



Antibacterial activity of extracts of *Vitex doniana* (black plum) stem bark

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ABSTRACT

Bacteria are a leading cause of diarrhoea in humans and the use of orthodox drugs in its treatment has been found to possess certain side effects as well as economic burden. This study aimed at evaluating the antibacterial activity of aqueous, methanol, ethylacetate and n-hexane extracts of *Vitex doniana* (black plum) stem bark on some diarrhoeal causing organisms. Fresh samples of *Vitex doniana* stem bark were collected from Kontagora Local Government Area of Niger State, Nigeria. Preparation, extraction, secondary metabolite screening and antibacterial activities of the different extracts of *Vitex doniana* stem bark were carried out following standard procedures. The results revealed that all the extracts contained different concentrations of flavonoids, alkaloids, tannins and phenols. Aqueous extract contained a significantly ($p < 0.05$) lower concentration of flavonoids (20.40 ± 0.04 mg/g) when compared to other extracts. However, there was a significantly ($p < 0.05$) higher concentration of alkaloids in the aqueous extract (40.67 mg/g) when compared to alkaloids concentration in the methanol (30.00 mg/g) extract. A significantly ($p < 0.05$) higher concentration of tannins was recorded in the methanol extract (0.99 mg/g) when compared to tannins concentration in ethylacetate (0.21 mg/g) extract. A significantly ($p < 0.05$) higher concentration of phenol was recorded in the aqueous extract (352.70 mg/g) as compared to the n-hexane (16.27 mg/g) extract. The result of the zone of inhibition for different extracts revealed that, at higher concentrations of 100% and 75%, the growth of *Escherichia coli*, *Pseudomonas spp*, *Staphylococcus aureus* and *Salmonella spp* were significantly ($p < 0.05$) inhibited, suggesting that the inhibitory activities of the extracts are dose-dependent. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the different extracts showed varying results across different extracts. In conclusion, the study demonstrated that both the aqueous and n-hexane extracts of *Vitex doniana* stem bark possess bacteriostatic effects whereas methanol and ethylacetate extracts exhibited bacteriocidal effects especially at higher concentrations.

1.0 Introduction

Diarrhoea remains an important health challenge in developed and developing countries, despite the economic advancement in public health [1]. It is one of the leading causes of mortality in children under the age of five years [2]. Microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella typhi* are the foremost causative agents of diarrhoea in humans

[3]. It is a condition that causes decrease in the intestinal absorptive and increased secretory function resulting in the alteration of faecal volume and flow rate within the gastrointestinal tract [4]. Diarrhoea is associated with irregular and repeated release of semisolid fluid from the bowel lasting for less than fourteen days depending on longevity of the epidemic which lasts for more than one month [5]. The disease has been occasioned by unhealthy environment which is evident in developing nations

like Nigeria. Over the years, efforts have been made both locally and globally by health-related institutions in controlling the disease but to no avail. According to Guerrant *et al.* [6], the disease results in increased volume of stool water content, leading to emaciation within few days of occurrence.

It is a known fact that, herbs generally, have been used for treating illnesses with 80% of people living in the third world countries make use of medicinal plants [6]. Herbal medications are time and again thought not to be hurtful since they are natural and free of negative consequences [7].

Vitex doniana (family: Lamiaceae), is a perennial shrub widely distributed in tropical West Africa. The plant is native to the afro-tropics. It produces green fruit at maturity, turning to dark brown or black when fully ripe. It is used in the treatment of inflammatory disorders, rheumatic pains, dysentery, cancer, high blood pressure and diarrhoea [8]. Also, it is highly rich in protein, carbohydrate, fat, vitamins, and phytochemical elements. The potential of *V. doniana* as source of antioxidants cannot be overemphasized because of its high abundance of flavonoids and other compounds [8,9]; and this has stimulated attention of various communities across the world to *V. doniana*.

Effective treatment of diarrhoea has been through the use of anti-diarrhoeal drugs such as adsorbents (kaolin, cholestyramine and activated charcoal), antimotility drugs (codeine, camphorated tincture of opium, loperamide and hydrochloride [1]). However, these orthodox drugs have been found to possess some side effects such as dry mouth, nausea, abdominal discomfort, rashes, constipation, headache, dizziness, addiction, metallic taste, eosinophilia, epigastric distress, allergic reactions, fever, and skin eruptions [10]. It is also important to note that aside these side effects, some of these drugs are not readily available to the poor people, who majorly reside in the rural areas [11].

