



## Antibacterial Activity of *Vitex doniana* (Black Plum) Fractions Against Diarrhoeogenic *Escherichia coli* Strains

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### ABSTRACT

The study was carried out to determine the antibacterial activity of *Vitex doniana* fractions against *Escherichia coli* strains. The antibacterial activity of *Vitex doniana* leaf and stem-bark fractions were also evaluated using agar well diffusion method. The minimum inhibitory and minimum bactericidal concentration was evaluated using broth tube dilution and pour plating methods respectively. The *Vitex doniana* aqueous stem-bark fraction with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 5.0 and 10.0 mg/mL emerged as the active fraction against 75% of the EAEC, while methanolic leaf fraction had MIC and MBC values of 5.0 and 10 mg/mL against 77% of the EHEC. The aqueous leaf fraction had MIC and MBC values of 10 mg/mL against 62.5% of the EIEC, while methanolic leaf fraction had MIC and MBC values of 5.0 and 10 mg/mL against 61% and 81% of the EPEC. The methanolic stem-bark fraction exhibited highest activity with MIC and MBC values of 5.0 and 10 mg/mL against 79% and 100% of the Enterotoxigenic *Escherichia coli*. The fractions of the plant could be promoted for search of new leads against enterobacterial pathogens having MIC values of 5.0-20 mg/mL. The result has justified their utilization by traditional medicine practitioners for the treatment of diarrhoea and other related ailments associated with *Escherichia coli* strains.

**Keywords:** Antibacterial, *Vitex doniana*, Extracts, Diarrhoeogenic *Escherichia coli*,

### INTRODUCTION

Diarrhoea can be defined as the passing out of three or more loose watery stools or one bloody loose stool for a period of 24-hours (Baba *et al.*, 2016). Bloody diarrhoea is that type of diarrhoea that manifested by loose or watery stools with the shedding of red blood due to inflammation of intestines (colitis). Diarrhoea is not a disease but a symptom of a number of illnesses (Galadima and Kolo, 2014).

Diarrhoeogenic *Escherichia coli* is the name given to *Escherichia coli* isolated from diarrhoeic stool. *Escherichia coli* is a Gram-negative, non-spore forming, facultative anaerobic and rod shaped bacterium. Bacteriological culture of the diarrhoeal stool of normal human and warm-blooded animals invariably yields *Escherichia coli* (Murray, 2011). *Escherichia coli* is the common pathogen responsible for a wide spectrum of diseases. The spectrum could range from diarrhoea, dysentery, urinary tract infection, wound infection, peritonitis, and neonatal meningitis to

haemolytic uremic syndrome (Jenkins *et al.*, 2018). *Escherichia coli* infection could be contracted through faecal-oral route, person to person in areas with poor sanitation and contaminated or non-chlorinated water has been implicated as the route of transmission (Galadima and Kolo, 2014).

It seems sense for illnesses that are induced by diet to have multiple potential origins. These may be caused by the contamination of food throughout any stage of production, including growing, harvesting, processing, storing, shipping, or even cooking the food. Infections can take place at any point. As well as the phenomenon known as cross-contamination, which refers to the movement of pathogenic organisms from one surface to another. This is especially problematic for items that are raw, ready to eat, such as various types of produce and

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salads. The instance of Mary Mallon, often known as "Typhoid Mary," an American cook who is thought to have infected 53 people with typhoid fever and caused the deaths of three of them, demonstrates that food vendors can also be the source of food poisoning (Akpota *et al.*, 2019). According to Aliyu *et al.* (2016), an unsanitary environment, adverse weather conditions, as well as the presence of animals and insects, can all have a significant role in the development of food-borne illnesses.

*Escherichia coli* comprise of five strains namely Enteropathogenic *Escherichia coli*, Enteroinvasive *Escherichia coli*, Enterotoxigenic *Escherichia coli*, Enteroaggregative *Escherichia coli* and Enterohaemorrhagic *Escherichia coli*. Jenkins *et al.* (2018) who reported that enterotoxigenic *Escherichia coli* causes' watery diarrhoea due to production of plasmid mediated toxin (LT, ST) in infants and adults popularly called traveller's diarrhoea, while Enteropathogenic *Escherichia coli* causes vomiting, fever and prolonged diarrhoea mainly in infants less than two years in most developing countries (Cheesbrough, 2006). Enteroinvasive *Escherichia coli* causes dysentery similar to shigellosis with symptoms of fever, colitis with blood, mucous and many pus cells in stool (Nataro *et al.*, 2006). Enterohaemorrhagic *Escherichia coli* causes life threatening haemorrhagic diarrhoea (colitis) in all ages without pus cells and fever, while enteroaggregative *Escherichia coli* causes chronic watery diarrhoea and vomiting in children (Orth *et al.*, 2010; Jenkins, 2018).

Plants that possess therapeutic properties or exert beneficial pharmacological effect on the human body are generally designated as medicinal plants (Melkamu *et al.*, 2018). Therefore, a medicinal plant is that plant in which one or more of its organs contain active ingredients which are used for therapeutic purposes or contain active ingredients that can be used for synthesis of drugs (Muhammad *et al.*, 2020).

