The Effect of Bitter Leaves (*Vernonia amygdalina*) Extract on *Salmonella typhi* and *Salmonella paratyphi*

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Abstract: Research Background: Antibiotic-resistant strains of *Salmonella* spp have been a major foodborne pathogen threat to human and amplifysignificant food safety hazard. The emergence of multiple drug resistance salmonella in foods has necessitated a search for new antimicrobial substance from other sources, including plants. This research aimed to evaluate the phytochemicals and anti-salmonella activity of ethanol and cold-water extracts of bitter leaves (*Vernonia amygdalina*) extract against *S. typhi* and *S. paratyphi*.

Experimental approach: Two species of *Salmonella* were collected from Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State. The plant leaf extracts were screened for their phytochemical component to evaluate the availability of active bio-compounds in the leaves. The leaf extractswere subjected to antimicrobial analysis by disc diffusion method using different concentration of the leaf's extracts.

Results and conclusion: The phytochemical evaluation showed that compounds such as Alkaloids, Cardiac glycosides, Flavonoids, Saponins, Steroids, Tannins, and Glycosides were present in different quantities. The extracts showed antibacterial effect against the bacteria with zone of inhibition ranging between 8.1 ± 0.14 mm (9.257 mg/mL) to 22.5 ± 0.24 mm (75 mg/mL) on *Salmonella typhi* and 7.3 ± 0.11 mm (9.257 mg/mL) to 23.3 ± 0.31 mm (75 mg/mL) on *Salmonella paratyphi*. Minimum Inhibitory Concentration was observed at 18.25 mg/mL and 37.5 mg/mL for ethanol and cold-water extracts of *V. amygdalina*, respectively, in both isolates. However, no significant difference in the extract's activity against the test isolates at $p < 0.05$ was observed. *V. amygdalina* cold-water and ethanolic extracts possess phytochemical with inhibitory activities on *Salmonella typhi* and *Salmonella paratyphi*.

Novelty and scientific contribution: This study showed that *V. amygdalina* possesses phytochemicals active against *Salmonella* and should be further explored to extract bioactive ingredient for use in *Salmonella* drug development and treatment regime.

Keywords: Antimicrobial, Bitter Leaves, Phytochemical, Zone of Inhibition

I. INTRODUCTION

The burden of reducing the effect of pathogenic microorganism on human health has been a global and major public health challenge[1]. Multidrug-resistant strains of bacteria are increasing at an alarming rate in developing countries due to the random use of antimicrobial drugs to treat infection[2]. Several synthetic antimicrobial drugs have been developed to counter the effect of these pathogens and their resistant strains, with few of these antimicrobial compounds having a significant effect on these pathogenic

bacteria. Therefore, the need to find novel and potent antimicrobial compound from various sources becomes paramount due to the realities of the rapid worldwide dissemination of these resistant strains of pathogenic bacteria and has forced researchers to explore new antibacterial substance from medicinal plants[3].

Plants have shown immense potential as natural sources of alternative antimicrobial agents with effective treatment ability against various bacterial infections. The World Health Organisation (WHO) has recognised medicinal plants as one of the best alternative sources of drugs to treat microbial infections[4]. Plants have been discovered to possess secondary metabolites and have been used as antimicrobial due to these phytochemicals synthesised from various medicinal plants. Secondary metabolites such as alkaloids, flavonoids, phenolic compound, terpenoids, steroids, quinones, among many other metabolites, have been found in-vitro with high-efficiency antimicrobial properties against pathogenic bacteria[5].

Vernonia amygdalina is a phototherapeutic plant used in folk medicine for several decades and is believed to have diverse active compounds that help treat and manage various diseases. Various parts of the plant have been locally used to treat disease conditions, and the leaves are used in ethno medicine to treat fever, kidney problems, gastroenteritis, vomiting, diarrhoea, dysentery, and several other conditions [6]. *V. amygdalina* roots, stem and leaves have been prepared using several solvents such as water and alcohol locally, and their extracts reportedly used as malaria and eczema cure. *V. amygdalina* leaves have been used in many West African countries to make soup, serve as appetizers, and have successfully been used as a supplement in weaning foods [7]. *V. amygdalina* leaves in animal experimentation have shown to possess hypoglycaemic and hypolipidemic properties; this can be potentially employed in managing disease conditions such as diabetes mellitus[8].