Therefore, this study was aimed at evaluating the secondary metabolite constituents and antibacterial potentials of the solvent extracts of *V. doniana* stem bark against some diarrhoeal causing microorganisms.

2.0 Materials and Methods

2.1 Collection of Plant Sample and Identification

Fresh samples of *V. doniana* stem bark were collected from Kontagora Local Government Area of Niger State, Nigeria (10.403°N latitude and 5.471°E longitude and an altitude of 287m) as determined using a handheld GPS receiver. The plant was thereafter taken to the Herbarium of the Department of Biological Sciences, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria, for identification.

2.2 Test Organisms and Identification

Clinical isolates of *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, obtained from Microbiology Unit of the Federal Medical Centre, Bida, Niger State, Nigeria were identified using Gram staining techniques, cultural characterization (Xylose Lysine Deoxycholate (XLD) agar for *S. typhi*, MacConkey agar for *E. coli*, nutrient agar for *P. aeruginosa* and mannitol salt (MS) agar for *S. aureus*) and biochemical characterization (key positive/negative test results). The pure isolates of the test organisms were inoculated in sterile slants containing nutrient agar at 4°C.

2.3 Preparation of Extracts

The stem bark of *V. doniana* collected were washed with tap water, air dried at ambient temperature and pulverized using a Waring Commercial Blender, model: CB-8231-L, USA. Distilled water, methanol, ethylacetate and n-hexane were solvents used for the extraction of the stem bark of *V. doniana*. The procedure described by Kallei *et al.* [12] for aqueous extraction. One hundred grammes of powdered stem bark was extracted for 3 days using 500 ml of distilled water in a sterile conical flask. The extract was filtered using Muslin cloth followed by Whatman filter paper No.1 and then concentrated on a water bath maintained at 40°C. The resulting filtrate (yield = 8.35 g; %yield = 8.35%) contained in universal bottles was then stored in the refrigerator at 4°C before being used. The extract was reconstituted in distilled water to give the required doses of 100, 200 and 400 mg/kg bodyweight [11]. For the organic extraction, a hundred gramme each of the powdered *V. doniana* stem bark was extracted in 500 ml of methanol, ethylacetate and n-hexane for 3 days; each mixture was filtered using Whatman No.1 filter paper and the extract was concentrated to dryness using rotary evaporator, model RE52-3. The resulting residues (yield from methanol extract = 5.20 g; %yield = 5.20%; yield from ethylacetate extract = 3.76 g; %yield = 3.76%; yield from n-hexane extract = 2.94 g; %yield = 2.94%) obtained were reconstituted in 5% of Dimethyl Sulphur Oxide at stock concentration, stored in the refrigerator at 4°C until used.

2.4 Qualitative and Quantitative Determination of Secondary Metabolites

The qualitative analyses of the secondary metabolites like alkaloids, flavonoids, saponins, tannins, phenols, steroids and terpenoids in the aqueous, methanol, ethylacetate and n-hexane extracts of *V. doniana* stem bark were carried out according to standard methods [13,14,15].

The quantitative analyses of alkaloids, flavonoids, tannins and phenols were determined spectrophotometrically according to standard methods [16,17,18, 19].

2.5 Antibacterial Assay

The antibacterial activity of the plant extracts was assessed using the agar well technique. In a sterile Petri dish, Mueller Hilton Agar was inoculated with 0.1 ml of the distinct standardized organism (0.5 McFarland), allowed to set, and then labelled. A sterile cork borer (6 mm) was then used to punch holes (i.e. 4 wells) in the inoculated agar and the agar was then removed. Different concentrations of the extract were added to the four wells created and then left on the bench for an hour to allow for sufficient diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition in each well were measured to the nearest millimetres [20].

2.6 Data Analysis

The data obtained from the analyses were subjected to analysis of variance (ANOVA) of a completely Randomized design (CRD) using SAS version 9.1. The means of the data obtained were presented using descriptive statistics and were separated using Duncan Multiple range test at 95% confidence level ($p < 0.05$).