Medicinal plants are used by traditional medicine practitioners' in the form of decoction, concoction, infusion and tisane. Decoction is when a medicinal plant is prepared in cold water brought to boil and then allowed to cool for about fifteen minutes mostly practice by Igbos' from Southeast of Nigeria, while concoction is a preparation of medicine in form of soup or drink, usually made from many ingredients mostly done by Yoruba tribe from Southwest of Nigeria

(Mann *et al.*, 2003). Moreso, infusion is usually made by pouring water on a specified plant material allowing the medicine to stand for about fifteen minutes mostly practice by Nupe tribe in North central of Nigeria, while tisane is a tea made by either decoction or infusion practice by Chinese (Mann *et al.*, 2003). Medicinal plants naturally synthesise and accumulate some secondary metabolites like alkaloids, steroids, tannins, terpenes, flavonoids, saponins, glycosides, cyanogenics, resins, lactones and carotenoids (Melkamu *et al.*, 2018).

In developing countries like Nigeria, Cameroon, Sudan, Niger, Republic of Benin and other Africa countries, more than 3.3 billion people use or utilize traditional medicine on regular basis for the treatment of various ailments such as malaria, typhoid fever, hepatitis, cancer, sexually transmitted diseases and others (Melkamu *et al.*, 2018). Moreso, World Health Organization (WHO), estimated that more than 80% of the world population still relies on traditional medicine for their primary health care needs (Sasidharan *et al.*, 2011). In recent years, medicinal plants have represented a primary health source for the pharmaceutical industry (Melkamu *et al.*, 2018). No less than 400 compounds derived from plants were reportedly used in the preparation of drugs such as vincristine and vinblastine used in the treatment of cancer, quinine and artemisinin used as antimalarial drugs (Muhammad *et al.*, 2020). The discovery of modern drugs such as quinine, vincristine, digoxin, digitoxin and artemisinin from medicinal plants signifies the huge potential that still exists for the production of more novel pharmaceuticals (Geyid *et al.*, 2005).

*Vitex doniana* commonly known as black plum and belongs to the *Verbernaceae* Family. The plant is called *Dinya* (Hausa), *Dinchi* (Nupe), *Orisi* or *Orinla* (Yoruba), *Efifi* (Igala) and *Olit* (Etsako). According to the report of Arokiyaraji *et al.* (2009), *Vitex doniana* has numerous applications in traditional medicine: Leaf sap was reported to be used as an eye drop for the treatment of conjunctivitis and other eye complaints. Leaf decoction of the plant is applied externally as a galactagogue and for the treatment of headache, stiffness, measles, body rash, fever, diarrhoea and chicken pox (Dash *et al.*, 2005). Based on the medicinal uses of the plant, the study was aimed at determining the antibacterial activity of *Vitex doniana* fractions against diarrhoeagenic *Escherichia coli* strains.

## METHODOLOGY

### Study Area

The study was carried out in Bida Niger State, Nigeria between July and December, 2022. Bida is the headquarters of Bida local Government.

Niger State is located in the central part (middle belt) of Nigeria. The state lies on latitude  $08^{\circ}$  to  $11^{\circ}30'$  North and longitude  $30^{\circ}$  to  $07^{\circ}40'$  east. It has a land mass of  $76,363\text{km}^2$  with a population of 4,082,558. Below are the maps Niger State showing the sampling site (Figure 1).