Extracts from some plants with proven bioactive components have been employed in alternative medicine to reduce the incidence of antibiotic resistance. Some research have suggested *V. amygdalina* leaves possesses some bioactive components such as alkaloids, saponins and terpenoids tannins, flavonoids and phenolic compound with a characteristic strong fragrant smell and slight pungency [9]. The study assessed the antisalmonella properties of *Vernonia*

amygdalina leaf extracts on selected human pathogenic bacteria and the phytochemical contents.

II. MATERIALS AND METHODS

Collection of *V. amygdalina* leaves

Vernonia amygdalina leaves were purchased from the Lafia Central Market, Nasarawa State, Nigeria. The plant's leaves were authenticated at the Botany Department, Federal University of Lafia, Nasarawa State, Nigeria. The process of preparation of the extract was done according to the procedure of [10] with modifications. Fresh leaves of *V. amygdalina* were air-dried in the shade at room temperature for two weeks, then grounded into fine uniform powder using a sterilised mortar and pestle under aseptic condition and stored for further use. About 50 g of the powdered leaf was soaked for 72 hours in 250 ml of ethanol and cold water at room temperature, then filtered first by using a clean muslin cloth and then followed by No.1 Whatman filter paper. The filtrate was concentrated to dryness at 45°C using a water bath to get a crude extract. These crude extracts were stored at a temperature of 4°C prior to antibacterial analysis.

Test organisms

The test organisms: *Salmonella typhi* and *Salmonella paratyphi* were collected from the Medical Laboratory of the Dalhatu Araf Specialist Hospital in Lafia, Nasarawa State, Nigeria. The organisms were stored at 37°C on *Salmonella Shigella* Agar (SSA) slants, and subcultures were made in selenite F broth and preserved until used.

Phytochemical Analysis of *Vernonia amygdalina* Extract

Phytochemical screening for bioactive components such as alkaloids, saponins, tannin, phlobatannins, anthraquinones and glycosides, steroids, flavonoids, proteins and sugars of the ethanol and cold water extracts of *Vernonia amygdalina* were determined as described by [9] with modification.

Sterility Test of Extract

The sterility of the filtered extracts *V. amygdalina* was tested as described by [11] with modification. One ml of each extract was introduced into 10 ml of freshly prepared nutrient broth and incubated for 24 hours at 37°C. Sterility was ascertained by observing the turbidity of the broth after the incubation.

Antibacterial Sensitivity Assay of Extracts

Antibacterial assay of the extracts was carried out using the disc diffusion method [12]. The test bacteria cultures were inoculated into Mueller Hinton agar (equivalent to 0.5 MacFarland) and distributed evenly to cover the surface of the plates. Sterilized 6 mm paper disc were dipped into different concentration of extracts (75 mg/mL, 37.5 mg/mL, 18.25mg/mL, and 9.125 mg/mL) for 2 minutes. These discs were placed aseptically on inoculated agar plates with the aid of sterile forceps. The inoculated plates were then inverted and incubated for 24 hours at 37°C, and inhibition zones around the disc were observed. For control, standard

Ciprofloxacin discs (commercially available) were used. Why ciprofloxacin: Because it has quick treatment response and shortened bacteraemia (Jin et al., 2019), fever disappears within 3 days and no significant adverse effects were reported in a study by Akhtar et al. (1992).

Determination of Minimum Inhibitory Concentration (MIC)

Stock solutions (150mg/mL) of each extract were half diluted serially to 75mg/mL, 37.50mg/mL, 18.25mg/mL and 9.125mg/mL by transferring and mixing equal volume (5mL each) of previous concentration with sterile nutrient broth. Each concentration was subsequently inoculated with 0.1mL of bacterial culture and incubated for 24 hours at 37°C. Each extract's minimum inhibitory concentration (MIC) was taken as the lowest concentration with the least turbidity, as described by [13].

