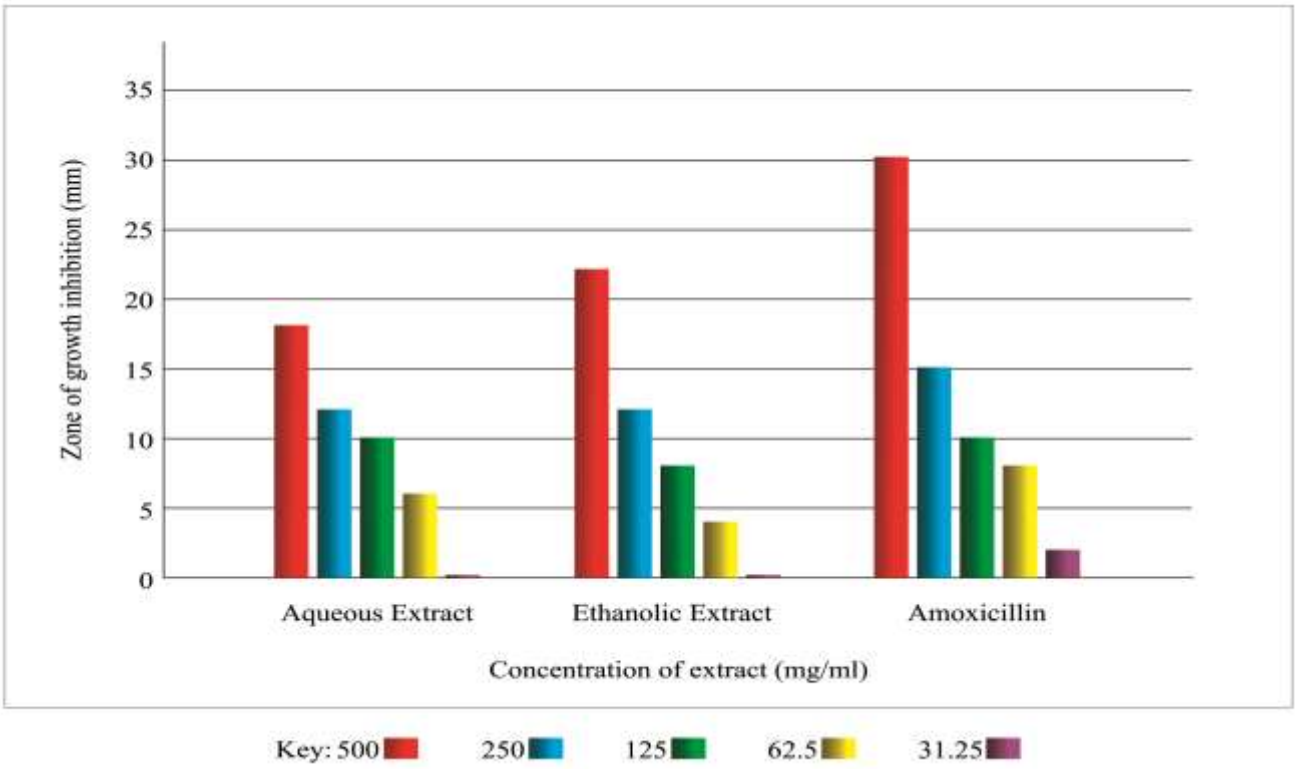

Figure 1: Bar chart showing growth inhibition of *S. typhi* by Aqueous and Ethanolic extracts of Vernonia amygdalina with Amoxicillin as control antibiotic

