UJMR, Conference Special Issue Vol. 9 No. 3. June, 2024, pp. 308 - 314

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.2493.037

Received: 13th Mar, 2024 **Accepted:** 17th June, 2024

Antibacterial Activity of Vernonia Amygdalina (Bitter Leaf) Extracts against Clinical Isolates of Salmonella Species

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Abstract

Salmonellosis is becoming a common illness in underdeveloped nations, and specifically, typhoid fever is a major public health concern due to its high likelihood of recurrence. Salmonella has become resistant to numerous drugs, making it necessary to look for effective novel antibacterial substances from alternative sources, including plants. The antibacterial activity of Vernonia amygdalina (bitter leaves) extracts was assessed in this study against ten (10) Salmonella species clinical isolates. The bitter leave was cleaned, dried, and ground into powder. The phytochemical content of the leaves was extracted using ethanol and aqueous solvent; the extracts' antibacterial activity was tested against clinical isolates of Salmonella. Alkaloids, cardiac glycosides, saponins, steroids, tannins, and glycosides were among the constituents present, according to the phytochemical analysis. The highest zone of inhibition was 11 mm for the ethanolic extract at 100mg/mL concentration demonstrated against SS 4 isolate, while 9 mm was the highest zone of inhibition for the aqueous extract at 100mg/mL concentration against SS 6 isolate. For both extracts, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of all Salmonella isolates tested were found at 50 mg/mL and 100 mg/mL concentrations, respectively. V. amygdalina has antibacterial activity against Salmonella and should be investigated further for potential application in developing anti-Salmonella medications and managing salmonellosis.

Keywords: Antibiotic resistance, Salmonella species, Vernonia amygdalina, Phytochemical, Antibacterial activity

INTRODUCTION

A Gram-negative bacterium called *Salmonella* is responsible for 41% of diarrhea-related deaths worldwide. *Salmonella enterica* and *Salmonella bongori* are the two species of *Salmonella* that are highly pathogenic. There are six subspecies of *Salmonella enterica-enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI)—represent 1500 of the more than 2600 serotypes that have been identified (Lee *et al.*, 2015). The causative serotypes of *Salmonella* differ from continent to continent. For example, non-typhoidal *Salmonella* (NTS) serovars are frequent in Africa, while typhoidal Salmonellae (serotypes Typhi and Paratyphi A) are common in Southeast Asia (WHO, 2016).

For years, *V. amygdalina* has been used as a folk medicine phototherapeutic plant. It is thought to contain a variety of active chemicals that aid in treating and managing a wide range of illnesses, including fever, renal issues, gastroenteritis, diarrhoea, and dysentery (Ali *et al.*, 2019). The leaf is an inexpensive, easily accessible plant widely available in all parts of Nigeria.

Of all the *Vernonia* species, *V. amygdalina* is most likely the one that is used medicinally. It is a highly significant medicinal plant, a highly significant medicinal plant, a commonly utilised antibacterial, antifungal, antiplasmodial, and antiparasite herb (Ijeh and Ejike, 2011). Local preparation of *V. amygdalina* roots, stems, and leaves has been done using a variety of solvents, including alcohol and water. Their extracts have purportedly been used to treat dermatitis and malaria. Throughout several West African nations, *V. amygdalina* leaves have been successfully incorporated into weaning foods, prepared into soups, and served as appetisers (Ali *et al.*, 2019).

It was discovered that the extracts from the leaves of *V. amygdalina* exhibited antibacterial activity against both Gram-negative and Gram-

positive bacteria, including *Klebsiella puemoniae, Pseudomonas aerugionosa, Salmonella typhi, Shigella dysenteriae,* and *Proteus vulgaricius*. Gram-positive bacteria included *Bacillus cereus, B. pumilus, B. Subtitlis, Staphylococcus aureus, Clostridium, Bacillus cereus, S. epidermidis, Micrococcus kristinae,* and *Streptococcus pyrogens* (Swee *et al.,* 2010). It has been demonstrated that the aqueous extracts of *V. amygdalina* leaves are effective against plant fungal infections without adversely impacting the plant's growth. However, it has been demonstrated that the phytochemical compounds of *V. amygdalina* exhibit activity against fungi like *Aspergillus flavus, Mucorhiemalis, Fusarium oxysporum, Penicillium notatum,* and *Aspergillus niger* in a way that is comparable to the standard medication Nystatin (at 0.1 mg/ml and above) (Ijeh and Ejike, 2011).