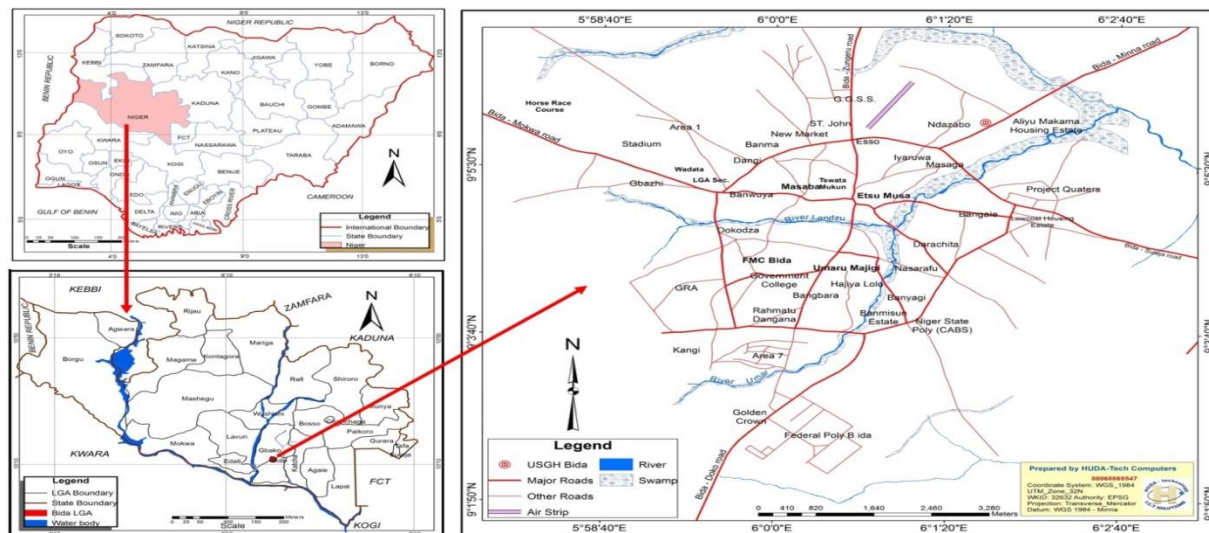


Fig 1: Map of Niger State showing Bida town and the sampling sites

### Collection and Identification of Plant Samples

Fresh leaves and stem-bark of *Vitex doniana* were collected within Bida Metropolis and were identified and authenticated at the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai, Niger State. A voucher specimen number VD/0145 was deposited at the Departmental Herbarium. The identification was done based on the literature and Atlas of medicinal plants by Mann *et al.* (2003).

### Preparation of Plant Sample

The leaves and stem-bark of *Vitex doniana* (Black plum) were rinsed with distilled water, cut into pieces with a sterile scalpel and air-dried in the laboratory for three weeks at room temperature ( $22^{\circ}\text{C}$ ) in a shaded area. The dried plant materials were grinded into particles by the aid of a clean mortar and pestle. The grinded particles were sieved with mesh sieve size (0.26mm) to obtain a fine powder.

### Extraction of Pulverized Plant Sample

Extraction of plant samples was carried out using Soxhlet extractor. Acetone, water, hexane and methanol were used as solvents of extraction. Methanol being a polar solvent has capacity of extracting hydrophilic compounds, while hexane

(non-polar) has capacity of extracting lipophilic compounds.

Twenty-five grams (25 g) of the powdered leaves of *Vitex doniana* were weighed and wrapped in a plain paper and placed in a Soxhlet extractor and extracted with 250 mL of water, acetone, hexane and methanol respectively for the period of 16 hours. The filtrate was transferred into a Porcelain dish and then allowed to dry. The extract obtained was labelled, weighed and kept for further analysis. The same procedure was followed for stem-bark sample (Hassan *et al.*, 2005).

### Fractionation of Plant Extracts

After preliminary antibacterial activity testing, the plant extracts with highest potency were considered for further fractionation using Tijjani *et al.* (2017).

The most active plant extract (*Vitex doniana* methanol leaf extract) (12.5g) was fractionated on a silica gel column chromatography using solvents such as hexane, acetone, methanol and distilled water. Three hundred grams (300g) silica gel 60-120 mesh was packed manually into a column (75cm x 3cm). The silica gel was allowed to hydrate in n-hexane and filled into the column with bedding height of 60cm and then

allowed to settle and packed. The methanol leaf extract of *Vitex doniana* was then dissolved in 9 mL of n-hexane, mixed with silica gel subsequently applied on the top of the column.

The methanol leaf extract of *Vitex doniana* was gradiently and successively eluted with n-hexane, acetone, and methanol 100% and aqueous at a flow rate of 1mL/min. Each eluent fraction (200 mL aliquots) was collected. The collected fractions were concentrated to dryness using rotary evaporator at 65°C and were subjected later to thin layer chromatography analysis by making slurry of silica gel 60G Merck, Germany on 20cm x 20cm x 7cm x 5cm pre-coated silica gel Thin layer chromatography plates. Fractions with similar R<sub>f</sub> values from the same solvents system were pooled together. The same procedure was followed for methanolic stem-bark extracts. The phytochemical testing of all the fractions was performed using qualitative methods of Ogukwe *et al.* (2004), Danlami, (2009) and Hassan *et al.* (2005).

#### **Reconstitution of the Plant Fractions**

The dried extracts (fractions) of *Vitex doniana* leaf and stem-bark fractions were weighed into McCartney bottles. Two hundred milligrams (200 mg) of *Vitex doniana* leaf and stem-bark fractions were dissolved or dispensed into 10 mL of dimethyl sulfur oxide (DMSO) to make a stock solution of 20 mg/mL respectively. Dimethyl sulfur oxide was used because it can dissolve both polar and non-polar compounds and can also be used to maintain stock solution of the test compounds with high boiling point. The serial dilution of 20 mg/mL was performed to yield 10 mg/mL, 5.0 mg/mL, 2.5 mg/mL and 1.25 mg/mL respectively (Muhammad *et al.*, 2020).