3.0 Results

Secondary metabolite composition of aqueous, methanol, ethylacetate and n-hexane extracts of *V. doniana* stem bark is shown in Table 1. The aqueous, methanol, ethylacetate and n-hexane extracts of *V. doniana* stem bark contained flavonoids, alkaloids, tannins and phenols while steroids and cardiac glycosides were not detected. All the solvent extracts of the stem bark contained different concentrations of flavonoids, alkaloids, tannins and phenols while steroids and cardiac glycosides were absent. Aqueous extract contained a significantly ($p < 0.05$) lower concentration (20.40 mg/g) of flavonoids when compared to the ethylacetate (40.23 mg/g) and n-hexane (41.00 mg/g) extracts of *V. doniana* stem bark. However, aqueous extract of the stem bark contained a significantly ($p < 0.05$) higher concentration (40.67 mg/g) of alkaloids when compared with methanol (30.00 mg/g), ethylacetate (30.30 mg/g), and n-hexane (30.18 mg/g) extracts of *V. doniana* stem bark. The concentration of tannins in the methanolic extract of *V. doniana* stem bark extract was significantly ($p < 0.05$) higher (0.99 mg/g) than the tannins in the ethylacetate (0.21 mg/g) extract of *V. doniana* stem bark. Phenol

concentration was significantly ($p < 0.05$) higher in aqueous extract (352.70 mg/g) of *V. doniana* stem bark than in the n-hexane (16.27 mg/g) extract of *V. doniana* stem bark.

For the aqueous extract, there was no zone of inhibition for *E. coli* except at higher concentrations of 100% and 75% which showed zones of inhibition for *P. aeruginosa*, *S. aureus* and *S. typhi*. (Table 2). Similarly, there was no zone of inhibition for *E. coli* with methanol extract of *V. doniana* stem bark. However, the 100%, 75% and 50% of methanol extract showed inhibition of *P. aeruginosa* growth whilst inhibitions were recorded at 100% and 75% concentrations of methanol extract of *V. doniana* stem bark for *S. aureus*. Only the 100% of methanol extract inhibited the growth of *S. typhi*. The ethylacetate and n-hexane extracts of *V. doniana* stem bark showed zones of inhibition for *E. coli* at 100% and 75%. At 100%, 75% and 50% of ethylacetate extract of *V. doniana* stem bark, there were zones of inhibition for *P. aeruginosa*, *S. aureus* and *S. typhi*.

The minimum inhibitory concentration (MIC) of aqueous extract of *V. doniana* stem bark was 85%, 70%, 85%, and 85% for *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhi* respectively (Table 3). The MIC of methanol extract of *V. doniana* stem bark was 70%, 70%, 85%, and 85% for *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhi* respectively. The MIC of ethylacetate extract of *V. doniana* stem bark was 70%, 85%, 70%, and 70% for *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhi* respectively. The MIC for n-hexane extract of *V. doniana* stem bark was 85%, 85%, 70%, and 70% for *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. typhi* respectively.

The minimum bactericidal concentration (MBC) of aqueous extract of *V. doniana* stem bark was 100%, 85%, 100%, and 100% for *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi* respectively. The MBC of methanol extract of *V. doniana* stem bark for *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi* was 85%, 85%, 100%, and 100% respectively. The MBC of ethylacetate extract of *V. doniana* stem bark was 85%, 100%, 85%, and 85% for *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi* respectively. The MBC of n-hexane extract of *V. doniana* was 85%, 100%, 85%, and 85% for *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi* respectively.

4.0 Discussion

Plants are valuable source of natural products for maintaining human health. Today, the use of plant extracts and phytochemicals is of great significance in therapeutic treatments [22]. The presence of phytochemicals such as alkaloids, phenols, tannins and flavonoids in this study is in tandem with the work of [11] who reported the

Table 1: Secondary metabolite constituents of solvent extracts of *Vitex doniana* stem bark

Extracts	Secondary Metabolites (mg/g)			
	Flavonoids	Alkaloids	Tannins	Phenols
Aqueous extract	20.40 ± 0.02 ^a	40.67 ± 0.24 ^b	0.41 ± 0.02 ^b	352.70 ± 0.42 ^d
Methanol extract	30.06 ± 0.01 ^b	30.00 ± 0.03 ^a	0.99 ± 0.01 ^d	36.00 ± 0.03 ^b
Ethylacetate extract	40.23 ± 0.13 ^c	30.30 ± 0.44 ^a	0.21 ± 0.01 ^a	43.67 ± 0.11 ^c
N-hexane extract	41.00 ± 0.20 ^c	30.18 ± 0.34 ^a	0.73 ± 0.12 ^c	16.27 ± 0.10 ^a
Cardiac glycosides	-	-	-	-
Steroids	-	-	-	-