One of the biggest global challenges facing public health is minimizing harmful microorganisms' impact on human health. As a result of the inappropriate application of antimicrobial medications to treat infections, multidrug-resistant bacterial strains are spreading alarmingly in underdeveloped nations. Though few synthetic antimicrobial agents have a major effect on these harmful bacteria, some have been produced to counteract the effects of these pathogens and their resistant variants. Typhoid fever is primarily more common in underdeveloped nations due to the presence of Salmonella species as its causal agent. Typhoid fever poses a severe public health risk due to its high likelihood of recurrence (Nitu, 2017). The reality of these pathogenic bacteria's rapid global spread makes it necessary to discover novel and potent antimicrobial compounds from various sources, which is why it's important to investigate new antibacterial substances from medicinal plants that are efficient, reasonably priced, and easily accessible. Testing the antibacterial activity of *V. amygdalina* leaf extract on *Salmonella* species is necessary due to the advent of antibiotic-resistant *Salmonella* species and the potential benefits of *V. amygdalina* leaves in treating bacterial illnesses. Accordingly, this study aimed to determine the antibacterial activity of *V. amygdalina* (bitter leaf) extract against clinical isolates of *Salmonella* species.

MATERIALS AND METHODS

Collection and authentication of *Vernonia amygdalina* leaf samples

Vernonia amygdalina leaves were collected from the central market of Kaduna, Kaduna State, Nigeria. The left sample was taken to the Department of Biological Science, Kaduna State University, to identify and authenticate them(Voucher number; KASU/BSH/684). The samples were then taken to the Department of Microbiology laboratory of the University for further analysis.

Preparation and Extraction of *V. amygdalina* leaves samples

Fresh *V. amygdalina* leaves were cleaned with distilled water, allowed to dry in the shade, and then crushed with a laboratory mortar and pestle into a fine powder (Udochukwu *et al.,* 2015).

Fifty (50) g of the grounded leaves were soaked in 250ml of ethanol and stirred. The leaves were then covered for 48 hours and filtered using Whatman's No1 filter paper. The filtrate in a beaker was placed in a water bath to remove ethanol from the filtrate. The pure residue was weighed and kept in the refrigerator until use (Udochukwu *et al.,* 2015).

Moreover, fifty (50) g of the grounded leaves were soaked in distilled water of about 500 ml. The leaves were then covered for 48 hours and filtered using Whatman's No1 filter paper. The filtrate in a beaker was placed in a water bath to remove the water content from the filtrate. The pure residue was weighed and kept in the refrigerator until use (Udochukwu *et al.,* 2015).

Determination of phytochemical components of *V.amygdalina* leaves

i. Alkaloid

Concentrated hydrochloric acid of 2 ml was added to 2ml of the plant extracts, followed by a few drops of Mayers reagent. The presence of alkaloids was revealed by the appearance of green colour (Evbuomwan *et al.,* 2018).

ii. Saponin

After diluting the extract with an equal volume of distilled water, the mixture was agitated in a graduated cylinder for fifteen minutes. The presence of saponins is indicated by the development of a 1 cm layer of foam (Evbuomwan *et al.*, 2018).

iii. Steroid

One ml of chloroform, a few drops of concentrated H2SO4, and 2 ml of acetic anhydride were added to 2 ml of the extract. The development of a reddish-brown colour signifies the presence of steroids (Evbuomwan *et al.*, 2018).