#### **Collection and Maintenance of Test Bacterial Isolates**

The eighty-one (81) pure culture of *Escherichia coli* strains were isolated from 480 diarrhoeic stool patients attending selected Hospitals in Niger State, Nigeria. The *Escherichia coli* strains used in the study include: Enteroaggregative *Escherichia coli* (EAEC), Enterohaemorrhagic *Escherichia coli* (EHEC), Enteroinvasive *Escherichia coli* (EIEC), Enteropathogenic *Escherichia coli* (EPEC) and Enterotoxigenic *Escherichia coli* (ETEC). The *E. coli* strains were subcultured into nutrient agar slants. Prior to inoculation, the sterility of agar slant was done

after preparation by incubating them for 24 hours at 37°C temperature.

#### **Preparation of inoculums**

A loopful of bacteria isolates was inoculated into 4 mL of peptone water and incubated at 37°C for four hours. The turbidity standard by actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units prepared by mixing 0.5 mL of 1.75% w/v barium chloride dehydrated with 99.5 mL of 1% v/v sulphuric acid. This turbidity was equivalent to approximately to  $1.2 \times 10^8$  colony forming unit per milliliter (cfu/ml). The two hours grown suspension was used for further analysis or testing (Cheesbrough, 2006).

#### **Determination of Antibacterial Activity of the Plant Fractions**

Before antibacterial susceptibility testing was carried out, the isolates were subcultured into fresh nutrient agar slants and incubated at 37°C for 24 hours. Broth suspension of the 24 hour culture was standardized to 0.5 McFarland standards. Eighteen milliliter (18 mL) of molten Mueller-Hilton agar was poured into sterile Petri plate and was allowed to solidify. The standardized suspension was used to inoculate the surface of agar plates using sterilized swab. A sterile standard cork borer of (6 mm diameter) was used to punch five wells aseptically into the agar and each well was filled with 0.2 mL of the extract and diffusion time of 45 minutes was allowed. The inoculated agar plates were then incubated at 37°C for 24 hours. The antibacterial activities were evaluated by measuring zones of inhibition using a transparent metric ruler (Junaid *et al.*, 2006).

#### **Determination of Minimum Inhibitory Concentration (MIC) of the Extracts**

The broth tube dilution method was adopted for the study. The stock solution of each fraction was first prepared by weighing 0.2 g of each fraction into the 10 mL of Mueller-Hinton broth to make the concentration of the stock to 20 mg/mL. The sterile capped test tubes were numbered 1-6. Approximately 5.0 mL of extract stock solutions (20 mg/mL) was placed into the first test tube, and 5.0 mL of sterile Mueller-Hinton broth was added to all other tubes. Exactly 5.0 mL was transferred from the first test tube that contains the extract and the broth to the second tube. The content of the second tube was mixed, using another sterile syringe and needle, 5.0 mL was again transferred to the third tube. The dilution

continued to tube 5, using a sterile syringe and needle at each stage of dilution. Exactly 5.0 mL was removed from tube 5 and discarded, while tube 6 contained the broth and inoculum only which served as the negative control. To all the test tubes, 0.1 mL of the bacterial suspension (inoculum) that conformed to 0.5 McFarland standards was added. All the tubes were incubated at 37°C for 24 hours. The tubes were examined for turbidity. The tube with the highest dilution without growth was taken as the MIC of the fractions.

#### **Determination of Minimum Bactericidal Concentration (MBC) of the Extracts**

The minimum bactericidal concentration was determined by sub-culturing the contents of the tubes that showed no growth in minimum inhibitory concentration tubes to extract free Mueller-Hinton agar plates, incubated at 37°C for 48 hours and then examined for bacterial growth. Three replicate plates were prepared for each fraction, and seven fractions were used, making total replicate plates of twenty-one per *Escherichia coli* strain. Therefore, a replicate plate or Mueller-Hinton agar plate with the highest dilution of extract (lowest concentration) that showed no growth was taken as the minimum bactericidal concentration (MBC) of the fraction.

### **RESULTS**

The results of susceptibility patterns of *Escherichia coli* strains to *Vitex doniana* fractions are presented in Table 1. The susceptibility pattern of the *Escherichia coli* strains to acetone leaf fraction of *Vitex doniana* (VDALF) indicated that 81.3% (n=13/16) of Enteroaggregative *Escherichia coli* (EAEC), 92.0% (n=12/13) of Enterohaemorrhagic *Escherichia coli* (EHEC), 75% (n=6/8) of Enteroinvasive *Escherichia coli* (EHEC), 85% (n=23/27) of Enteropathogenic *Escherichia coli* (EPEC) and 88% (n=15/17) of Enterotoxigenic *Escherichia coli* (ETEC), while aqueous stem-bark fraction of *Vitex doniana* (VDSBF) showed that 88.0% (n=14/16) of Enteroaggregative *Escherichia coli* (EAEC), 69.0% (n=9/13) of Enterohaemorrhagic *Escherichia coli* (EHEC), 38% (n=3/8) of Enteroinvasive *Escherichia coli* (EHEC), 82% (n=22/27) of Enteropathogenic *Escherichia coli* (EPEC) and 88% (n=15/17) of Enterotoxigenic *Escherichia coli* (ETEC).