Data represent means ± standard deviation of triplicate determinations. Means with the same superscript down the column are not significantly different (p<0.05)

Table 2: Zones of inhibition of solvent extracts of *Vitex doniana* stem bark

Sample (%)	<i>Pseudomonas aeruginosa</i>				<i>Escherichia coli</i>				<i>Staphylococcus aureus</i>				<i>Salmonella typhi</i>			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
A	14.0	10.0	-	-	-	-	-	-	18.0	12.0	-	-	15.0	12.0	-	-
B	15.0	10.0	8.0	-	-	-	-	-	11.0	9.0	-	-	9.0	-	-	-
C	20.0	15.0	13.0	-	9.0	6.0	-	-	10.0	9.0	7.0	-	15.0	13.0	9.0	-
D	18.0	15.0	13.0	-	9.0	6.0	-	-	18.0	15.0	11.0	-	18.0	14.0	10.0	-

Key: - = Resistance values = susceptibility, A = Aqueous extract, B = Methanol extract, C = Ethylacetate extract, D = n-hexane extract

Table 3: Minimum inhibitory concentration of solvent extracts of *Vitex doniana* stem bark

Sample (%)	<i>Pseudomonas aeruginosa</i>				<i>Escherichia coli</i>				<i>Staphylococcus aureus</i>				<i>Salmonella typhi</i>			
	100	85	70	55	100	85	70	55	100	85	70	55	100	85	70	55
A	-	-	+	+	-	-	-	+	-	-	+	+	-	-	+	+
B	-	-	-	+	-	-	-	+	-	-	+	+	-	-	+	+
C	-	-	-	+	-	-	+	+	-	-	-	+	-	-	-	+
D	-	-	-	+	-	-	+	+	-	-	-	+	-	-	-	+

Key: - = Resistance values, + = susceptibility, A = Aqueous extract, B = Methanol extract, C = Ethylacetate extract, D = n-hexane extract

Table 4: Minimum bacteriocidal concentration of solvent extracts of *Vitex doniana* stem bark

Sample (%)	<i>Pseudomonas aeruginosa</i>				<i>Escherichia coli</i>				<i>Staphylococcus aureus</i>				<i>Salmonella typhi</i>			
	100	85	70	55	100	85	70	55	100	85	70	55	100	85	70	55
A	-	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+
B	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	+
C	-	-	+	+	-	+	+	+	-	-	+	+	-	-	+	+
D	-	-	+	+	-	+	+	+	-	-	+	+	-	-	+	+

Key: - = Resistance values, + = susceptibility, A = Aqueous extract, B = Methanol extract, C = Ethylacetate extract, D = n-hexane extract

presence of tannins, flavonoids and phenols in the aqueous extract of *V. doniana* stem bark. However, the presence of alkaloids in the aqueous extract of *V. doniana* stem bark in this study negates the finding of Amuzat *et al.* [11] who reported the absence of alkaloids in the aqueous extract of *Vitex doniana* stem bark. The disparity between what was observed in this study when compared with work of Amuzat *et al.* [11] maybe due to the difference in the locations from where the plant materials were collected as was reported earlier by Lawal *et al.* [23], that not all plants contain all phytochemicals and that those present vary according to the location.

Plant extracts essentially used in food preservation are potential sources of novel antimicrobial compounds especially against bacterial pathogens [24]. Secondary metabolites of plants such as alkaloids, tannins, phenols are responsible for the antimicrobial activities [25], free radical scavenging activities as well as its ability to hinder/reduce/ameliorate cardiovascular complications [26]. Flavonoids, important polyphenolic secondary metabolites, detected in the stem bark extract of *V. doniana* in this study have been reported to have anti-oxidant, anti-parasitic, analgesic and cytostatic activities [27]. Furthermore, tannins have equally been reported to exhibit anti-diarrhoeal, anti-parasitic, antioxidant and anti-inflammatory activities [27]. Alkaloids have been shown to possess antiviral, antiulcer, analgesic, and anti-inflammatory activity [28]. The variations in the concentration of secondary metabolites among different solvent extracts of aqueous, methanol, ethylacetate, and n-hexane in the *V. doniana* stem bark in the present study may be attributed to the differences in solvent polarity, which influenced the solubility and extraction efficiency of the secondary metabolites.