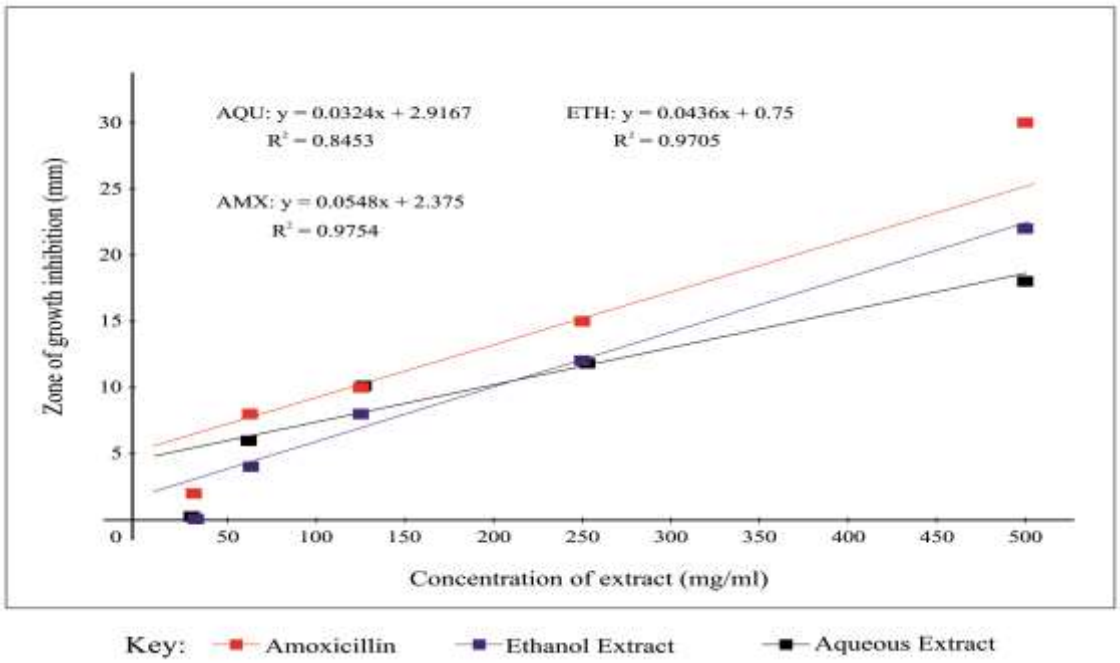

Figure 2: Dose-response curve showing zone of growth inhibition (mm) against concentration of Vernonia amygdalina (mg/ml)

DISCUSSION

The purpose of this study was to assess Vernonia amygdalina's antibacterial efficacy against Salmonella typhi. According to the results of the tests, extracts from Vernonia amygdalina leaves have a high degree of antibacterial activity and contain phytochemicals such as alkaloids, flavonoids, tannins, and saponins.

Therefore, it may be said that Vernonia amygdalina's phytochemical components play a significant role in its antibacterial qualities. This assertion supports the findings of [16], who demonstrated in his study on the antibacterial properties of leaf extracts of Vernonia amygdalina, that the bioactive components found in plants, including alkaloids, saponins, tannins, flavonoids, and others, were responsible for the antimicrobial activity in plants.

It was discovered that the plant's ethanolic and aqueous extracts both have varied degrees of antibacterial activity. Both extracts were effective against the test organism, according to the susceptibility test, but Salmonella typhi was more vulnerable to the ethanolic extract. Additionally, this result is consistent with earlier research showing that ethanolic extracts of fresh and dried Vernonia amygdalina are more effective than aqueous extracts on the test organisms under investigation [17]. According to [10], ethanol extracts exhibited greater activity against the bacterial isolates than aqueous extracts. This could be because ethanol has a higher volatility than water, which tends to extract more active chemicals from the samples.

Salmonella typhi was shown to be susceptible, confirming the substantial antibacterial action of Vernonia amygdalina. This was demonstrated by the level of inhibition caused by the extract's presence at different concentrations. The capacity of Vernonia amygdalina to suppress microbial growth as investigated in this study is consistent with a number of reports, such as that of [18], who reported that ethanol and aqueous extracts of V. amygdalina leaves exhibited antimicrobial effects against Candida albicans, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella spp.

This study demonstrates that Vernonia amygdalina's antibacterial activity is concentration-dependent (dose dependent), meaning that higher extract concentrations cause more noticeable growth suppression than later diluted concentrations. This might be because there are less phytochemicals available to prevent microbial growth at lower extract concentrations.

Additionally, the results demonstrated that the minimum bactericidal concentrations for the ethanolic and aqueous extracts of Vernonia amygdalina were 250 mg/ml and 500 mg/ml, respectively, while the lowest inhibitory concentrations for both extracts were 250 mg/ml.

The limitation of the study is that the extract is only used on Salmonella Typhi; further research work on the antimicrobial activity of *Vernonia amygdalina* should be carried out on gram positive bacteria and other pathogenic microbes including fungi and protozoans to test for its broad-spectrum activity and assess its ability to function in wide range areas outside its use as an antibiotic. The potential of Vernonia amygdalina to serve as an antibiotic by preventing bacterial growth should be communicated to the public community; this would enhance its medicinal use in orthodox medicine and as well as its typical traditional use.

CONCLUSION

Extracts of Vernonia amygdalina have great antibiotic effect on Salmonella typhi. Salmonella typhi responds well to extracts of Vernonia amygdalina. However, in comparison to the ethanolic extract, the aqueous extract has a smaller effect. Additionally, the concentration of the extract is directly proportional to its activity, as higher concentrations demonstrated stronger antibacterial activity; the Vernonia amygdalina extract should be used in larger doses for optimal and efficient outcomes.

RECOMMENDATION

The potential of Vernonia amygdalina to serve as an antibiotic by preventing bacterial development should be made known to the public. This would enhance its medicinal potential use in orthodox medicine and as well as its typical traditional use. Vernonia Amygdalina extract should be used in high concentration in order to achieve its bactericidal or bacteriostatic effects.

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