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractions against Enteroaggregative *Escherichia coli* strains are presented in Table 2. *Vitex doniana* acetone leaf fraction (VDALF) had an MIC value of 5.0 mg/mL against 25% (n=4/16) of Enteroaggregative *Escherichia coli* strains, MIC value of 10 mg/mL against 43% (n=7/16) of the Enteroaggregative *Escherichia coli* strains and MIC of 20 mg/mL against 12.5% (n=2/16) of the Enteroaggregative *Escherichia coli* strains respectively. However, against 43.8% (n=7/16) of Enteroaggregative *Escherichia coli* strains and MIC value of 20 mg/mL against 12.5% (2/16) of Enteroaggregative *Escherichia coli* strains, while *Vitex doniana* acetone leaf fraction (VDALF) had an MBC value of 10 mg/mL against 43% (n=7/16) of the Enteroaggregative *Escherichia coli* strains and MBC value of 20 mg/mL against 31.3% (n=5/16) of Enteroaggregative *Escherichia coli* strains. On the other hand, *Vitex doniana* aqueous stem-bark fraction had an MIC values of 5.0 mg/mL and 10 mg/mL against 75% (n=12/16) and 25% (n=4/16) of the Enteroaggregative *Escherichia coli* strains respectively. However, the same fraction had an MBC values of 5.0 mg/mL and 10 mg/mL against 25% (n=4/16) and 75% (n=12/16) of the Enteroaggregative *Escherichia coli* strains respectively.

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractions against Enterohaemorrhagic *Escherichia coli* strains are presented in Table 3. *Vitex doniana* acetone leaf fraction (VDALF) had an MIC value of 5.0 mg/mL against 46.2% (n=6/13) of the Enterohaemorrhagic *Escherichia coli* strains and MIC 10 mg/mL against 26.7% (n=4/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively. However, Acetone leaf fraction had an MBC value of 10mg/mL against 76.9% (n=10/13) of the Enterohaemorrhagic *Escherichia coli* strains.

*Vitex doniana* aqueous stem-bark fraction had an MIC values of 5.0 mg/mL and 10 mg/mL against 15.4% (n=2/13) and 46.2% (n=6/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively. However, the same fraction had an MBC value of 10 mg/mL against 61.5% (n=8/13) of the Enterohaemorrhagic *Escherichia coli* strains respectively (Table 3).

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractions against Enteroinvasive *Escherichia coli* strains are presented in Table 4. *Vitex doniana* acetone leaf fraction (VDALF) had an MIC value of 5.0 mg/mL against 25% (n=2/8) of the Enteroinvasive *Escherichia coli* strains, MIC value of 10 mg/mL against 62.5% (n=5/8) of the Enteroinvasive *Escherichia coli* strains and MIC of 20 mg/mL against 12.5% (n=1/8) of the Enteroinvasive *Escherichia coli* strains. However, Acetone leaf fraction had an MBC of 10 mg/mL and 20 mg/mL against 75% (n=6/8) and 25% (n=2/8) of the Enteroinvasive *Escherichia coli* strains respectively. On the other hand, *Vitex doniana* aqueous stem-bark fraction had an MIC value of 10 mg/mL against 37.5% (n=3/8) of the Enteroinvasive *Escherichia coli* strains. However, the same fraction had an MBC value of 10 mg/mL against 37.5% (n=3/8) of the Enteroinvasive *Escherichia coli* strains.

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractions against Enteropathogenic *Escherichia coli* strains are presented in Table 5. *Vitex doniana* acetone leaf fraction (VDALF) had an MIC value of 5.0 mg/mL against 26% (n=7/27) of the Enteropathogenic *Escherichia coli* strains, MIC 10 mg/mL against 48.1% (n=13/27) of the Enteropathogenic *Escherichia coli* strains and MIC of 20 mg/mL against 7.4% (n=2/27) of the Enteropathogenic *Escherichia coli* strains respectively. However, Acetone leaf fraction had an MBC values of 10 mg/mL and 20 mg/mL

against 63% (n=17/27) and 11.1% (n=3/27) of the Enteropathogenic *Escherichia coli* strains respectively

*Vitex doniana* aqueous stem-bark fraction had an MIC values of 5.0 mg/mL and 10 mg/mL against 37% (n=10/27) of the Enterohaemorrhagic *Escherichia coli* strains respectively. However, the same fraction had an MBC values of 5.0 mg/mL and 10 mg/mL and 20 mg/mL against 3.7% (n=1/27), 55.6% (n=15/27) and 14.8% (n=4/27) of the Enteropathogenic *Escherichia coli* strains respectively (Table 5).

The results of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of *Vitex doniana* fractions against Enterotoxigenic *Escherichia coli* strains are presented in Table 6. *Vitex doniana* acetone leaf fraction (VDALF) had an MIC values of 5.0mg/mL and 10mg/mL against 23.5% (n=4/17) and 64.7% (n=11/17) of the Enterotoxigenic *Escherichia coli* strains respectively. However, Acetone leaf fraction had an MBC values of 10 mg/mL and 20 mg/mL against 58.8% (n=10/17) and 29.4% (n=5/17) of the Enterotoxigenic *Escherichia coli* strains respectively.