Flavonoids are known for their strong antioxidant, anti-inflammatory, and antimicrobial properties, contributing to the medicinal value of *V. doniana* stem bark in traditional therapy [29]. The concentration of flavonoids in this study range from 20.40 mg/g in the aqueous extract to 41.00 mg/g in the n-hexane extract with n-hexane and ethyl acetate extracts showing relatively higher flavonoid contents (41.00 and 40.23 mg/g, respectively), which is an indication that the moderately non-polar solvents efficiently extracted flavonoid compounds. This observation agreed with the findings of Adebayo *et al.* [30] and Edeoga *et al.* [31], who reported that the type of solvent significantly affects the extraction of flavonoids, with less polar solvents favouring the recovery of certain aglycone flavonoids.

Alkaloids are nitrogenous compounds with diverse pharmacological activities such as analgesic, antimalarial, and antihypertensive effects [32]. In this study, the alkaloid content which ranged from 30.00 mg/g (methanol extract) to 40.67 mg/g

(aqueous extract) with the aqueous extract exhibiting the highest alkaloid concentration, suggests that these compounds were more soluble in polar solvents. The relatively high alkaloid concentration in the aqueous extract may explain the use of *V. doniana* aqueous preparations in traditional medicine for treating infections including diarrhoea. This is in line with the reports of Btissam *et al.* [33] who reported that aqueous and ethanolic extracts of Lamiaceae contained alkaloids and demonstrated antibacterial activity.

Tannins are known for their astringent, antimicrobial and antioxidant properties, and play a vital role in plant defense mechanisms [34]. The concentrations of tannins in this study which was relatively low across all the solvent extracts, ranging from 0.21 mg/g in ethylacetate extract to 0.99 mg/g in methanol extract, suggests that methanol as a solvent extracted tannins the most and is consistent with the polarity of both the solvent and the polyphenolic nature of tannins. The moderate presence of tannins in the methanol extract of plant material supports its potential use in wound healing and antimicrobial formulations [35].

The abundance of phenolics is associated with strong antioxidant capacity, which neutralize free radicals and prevent oxidative stress-related disorders. In this study, a striking difference, in the phenolic contents of different solvent extracts (aqueous extract, 352.70 mg/g; n-hexane extract, 16.27 mg/g), indicates that phenolic compounds in *V. doniana* stem bark are highly polar and therefore more extractable in water. This finding corroborates the reports of Oladunmoye [36] and Wiegand *et al.* [37], who noted that aqueous and methanol solvents were more efficient in recovering phenolic compounds due to their high polarities.

The *in vitro* studies in this work showed that the different plant extracts of *V. doniana* stem bark inhibited the growth of *P. aeruginosa*, *E. coli*, *S. aureus* and *S. typhi* which are ubiquitous in the environment and are intrinsically resistant to a number of antibiotics [38, 39, 40, 41] at different rate. The ethylacetate and n-hexane extract of *V. doniana* stem bark produced the best antimicrobial activity with the highest zones of inhibition, least minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MIB). This may be due to the high level of important chemical compounds such as alkaloids and flavonoids in both extracts. This is in line with the work of Poole [38] who reported that ethanol and ethylacetate extracts of fruit of sweet fruit have better antimicrobial activity but the ethanol extract of the leaves performed better than the ethylacetate extract. The findings here also suggests that the antimicrobial components of the extracts are concentration-dependent and species-specific in their activity.

Pseudomonas aeruginosa is a Gram-negative bacterium known for its intrinsic resistance to many

antibiotics due to its impermeable outer membrane and active efflux systems [38]. The inhibition at higher concentrations of all the extracts of *V. doniana* stem bark suggests that the extracts contained some chemical compounds that are capable of disrupting bacterial cell membranes or interfering with its metabolic enzymes. The findings here are similar to those previously reported by Airaodion *et al.* [42] and Nwinyi *et al.* [43], who found that the extracts of *V. doniana* stem bark and related medicinal plants displayed moderate to strong activity against *P. aeruginosa* at high concentrations.