iv. Tannins

Two ml of the plant extract was added to 5% ferric chloride, and the presence of dark blue indicates tannins (Evbuomwan *et al.*, 2018).

v. Glycoside

Two (2) millilitres of the extract was mixed with 3 ml of chloroform and a 10% ammonia solution. The development of a pink colour indicates the presence of glycosides (Evbuomwan *et al.*, 2018).

vi. Quinones

One (1) millilitre of concentrated sulfuric acid was combined with 1 ml of the extract. The presence of quinones is indicated by the development of red colour (Evbuomwan *et al.*, 2018)

vii. Phenol

Two (2) ml of distilled water was added to 1 ml of the plant extract. A few drops of 10% ferric chloride were added, and the development of blue-green signifies the presence of phenols (Evbuomwan *et al.*, 2018).

viii. Terpenoids

Two (2) ml chloroform and 2 ml sulfuric acid were added to 0.5 ml of the extract and agitated. The presence of terpenoids was indicated by the appearance of yellow colour (Evbuomwan *et al.*, 2018).

ix. Cardiac Glycoside

Two (2) ml glacial acetic acid, 5% ferric chloride, and 1ml sulfuric acid were added to 2 ml of the extract. Concentrated hydrochloric acid was added, and the appearance of blue indicates cardiac glycoside's presence (Evbuomwan *et al.*, 2018).

x. Phytosterol

One (1) ml of chloroform and a few drops of concentrated H_2SO_4 were added to 2 ml of the extract, and the development of bluish green colour signifies the presence of phytosterol (Evbuomwan *et al.*, 2018).

Collection and Confirmation of Clinical Isolates

The clinical isolates of *Salmonella* species were obtained from Barau Dikko Teaching Hospital Kaduna state. The isolates were reconfirmed based on their colonial morphology on *Salmonella-Shigella* agar (SSA), their Gram reaction, and biochemical reaction (citrate utilization test, Methyl Red, indole, and sugar fermentation on triple sugar iron tests, as reported by Chessbrough (2005). The isolates were stored on a nutrient agar slant until further use (Kate, 2012).

Antibacterial Activity Testing of Leaf Extracts against *Salmonella* Species

Preparation of McFarland Standard

The 0.5 McFarland standard was prepared by carefully mixing 99.5ml of 1% sulphuric acid with 0.5ml of 1% barium chloride (NaCl) solution. The prepared McFarland standard was transferred into a bijou bottle and capped (Chessbrough, 2005).

Preparation of standard inoculum

A discrete colony of each of the confirmed isolates of *Salmonella* spp was inoculated in distilled water till the turbidity matched that of 0.5 McFarland standard (Ali and Yahaya, 2017).

Preparation of the *V. amygdalina* leaves extract concentrations.

To get a 100 mg/mL concentration for the stock solution, 1g of aqueous and ethanolic extracts were separately dissolved in 10 mL of sterile dimethyl sulfoxide (DMSO). To achieve a 50 mg/ml concentration, 1 mL of the stock solution was serially half-diluted with 1 mL of sterile DMSO. The process continued to get

25 mg/ml concentrations and 12.5 mg/mL (Manandhar *et al.*, 2019).

Susceptibility testing of the organism using the agar diffusion method

Mueller Hinton agar plates were uniformly streaked with an inoculum size of the confirmed isolates of the test isolates matching the 0.5 McFarland standard. A cock borer of 4mm was used to create four wells on the surface of the agar. The ethanoic and aqueous extract of *V. amygdalina* in various concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml) were added (2 drops) to the wells. Inhibition zones were measured and recorded after incubating the plates for 24 hours at 37°C. A Cefoxitin disc (10µg) was used as the positive control (Gobezie *et al.*,2020).

Determination of Minimum Inhibitory Concentration (MIC)

A loopful of the test organisms was introduced into test tubes that contained five millilitres of sterile nutritional broth. Each test tube was filled with 1ml of the various concentrations: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml, and the tubes were then incubated at 37°C for 24 hours. A test tube with just broth and extract was employed as a control. The MIC was determined as the lowest concentration inhibiting the bacteria's visible growth (Evbuomwan*et al.*, 2018).