*Vitex doniana* aqueous stem-bark fraction had an MIC values of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 64.7% (n=11/17), 17.6% (n=3/17) and 5.9% (n=1/17) of the Enterotoxigenic *Escherichia coli* strains respectively. However, the same fraction had an MBC values of 5.0 mg/mL, 10 mg/mL and 20 mg/mL against 5.9% (n=1/17), 76.5% (n=13/17) and 5.9% (n=1/17) of the Enterotoxigenic *Escherichia coli* strains respectively.

Table 1: The Percentage Susceptibility Pattern of *Escherichia coli* Strains to *Vitex doniana* Fractions.

Plant fraction	EAEC (n=16)	EHEC (n=13)	EIE C (n=8)	EPEC (n=27)	ETEC (n=17)
VDALF	13 (81.30)	12 (92.00)	06 (75.00)	23 (85.00)	15 (88.00)
VDASBF	08 (50.00)	05 (39.00)	03 (38.00)	16 (59.00)	09 (53.00)
VDHLF	11 (69.00)	10 (77.00)	05 (63.00)	26 (96.00)	15 (88.00)
VDMLF	15 (94.00)	13 (100.0)	04 (50.00)	27 (100.0)	17 (100.0)
VDMSBF	12 (75.00)	13 (100.0)	05 (63.00)	25 (93.00)	14 (82.00)
VDQLF	12 (75.00)	12 (92.00)	04 (50.00)	24 (89.00)	14 (82.00)
VDQSBF	14 (88.00)	09 (69.00)	03 (38.00)	22 (82.00)	15 (88.00)

Key: EAEC=Enterococcal *Escherichia coli*; EHEC Enterohaemorrhagic *Escherichia coli* strains; EIEC=Enteroinvasive *Escherichia coli*; EPEC=Enteropathogenic *Escherichia coli*; ETEC=Enterotoxigenic *Escherichia coli*; VDALF=*Vitex doniana* acetone leaf extract; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition.



Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Vitex doniana* Fractions against Enteroaggregative *Escherichia coli* isolates (n=16).

<i>Vitex doniana</i> Fraction	MIC (mg/mL)			MBC (mg/mL)		
	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	4(25.0)	7(43.8)	2(12.5)	-(0.0)	7(43.8)	5(31.3)
VDASBF	-(0.0)	6(37.5)	1(6.3)	-(0.0)	4(25.0)	2(12.5)
VDHLF	2(12.5)	5(31.3)	3(18.8)	-(0.0)	5(31.3)	5(31.3)
VDMLF	8(50.0)	3(18.8)	1(6.3)	-(0.0)	9(56.3)	2(12.5)
VDMSBF	7(43.8)	3(18.8)	-(0.0)	2(12.5)	8(50.0)	-(0.0)
VDQLF	1(6.3)	7(43.8)	4(25.0)	1(6.3)	5(31.3)	6(37.5)
VDQSBF	12(75.0)	4(25.0)	-(0.0)	4(25.0)	12(75.0)	-(0.0)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana*

Table 3: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBCs) of *Vitex doniana* Fractions against Enterohaemorrhagic *Escherichia coli* isolates (n=13).

<i>Vitex doniana</i> Fraction	MIC (mg/mL)			MBC (mg/mL)		
	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	6(46.2)	4(30.8)	-(0.0)	-(0.0)	10(76.9)	-(0.0)
VDASBF	4(30.8)	2(15.4)	-(0.0)	1(7.7)	5(38.5)	-(0.0)
VDHLF	8(61.5)	2(15.4)	-(0.0)	1(7.7)	9(69.2)	-(0.0)
VDMLF	10(76.9)	3(20.2)	-(0.0)	3(20.2)	10(76.9)	-(0.0)
VDMSBF	8(61.5)	5(38.5)	-(0.0)	-(0.0)	12(92.3)	1(7.7)
VDQLF	7(53.8)	4(30.8)	-(0.0)	1(7.7)	11(84.6)	-(0.0)
VDQSBF	2(15.4)	6(46.2)	-(0.0)	-(0.0)	8(61.5)	-(0.0)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitory concentration; MBC=Minimum bactericidal concentration.

Table 4: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Vitex doniana* Fractions against Enteroinvasive *Escherichia coli* (n=8).

<i>Vitex doniana</i> Fraction	MIC (mg/mL)			MBC (mg/mL)		
	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	2(25.0)	5(62.5)	1(12.5)	-(0.0)	6(75.0)	2(25.0)
VDASBF	2(25.0)	1(12.5)	-(0.0)	-(0.0)	3(37.5)	-(0.0)
VDHLF	1(12.5)	4(50.0)	-(0.0)	-(0.0)	5(62.5)	-(0.0)
VDMLF	1(12.5)	2(25.0)	1(12.5)	-(0.0)	2(25.0)	2(25.0)
VDMSBF	3(37.5)	2(25.0)	-(0.0)	1(12.5)	3(37.5)	1(0.0)
VDQLF	1(12.5)	3(37.5)	-(0.0)	1(12.5)	4(50.0)	-(0.0)
VDQSBF	-(0.0)	3(37.5)	-(0.0)	-(0.0)	3(37.5)	-(0.0)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitory concentration; MBC=Minimum bactericidal concentration.