Among all the samples, only ethylacetate and n-hexane extracts of *V. doniana* stem bark showed inhibition against *E. coli*, with zones of 9.0 mm and 6.0 mm at 100% and 75% concentrations, respectively. Aqueous and methanol extracts showed no inhibitory effect, indicating that *E. coli* may be more resistant to the chemical compounds extracted by the water and methanol solvents. This moderate susceptibility is consistent with reports of Edeoga *et al.* [31] and Okeke *et al.* [44], who reported that *E. coli* resisted plant-derived antimicrobials unless in concentrated forms. The reduced activity could be due to the complex lipopolysaccharide layer of Gram-negative bacteria, which might have limited the penetration of the hydrophobic chemical compounds [45].

The results also showed strong inhibitory activity against *S. aureus*, especially at higher extract concentrations. Aqueous, ethylacetate and n-hexane extracts of *V. doniana* stem bark demonstrated zones of inhibition between 10.0–18.0 mm, while methanol extract exhibited moderate inhibition (11.0 mm) at 100%. The activity decreased proportionally with dilution and was lost completely at 25%. The higher susceptibility of *S. aureus* may be due to its Gram-positive nature, characterized by a peptidoglycan-rich cell wall that allows easier penetration of chemical compounds [46].

Flavonoids, tannins, phenolics, and alkaloids previously reported in *V. doniana* stem bark by Amuzat *et al.* [11] could be responsible for this antibacterial activity, as these compounds have been reported to inhibit bacterial enzymes, disrupt cell walls, and inactivate nucleic acids [36, 47]. All the solvent extracts of *V. doniana* stem bark displayed measurable inhibitory zones against *S. typhi* at higher concentrations (100% and 75%). The n-hexane extract of *V. doniana* stem bark showed the highest inhibition (18.0 mm), followed by ethylacetate extract (15.0 mm), and aqueous, extract (15.0 mm). This indicated that the chemical compounds in the extracts were effective against enteric pathogens including *S. typhi*, possibly through interference with bacterial cell membrane concentration in all the samples investigated. The absence of inhibition at higher concentrations (100%–70%) suggests that *E. coli* may have a

integrity or inhibition of protein synthesis. Similar patterns of inhibition were reported by Owolabi *et al.* [29] and Kolawole and Akinpelu [48], where ethanol and methanol extracts of *V. doniana* stem bark exhibited significant antibacterial activity against *S. typhi* and *S. paratyphi*. Overall, ethylacetate and n-hexane extracts consistently demonstrated stronger antibacterial activity against all the test organisms and especially at 100% and 75% concentrations. The trend of activity was generally concentration-dependent, confirming that dilution reduced the concentrations of these active chemical compounds responsible for inhibition.

Gram-positive bacteria (*S. aureus*) were generally more susceptible than Gram-negative bacteria (*E. coli*, *P. aeruginosa* and *S. typhi*), a pattern consistent with earlier reports of Cowan [45] and Savoia [46]. The results substantiated the potential of these extracts as sources of natural antimicrobial agents and supported the ethnomedicinal use of *V. doniana* for the treatment of bacterial infections including diarrhoea.

The Minimal Inhibitory Concentration (MIC) is the lowest concentration of chemical compounds/an extract required to inhibit visible bacterial growth [49]. The results obtained on the extracts of *V. doniana* stem bark in the present study against the four test organisms (*P. aeruginosa*, *E. coli*, *S. aureus*, and *S. typhi*) showed varying levels of susceptibility to the microorganisms. The differences in the inhibition patterns by the extracts and bacterial species indicated variability in the concentration and potency of the active chemical constituents. The MIC revealed that *P. aeruginosa* exhibited susceptibility only at the lowest concentrations (70% and 55%) for all the solvent extracts of *V. doniana* stem bark. However, none of the extracts inhibited *P. aeruginosa* at higher concentrations (100% and 85%). This pattern may be due to concentration-dependent interactions by the secondary metabolites, where excessive amounts could cause aggregation or reduced bioavailability of the active compounds, whereas moderate dilution might have enhanced their activity [45]. The susceptibility of *P. aeruginosa* at 70-55% concentrations indicated that the extracts of *V. doniana* stem bark contained compounds capable of interfering with its membrane integrity or metabolic enzymes despite the well-established multidrug resistance mechanisms [38]. Similar findings were reported by Airaodion *et al.* [42] and Owolabi *et al.* [29], where *V. doniana* stem bark extracts exhibited measurable inhibitory effects against *P. aeruginosa* at moderate concentrations. The findings in the present study showed that *E. coli* was susceptible to the extracts only at 55% stronger resistance barrier, possibly due to its outer lipopolysaccharide membrane layer which limited the diffusion of hydrophobic compounds [47]. The