Determination of Minimum Bactericidal Concentration (MBC)

The MIC tubes showing no visible growth were subcultured onto freshly prepared Muller Hinton agar plates. The plates were then incubated at 37℃ for 48 hours. The lowest concentration at which the organism did not recover and grow was MBC (Evbuomwan *et al.*, 2018).

RESULTS

The determination of bioactive components of *Vernonia amygdalina* leaves showed that the ethanolic extracts contain Saponins, tannins, alkaloids, quinones, phenol, terpenoids, and steroids, while the aqueous extract contained tannins, saponins, quinones, phenol and phytosteroids (Table 1).

Table 1: Phytochemical components of *Vernonia amygdalina* leaf extract

Bioactive constituents	Ethanolic extract	Aqueous extract
Tannins	+	+
Saponins	+	+
Alkaloids	+	-
Glycosides	-	-
Quinones	+	-
Phenol	+	+
Terpenoids	+	-
Cardiac glycosides	-	-
Phytosterols	-	+
Steroids	+	-

Key: +: Present; -: Absent

The ethanolic extracts of *V. amygdalina* demonstrated a larger zone of inhibition than the aqueous extract against the *Salmonella* species. Compared to the aqueous extract, the ethanolic extracts showed zones of inhibition as high as 11 mm by SS 4 isolate, while the aqueous extract showed a zone of inhibition as high as 9 mm by SA 6 (Table 3 and Table 4).

Table 3: Antibacterial activity of ethanolic extracts of *Vernonia amygdalina* leaves against *Salmonella* species

	Concentration / Zone of inhibition				
Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Cefoxitin
SS 1	8.4	7	4.3	NI	3.7
SS 2	NI	NI	NI	NI	NI
SS 3	9.6	8.2	5	4.4	3.8
SS 4	11	9.3	6	4.8	4
SS 5	NI	NI	NI	NI	NI
SS 6	10	8.5	6	4.5	4.8
SS 7	8	6.4	4.9	NI	4
SS 8	NI	NI	NI	NI	NI
SS 9	7.2	6	5.2	4.8	3.7
SS 10	NI	NI	NI	NI	NI

Key: SS: *Salmonella* species'; NI: No inhibition

The lowest concentration of the leaf that inhibited the growth of the test bacteria after a 24-hour incubation period was determined to be the minimum inhibitory concentration (MIC), and the lowest concentration of the extract that the organism didn't recover and grow was determined to be the minimum bactericidal concentration (MBC). For the ethanolic and aqueous extract, the MIC and MBC were found to be at 50 mg/ml and 100 mg/mL, respectively, for all the *Salmonella* isolates tested (Table 5).

Table 4: Antibacterial activity of aqueous extracts of *Vernonia amygdalina* leaves against *Salmonella* species

	Concentration / Zone of Inhibition (mm)				
Isolates	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Cefoxitin (10µg)
SS 1	8	6	4.7	NI	3.6
SS 2	NI	NI	NI	NI	NI
SS 3	8.3	6	5.6	4.3	3.8
SS 4	6	5.6	4.3	NI	4.1
SS 5	NI	NI	NI	NI	NI
SS 6	9	7.6	6	4.8	4
SS 7	7	6.4	5.2	4.4	4
SS 8	NI	NI	NI	NI	NI
SS 9	5	4.3	NI	NI	3.9
SS 10	NI	NI	NI	NI	NI

KEY; SS: *Salmonella* species NI:No inhibition

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the *V. amygdalina* Leaves Extract against *Salmonella* species

	Ethanolic extract		Aqueous extract	
Isolates	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
SS 1	50	100	50	100
SS 2	50	100	50	100
SS 3	50	100	50	100
SS 4	50	100	50	100
SS 5	50	100	50	100
SS 6	50	100	50	100
SS 7	50	100	50	100
SS 8	50	100	50	100
SS 9	50	100	50	100
SS 10	50	100	50	100