Statistical Analysis

The zones of inhibition were presented as mean±standard deviation. One-way analysis of variance (ANOVA) was used to analyse all data at the significance level of $p < 0.05$ using the IBM SPSS statistical package version 25 (2017).

III. RESULTS

Phytochemical Analysis of *Vernonia amygdalina* Extract

The phytochemical analysis of ethanol and cold-water plant extracts reveals, the presence of Alkaloids, Cardiac glycosides, Steroids, Flavonoids, Saponin glycosides, Saponins, Tannins and Glycosides in both cold-water and ethanolic leaf extracts, as shown in Table 1. Anthraquinones were not detected in both extracts assayed.

Table 1: Qualitative phytochemical analysis of *Vernonia amygdalina* leaf extract

Phytochemical	Ethanol Extracts	Cold Water Extracts
Alkaloids	+	+
Anthraquinone	-	-
Cardiac glycosides	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	+	+
Tannins	+	+
Glycosides	+	+

Key: + = present, - = absent

Antisalmonella Sensitivity Assay of Extracts

Antisalmonella assay of *Vernonia amygdalina* leaf showed that the zone of inhibition expressed by extracts on isolates depends on the extracts' concentration. Although, as shown in Table 2, both extracts had inhibitory effects on the bacterial isolates, it was observed that the inhibitory activity was concentration-dependent. In addition, the ethanolic extract

was observed to have higher inhibitory activity than water extract.

Table 2. Average zone of inhibition (mm) of Antibacterial Sensitivity Assay of *Vernonia amygdalina* Leaf Extract on *Salmonella typhi* and *Salmonella paratyphi*

Extr acts	Concentration (mg/mL)							
	<i>Salmonella typhi</i>				<i>Salmonella paratyphi</i>			
	9.257	18.25	37.5	75.0	9.25 7	18.25	37.5	75.0
Col d wa te r	8.1±0 .14	10.2± 0.12	12.6± 0.22	15.8± 0.16	7.3± 0.11	11.4± 0.17	12.8± 0.20	14.1± 0.22
Etha nol	12.5± 0.23	17.1± 0.18	20.0± 0.11	22.5± 0.24	9.7± 0.15	13.2± 0.19	18.0± 0.15	23.3± 0.31

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration for the ethanol extracts was observed for both microorganisms at a concentration of 18.25 mm/mL, while 37.5 mm/mL concentration was observed to be the minimum inhibitory concentration for cold water extracts of the same plant leaf; this is as shown in Table 3.

Table 3: Minimum inhibitory concentration of *Vernonia amygdalina* leaf extract

Extracts	Concentrations (mg/mL)							
	<i>Salmonella typhi</i>				<i>Salmonella paratyphi</i>			
	9.257	18.25	37.5	75.0	9.257	18.25	37.5	75.0
Cold water	-	-	+	+	-	-	+	+
Ethanol	-	+	+	+	-	+	+	+

Key: + = Low turbidity, - = High turbidity.

IV. DISCUSSION

The main objective of this study was to determine the effects and efficacy of *Vernonia amygdalina* leaf extracts on *Salmonella typhi* and *Salmonella paratyphi* in-vitro using two solvents of extraction (ethanol and water). Phytochemicals analysis of *Vernonia amygdalina* showed that Alkaloids, Cardiac glycosides, Flavonoids, Saponin glycoside, Saponins, Steroids, Tannins and Glycosides were present in both cold water and ethanol extracts; corroborating earlier findings in research carried out by [6,14]. Anthraquinones was not present as phytochemical in this research, contradicting the report of [9], which report anthraquinones to be present in the plant in both ethanol and aqueous extracts. Several research studies have reported the importance of these phytochemicals with distinct functions and collectively providing antimicrobial, anti-inflammatory, and antioxidant benefits [15–18].