findings also aligned with the reports of Edeoga *et al.* [31] and Okeke *et al.* [44], who hinted that Gram-negative bacteria like *E. coli*, often require higher extract potency or synergistic compound interactions to achieve inhibition. This implies that while *V. doniana* stem bark extracts possess antibacterial agents effective against *E. coli*, the efficacy depends largely on optimal concentration and the extractant, which influences chemical compound solubility and by extension, bioavailability. Among the tested organisms, *S. aureus* showed consistent susceptibility with all the extracts investigated at the concentrations of 70% and 55%. This indicated that *S. aureus* was more sensitive to the active chemical components of *V. doniana* stem bark when compared with the Gram-negative organisms. Similarly, the higher susceptibility of *S. aureus* may be attributed to its Gram-positive cell wall structure, which might have allowed/promoted the smooth penetration of chemical compounds like the phenolics, flavonoids, and tannins [46, 47]. This finding corroborated the earlier outcome on the agar diffusion method, where *S. aureus* displayed the highest zones of inhibition [30]. Furthermore, similar patterns of sensitivity of *S. aureus* to *V. doniana* stem bark have been reported by Akinpelu and Kolawole [48]. *S. typhi* also exhibited susceptibility at 70% and 55% for all the extracts, similar to the pattern displayed by *P. aeruginosa* and *S. aureus*. The consistent inhibition at these concentrations indicated that the chemical compounds with the antibacterial activity in *V. doniana* stem bark remained active even upon dilution, suggesting strong potency and stability. Previous studies have linked the antibacterial activity of *V. doniana* stem bark to its rich content of phenolic compounds, alkaloids, and flavonoids, which could have disrupted microbial membranes and inhibited DNA gyrase activity [42, 49]. The comparable activity against *S. typhi* aligned with the reports of Owolabi *et al.* [29], who documented moderate MIC values for *V. doniana* essential oils against enteric pathogens. All the solvent extracts of *V. doniana* stem bark demonstrated MIC values at 70–55%, indicating that the minimal concentration required for inhibitory activity lies within this range. This suggests that the active chemical compounds in the plant extract were effective at moderate concentrations but may become less efficacious at higher concentrations (100%). The consistent response for all the bacterial species highlights the broad-spectrum antibacterial potential of *V. doniana* stem bark. The stronger inhibition of Gram-positive *S. aureus* compared to Gram-negative strains further confirmed that the composition of the cell wall might have a role to play in the antimicrobial susceptibility [45]. These findings supported the ethnomedicinal use of *V. doniana* in treating infections caused by *S. aureus*, *S. typhi*, and *P. aeruginosa*, and suggests potential for further

purification and formulation into antimicrobial agents.

The Minimal Bactericidal Concentration (MBC) represents the lowest concentration of an antimicrobial agent required to kill a bacterial population, rather than merely inhibiting its growth. The MBC values obtained for the various extracts of *V. doniana* stem bark against *P. aeruginosa*, *E. coli*, *S. aureus*, and *S. typhi* in this study showed variable bactericidal effects at different extract concentrations. The variations are likely to be due to differences in solvent polarity, chemical compounds solubility, and bacterial cell wall structure [50]. All the tested solvent extracts in this study exhibited bactericidal activity against *P. aeruginosa* at concentrations of 70% and 55%, while no activity was recorded at higher concentrations (100% and 85%) for most extracts. This trend suggests that the chemical compounds with bactericidal action in *V. doniana* stem bark may be more effective at moderate concentrations. Higher extract concentrations may promote compound aggregation or reduce membrane permeability, thereby diminishing antibacterial efficiency [45, 51].