DISCUSSION

The findings of this study have shown that the ethanolic and aqueous extracts of *V. amygdalina* possess antibacterial activity against *Salmonella* species in a concentration-dependent manner, similar to the findings of Ali *et al.* (2019). The antibacterial activity of this plant is due to the presence of phytochemicals such as tannins, steroids, saponins, and phenol. These complex bioactive components of the leaves account for their pharmacological usefulness in ethno-medicine (Uzoigwe *et al.*, 2011). Studies by Udochukwu *et al.* (2015) and Ali *et al.* (2019) also detect saponins, alkaloids, tannins, phenolics, terpenes, and steroidal glycosides as bioactive components of *V. amygdalina* leaf extract. The study also revealed that *V. amygdalina* leaves contain many of these bioactive components. It is thought that these bioactive elements are what give the plant its bioactivities against microorganisms. The bioactive ingredients of *V. amygdalina* may work alone or in concert to provide antimicrobial effects (Ijeh*et al.*, 2011).

The antibacterial activity of *V. amygdalina* leaf extracts has been the subject of numerous studies (Akujobi *et al.*, 2004; Ijeh and Adedokun, 2006; Zubairu *et al.*, 2019). Their findings demonstrate that the extracts' levels of antibacterial activity varied. The type of extraction solvent and extract concentration were found to have an impact on the antibacterial activity of the extract, according to their findings. This study confirmed that compared to water extracts, with 9 mm as the highest zone of inhibition, the ethanolic extract exhibited greater activity, with 11 mm as the highest zone of inhibition against the bacterial isolates. This could be because ethanol extracts more active chemicals from the leave samples than water. This study's findings were consistent with those of Ali et al. (2019), who discovered that *V. amygdalina* leaf ethanol extract was efficient against several bacterial strains. This result also justifies the finding of Zubairu*et al.*, 2019, who found *V. amygdalina* leaf extract effective against *E. coli*, *S. aureus*, and *S. typhi*.

On the other hand, the result contradicts the finding of Ogundare (2011) who found no activity

of *V. amygdalina* leaf extract against *E. coli* and *Salmonella typhi*. Therefore, bioactive components in the extracts could explain their antibacterial activities, as observed in this study. This study observed that bioactive components are abundant in these leaves and that the ethanol extracts exhibited higher inhibitory activity on the test organisms than the aqueous extracts. This might be due to the ability of ethanol to extract more of the bioactive components than the aqueous extracts.

The leaf extract of *V.amygdalina* showed a reasonable antibacterial effect against most of the *Salmonella* species isolates collected. However, some isolates showed no zone of inhibition from both the extracts and the control, indicating resistance. Ogundare *et al.* (2011) also reported similar findings in their research, where no activity of *V.amygdalina* leaf extract was detected against *Salmonella*. The minimum inhibitory concentration for ethanolic and aqueous extracts was 50mg/ml, while the minimum bacteriocidal concentration was 100 mg/ml. Although Udochukwu *et al.* (2015) *reported antibacterial activity of V. amygdalina against Salmonella, earlier work of* Adetunji et al. (2013) *reported that the plant only inhibits the growth but does* not kill the bacteria. One limitation of this work is that it only tests the antibacterial effect of the crude extract of the plant. It is expected that there might be an increase in the antibacterial activity of the extract when purified. Further research could be done on the antimicrobial activity of *V.amygdalina* to establish a strong basis for using the plant to produce drugs.

CONCLUSION

The aqueous and ethanolic extracts contained various bioactive components such as tannins, saponins, alkaloids, and phenol; both demonstrated antibacterial activity against *Salmonella*. The MIC and MBC of both extracts were found to be at 50 mg/mL and 100 mg/mL for all *Salmonella* tested in this research. This study highlights the possibility of exploring *V. amygdalina* leaves as a natural product source for future disease management.

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