Table 5: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Vitex doniana* Fractions against Enteropathogenic *Escherichia coli* (n=27).

<i>Vitex doniana</i> Fraction	MIC (mg/mL)			MBC (mg/mL)		
	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	7(25.9)	13(48.1)	2(7.4)	-(0.0)	17(63.0)	3(11.1)
VDASBF	6(22.2)	9(33.33)	-(0.0)	1(3.7)	10(37.0)	4(14.8)
VDHLF	7(25.9)	16(59.2)	1(3.7)	3(11.1)	17(63.0)	4(14.8)
VDMLF	15(55.6)	12(44.4)	-(0.0)	1(3.7)	24(88.9)	2(7.4)
VDMSBF	18(66.7)	16(59.2)	1(3.7)	1(3.7)	22(81.5)	2(7.4)
VDQLF	5(18.5)	17(63.0)	1(3.7)	-(0.0)	18(66.7)	5(18.5)
VDQSBF	10(37.0)	10(37.0)	-(0.0)	-(0.0)	15(55.6)	4(14.8)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitory concentration; MBC=Minimum bactericidal concentration.

Table 6: Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs) of *Vitex doniana* Fractions against Enterotoxigenic *Escherichia coli* (n=17).

<i>Vitex doniana</i> Fraction	MIC (mg/mL)			MBC (mg/mL)		
	5.0	10.0	20.0	5.0	10.0	20.0
VDALF	4(23.5)	11(64.7)	-(0.0)	-(0.0)	10(52.9)	5(18.5)
VDASBF	3(17.6)	5(18.5)	1(5.9)	-(0.0)	7(41.2)	2(11.8)
VDHLF	1(5.9)	14(82.4)	-(0.0)	-(0.0)	11(64.7)	4(23.5)
VDMLF	11(64.7)	16(94.1)	-(0.0)	-(0.0)	17(100)	-(0.0)
VDMSBF	14(82.4)	-(0.0)	-(0.0)	5(18.5)	9(52.9)	-(0.0)
VDQLF	5(18.5)	7(41.2)	2(11.8)	-(0.0)	9(52.9)	5(18.5)
VDQSBF	11(64.7)	3(17.6)	1(5.9)	1(5.9)	13(76.5)	1(5.9)

Key: VDALF=*Vitex doniana* acetone leaf fraction; VDASBF=*Vitex doniana* acetone stem-bark fraction; VDHLF=*Vitex doniana* hexane leaf fraction; VDMLF=*Vitex doniana* methanol leaf fraction; VDMSBF=*Vitex doniana* methanol stem-bark fraction; VDQLF=*Vitex doniana* aqueous leaf fraction; VDQSBF=*Vitex doniana* aqueous stem-bark fraction; - =No inhibition; MIC=Minimum inhibitory concentration; MBC=Minimum bactericidal concentration.

## DISCUSSION

From the result of MIC and MBC test of *Vitex doniana* fractions against Enterotoxigenic *Escherichia coli*. Isolates of Enterotoxigenic *Escherichia coli* were susceptible to the fractions at MIC of 5.0 mg/mL especially aqueous stem-bark fraction having inhibitory effect against 75% isolates. The reason may be as a result of anthraquinones present in the fraction. It was reported that anthraquinones act by inhibiting two enzymes involved in bacterial DNA synthesis; topoisomerase II and DNA gyrase which block DNA replication thereby impair cell division (Omo-Kalu and Nonye, 2015).

Resistance exhibited by some of the Enterotoxigenic *Escherichia coli* strains to *Vitex doniana* fractions might be due to some mutant EAEC strains that possess genes called *gyrA*, *gyrB*, *ParC*<sup>1</sup> and *ParC*<sup>2</sup> which are capable

of altering their efflux pump to unspecific compounds or substrates (Cushnie and Lamb, 2005).

*Vitex doniana* fractions had an MIC of 5.0 and 10 mg/mL against Enterohaemorrhagic *Escherichia coli*. The *Vitex doniana* methanol leaf fraction had an MIC of 5.0 mg/mL against 76.9% (n=10/13) of the Enterohaemorrhagic *Escherichia coli*. This could be attributed to the presence of tannins in the methanol leaf fraction. Tannins are polyphenols with pronounced autolytic enzymes of microbial metabolism such as proteolytic macerating enzymes (Obase *et al.*, 2010).