Pseudomonas aeruginosa are known for their multidrug resistance and biofilm formation, which limit antibiotic penetration [38]. Hence, the MBC at moderate concentrations implied that *V. doniana* stem bark contained chemical compounds capable of disrupting bacterial integrity even in resilient organisms such as *P. aeruginosa*. These findings corroborated that of Owolabi *et al.* [29], who reported significant antibacterial effects of *V. doniana* essential oils against *Pseudomonas aeruginosa*. The bactericidal activity against *E. coli* was evident at 70% and 55% concentrations for all the extracts, with few displaying additional activity at 85%. The absence of bactericidal action at 100% concentration suggests that dilution enhanced compound diffusion or synergism, leading to better bacterial membrane disruption [47]. The findings here aligned with the reports of Edeoga *et al.* [31] and Airaodion *et al.* [42], who hinted that Gram-negative bacteria such as *E. coli* often require higher exposure or optimal extract concentrations due to their complex outer membrane-rich in lipopolysaccharides, which might have acted as permeability barrier. The bactericidal potential of *V. doniana* stem bark against *E. coli* provided scientific backing for its traditional use in the treatment of gastrointestinal infections, including diarrhoea. *Staphylococcus aureus* exhibited bactericidal susceptibility at 85%, 70%, and 55% concentrations by all the extracts, with no effect recorded at 100%. This indicated that *S. aureus* was highly sensitive to *V. doniana* stem bark extracts, particularly at moderate concentrations. The higher susceptibility of this Gram-positive organism is likely to be due to its relatively simple cell wall, which might have allowed penetration of hydrophobic and phenolic compounds [46,47]. The findings corroborated those of Adebayo *et al.* [30] and Oladunmoye [36], who

reported that *V. doniana* stem bark extracts showed pronounced bactericidal effects against Gram-positive bacteria. The activity of *V. doniana* stem bark extracts against *S. aureus* further supported the presence of potent antibacterial secondary metabolites such as alkaloids, flavonoids, and phenolic acids, as revealed in the present study. For *Salmonella typhi*, all the extracts demonstrated bactericidal activity at 85%, 70%, and 55% concentrations, with no effect at 100%. This suggests that the chemical compounds with antibacterial activity were effective in killing *S. typhi*, a common foodborne pathogen. This finding was supported by the reports of Owolabi *et al.* [29] and Airaodion *et al.* [42], who found that *V. doniana* stem bark extracts possessed significant antibacterial effects against enteric bacteria. The MBC data suggests that these secondary metabolites in *V. doniana* stem bark, notably phenols, flavonoids, and alkaloids, could disrupt bacterial cell membranes and interfere with metabolic enzymes, leading to cell death. Comparatively, *S. aureus* and *S. typhi* were more susceptible to the extracts of *V. doniana* stem bark than *P. aeruginosa* and *E. coli*. This differential susceptibility is consistent with the school of thought trend that Gram-positive bacteria were more sensitive to plant-derived antimicrobials than the Gram-negative bacteria due to differences in cell wall composition [45]. The MBC values of *V. doniana* stem bark extracts recorded at 70% and 55% concentrations for most organisms suggests in this study that these were the minimal levels for bactericidal action of *V. doniana* stem bark extracts. The lower concentrations-enhanced activity may also imply synergistic interactions among secondary metabolites when properly diluted. Overall, the results confirm that *Vitex doniana* stem bark possesses broad-spectrum bactericidal activity and justifies its traditional use for treating bacterial infections. Its efficacy at relatively moderate concentrations might be an indication that can be explored for the formulation of natural antimicrobial agent [50].

In conclusion, the findings of this study demonstrated that *Vitex doniana* stem bark contains significant amounts of secondary metabolites, including flavonoids, alkaloids, tannins, and phenols, which contributed to the antibacterial activity of *Vitex doniana* stem bark extract. Although, all the solvent extracts inhibited bacterial growth to varying degrees, the ethylacetate and n-hexane extracts produced the strongest antibacterial effects. These results support the traditional use of *V. doniana* in managing diarrhoea and highlight its potential as a source of natural antibacterial agent. Further research, including *in vivo* studies and isolation of specific bioactive compounds, is recommended to optimize its therapeutic applications.

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Disclosure of conflict of interest

No conflicts of interest among the authors as all authors have contributed to both the laboratory work and the drafting of the manuscript.

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None

Authors Contributions

AOA – Conceptualization and Supervision; SU – Data curation, Formal analysis and Resources; YN – Methodology and Writing – original draft, review & editing; HMA – Data curation; AOA - Writing – review & editing; RSS - Writing – review & editing.

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