Extracts of *V. amygdalina* leaves in this study showed varied antimicrobial potential against the bacterial isolates. The findings in this study concur with several pieces of research as reported by [9,19,20]. The type of solvent used to extract the *V. amygdalina* leaves showed different antibacterial activity

against the isolates – ethanol extract showed higher activity against the isolates than cold water extracts. The efficacy of both ethanolic and cold-water extracts was also observed to be concentration-dependent; the higher the concentration, the higher the zone of inhibition. The volatility of ethanol might be why it is a better solvent for extracting the active ingredient than cold water. Similar findings were also observed and reported by [21,22].

Minimum inhibitory concentration analysis varied amongst extracts. The minimum inhibitory concentration was observed at a concentration of 18.25 mg/mL and 37.5 mg/mL for ethanol and cold-water extracts of *V. amygdalina*, respectively, in both isolates. The findings of this research showed that ethanol extract of *V. amygdalina* was more effective in inhibiting *S. typhi* and *S. paratyphi* at a lower concentration than cold water extracts. This agrees with the previous finding of [7], whose research result claimed that ethanol extracts of *V. amygdalina* are effective in inhibiting the growth of some bacteria isolates.

V. CONCLUSION

Result of this research confirmed the presence of some phytochemicals in *V. amygdalina* leaves analysed. Cold water and ethanol extracts of *V. amygdalina* leaves displayed antibacterial effect on the isolates, with ethanol extract having the highest inhibitory activity; this effect may be bacteriostatic or bactericidal. Antisalmonella activity of *V. amygdalina* against the test isolates is due to the diverse phytochemicals present in it. This plant extracts can be further researched to evaluate their potential against multidrug-resistant strains of bacteria and purify bioactive secondary metabolites with potential for antibacterial drug formulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Authors FGA and OOO developed the concept and design of this study, all authors were involved in performing the analysis and collection of data, authors OAO and BSE analysed and interpreted all data, authors OAO, BSE and FYA drafted the article, authors FGA and OOO critically reviewed the article. All authors approved the final version to be published.

REFERENCES

- [1] Bhatia R, Narain JP. The growing challenge of antimicrobial resistance in the South-East Asia Region - are we losing the battle? *Indian J Med Res* 2010;132:482–6. <https://doi.org/10.4103/0971-5916.73313>.
- [2] Nitu S. Evaluation of Antimicrobial Activity Determination and Phytochemical Investigation in Selected Plants. Available Online *Www.IjpprComInt J Pharmacogn Phytochem Res* 2017;9:1429–34. <https://doi.org/10.25258/phyto.v9i11.11187>.