The result indicated that 53.85% (n=7/13) of the Enterohaemorrhagic *Escherichia coli* were resistant to the methanol leaf fraction. This might



be due to some mutant strains that possess genes that rendered the fractions inactive. It was reported that some mutant EHEC and EPEC possess an enzyme called Beta-lactamase enzyme and this contributed to high level of resistance to phytochemical constituents such as flavonoids, tannins and antibiotics like ampicillin and penicillin (Cushnie and Lamb, 2005; Omo-Kalu and Nonye, 2015)

The result indicated that *Vitex doniana* methanol stem-bark fraction had an MIC of 5.0 mg/mL, while acetone leaf fraction had an MIC of 10.0 mg/mL against 37.5% (n=3/8) and 62% (n=5/8) of the Enteroinvasive *Escherichia coli*. The inhibitory effect of the fractions against Enteroinvasive *Escherichia coli* might be due to the presence of saponins and terpenoids in the *Vitex doniana* methanol stem-bark and *Vitex doniana* acetone leaf fraction respectively. Saponins might act by altering the permeability of the cell membrane and hence exerting toxicity on all organised tissues. They exert some bacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Cushnie *et al.*, 2014).

However, some strains of Enteroinvasive *Escherichia coli* were resistant to saponins. This may be attributed to the fact that mutant EIEC possess an enzyme that is capable of altering mode of action of terpenoids that act on beta-hydroxylacyl-acyl carrier protein dehydrates activities, thus the RNA synthesis will not be impaired (Cushnie and Lamb, 2005).

Methanol stem-bark and aqueous leaf fractions of *Vitex doniana* had an MIC of 5.0 mg/mL and 10.0 mg/mL against 66.7% (n=18/27) and 63% (n=17/27) of the Enteropathogenic *Escherichia coli*. The reason may be as a result of glycosides and flavonoids detected in the methanol stem-bark and aqueous leaf fractions respectively. Flavonoids have been reported to form complexes with both extracellular and soluble proteins as well as bacterial cell walls (Hanson, 2010). The glycosides have been also reported to inhibit protein synthesis by binding with high affinity to the A-site on the 16s ribosomal RNA of the 30s ribosome (Cushnie and Lamb, 2005).

However, some strains of Enteropathogenic *Escherichia coli* were resistant to phytochemical constituents detected in the fractions. It was reported that some mutant EPEC possess an enzyme called Beta-lactamase enzyme and this

contributed to high level of resistance to phytochemical constituents such as flavonoids, tannins and antibiotics like ampicillin and penicillin (Cushnie and Lamb, 2005; Omo-Kalu and Nonye, 2015).

*Vitex doniana* methanol leaf fraction had an MIC and MBC values of 5.0 mg/mL respectively against 82.4% (n=14/17) and 15.8% (n=5/17) of the Enterotoxigenic *Escherichia coli*, while methanol leaf fraction had MIC value of 5.0 mg/mL against 94.1% (n=16/17) of the Enterotoxigenic *Escherichia coli*. This might be attributed to the presence of alkaloids and flavonoids in the fraction respectively. According to Omo-Kalu and Nonye (2015), alkaloids such as pergulamine and tylophorinidine have effect on cell division by inhibiting dihydrofolate reductase which is responsible in the production of purine and pyrimidine precursors for amino acids, RNA and DNA biosynthesis. Flavonoids on the other hand have been reported to form complexes with both extracellular and soluble proteins as well as bacterial cell walls (Hanson, 2010).

However, some strains of Enterotoxigenic *Escherichia coli* were resistant to phytochemical constituents detected in *Vitex doniana* fractions. This may be due to mutation that might have occurred within some strains of Enterotoxigenic *Escherichia coli*.

The result of an antibacterial susceptibility study of the fractions against the *Escherichia coli* strains in this study indicated that they had activity against almost all the *Escherichia coli* strains. This activity is attributed to phytochemical constituents that were found present in the plant extract which have been reported to have activity against bacteria. Anthraquinones were reported to inhibit DNA gyrase thereby preventing DNA synthesis, Saponins might act by altering the permeability of the cell membrane and hence exerting toxicity on all organised tissues. They exert some bacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Cushnie *et al.*, 2014). Steroids have been reported to have synergistic effect with saponins (Hanson, 2010; Malziga, 2010). Tannins are polyphenols with pronounced autolytic enzymes of microbial metabolism such as proteolytic macerating enzymes (Malziga, 2010).

## Conclusion

The test *Vitex doniana* fractions in this study revealed or have been confirmed to be very effective or active against diarrhoeagenic *Escherichia coli* strains especially the *Vitex doniana* methanol leaf and stem-bark fractions that had an MIC and MBC values of 5.0 and 10.0 mg/mL respectively against 87% of the tested diarrhoeagenic *Escherichia coli* strains. The efficacy of these fractions might be attributed to phytochemical constituents present in the fractions and this has justified their utilization by traditional medicinal practitioners for the treatment of diarrhoea and other related ailments associated with *Escherichia coli* strains.

## Recommendation

It is therefore, recommended that further study is needed to purify, characterize and elucidate the structure of bio-active compounds present in the fractions which were responsible for antibacterial activity with a view to supplement conventional drug development.

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