- [3] Davies J. Origins and evolution of antibiotic resistance. *Microbiologia* 1996;12:9–16. <https://doi.org/10.1128/mmbr.00016-10>.
- [4] Manandhar S, Luitel S, Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. *J Trop Med* 2019;2019. <https://doi.org/10.1155/2019/1895340>.
- [5] Djeussi DE, Noumedem JAK, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complement Altern Med* 2013;13. <https://doi.org/10.1186/1472-6882-13-164>.
- [6] Ali M, Diso SU, Waiya SA, Abdallah MS. Phytochemical Screening and Antibacterial Activity of Bitter Leaf (*Vernoniaamygdalina*). *Ann Microbiol Infect Dis* 2019;2:1–07.
- [7] Gberikon GM, Iyola FI, Ichor ST. Effect of Bitter Leaves Extract on Bacteria Isolated from Soils of Some Mechanic and non-Mechanic Sites in Makurdi Metropolis. *Int J ApplSciBiotechnol* 2019;7:81–7. <https://doi.org/10.3126/ijasbt.v7i1.23302>.
- [8] Akah P, Njoku O, Nwanguma A, Akunyili D. Effects Of Aqueous Leaf Extract Of *VernoniaAmygdalina* On Blood Glucose And Triglyceride Levels Of Alloxan-Induced Diabetic Rats (*Rattusrattus*). *Anim Res Int* 2008;1. <https://doi.org/10.4314/ari.v1i2.40748>.
- [9] Evbuomwan L, Chukwuka EP, Obazenu EI, Ilevbare L. Antibacterial activity of *Vernoniaamygdalina* leaf extracts against multidrug resistant bacterial isolates. *J ApplSci Environ Manag* 2018;22:17. <https://doi.org/10.4314/jasem.v22i1.4>.
- [10] Kate I. Effects of *Vernoniaamygdalina* Del. Extract on Cholesterol Level and Lipid Peroxidation Status in Rats Given Red Dye Adulterated Palm Oil Diets. *Br J Pharm Res* 2012;2:98–107. <https://doi.org/10.9734/bjpr/2012/1095>.
- [11] Oluyele O, Oladunmoye M. Susceptibility Patterns of *Staphylococcus aureus* Isolated from Wounds Swabs to Extracts of *Vernoniaamygdalina*. *J Adv Med Pharm Sci* 2017;13:1–11. <https://doi.org/10.9734/jamps/2017/33837>.
- [12] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J ClinPathol* 1966;45:493–6.
- [13] Ibekwe VI, Nnanyere NF, Akujobi CO. Studies on antibacterial activity and phytochemical qualities of extracts of orange peels. *Int J Environ Heal Hum Dev* 2001;21:41–6.
- [14] Akinjogunla OJ, Ekoi OH, Odeyemi AT, Akinjogunla OJ, C.A. E, C.E O. Preliminary phytochemistry and in-vitro antibacterial efficacy of Hydro-Ethanollic leaf extracts of *Psidiumguajava* on common urinary tract bacterial pathogens. *Bioresource* 2011;5:329–36.
- [15] Erasto P, Grierson DS, Afolayan AJ. Evaluation of antioxidant activity and the fatty acid profile of the leaves of *Vernoniaamygdalina* growing in South Africa. *Food Chem* 2007;104:636–42. <https://doi.org/10.1016/j.foodchem.2006.12.013>.
- [16] Rabi T, Bishayee A. Terpenoids and breast cancer chemoprevention. *Breast Cancer Res Treat* 2009;115:223–39. <https://doi.org/10.1007/s10549-008-0118-y>.
- [17] Toyang NJ, Verpoorte R. A review of the medicinal potentials of plants of the genus *Vernonia* (Asteraceae). *J Ethnopharmacol* 2013;146:681–723. <https://doi.org/10.1016/j.jep.2013.01.040>.
- [18] Ali M, Yahaya A, Zage A, Yusuf Z. In-vitro Antibacterial Activity and Phytochemical Screening of *Psidiumguajava* on Some Enteric Bacterial Isolates of Public Health Importance. *J Adv Med Pharm Sci* 2017;12:1–7. <https://doi.org/10.9734/jamps/2017/31126>.
- [19] Salami OO, Georgia CA. The assessment of antimicrobial activities of *ocimumgratissimum*(wild basil)and *vernoniaamygdalina* (bitter leaf)on some enteric pathogen causing dysentery or diarrhea in patients. *Int J EngSci* 2013;2:83–96.
- [20] Ogundare A. Antibacterial properties of the leaf extracts of *Vernoniaamygdalina*, *Ocimumgratissimum*, *Corchorousolitorius* and *Manihot palmate*. *J MicrobiolAntimicrob* 2011;3:77–86.
- [21] Udochukwu U, Omeje FI, Uloma IS, Oseiwe FD, Udochukwu U. PHYTOCHEMICAL ANALYSIS OF *Vernoniaamygdalina* AND *Ocimumgratissimum* EXTRACTS AND THEIR ANTIBACTERIAL ACTIVITY ON SOME DRUG RESISTANT BACTERIA. *Am J Res Commun* 2015;3:225–35.
- [22] Zubairu AY, Mukhtar M, Saidu I, Ibrahim Z, Isah S, Garga MA, et al. Antibacterial activity of methanolic extract of bitter leaf (*Vernoniaamygdalina*) from various component fractions using column chromatography. *GSC Biol Pharm Sci* 2019;7:016–21. <https://doi.org/10.30574/gscbps.2019.7.2.0095>.