



RESEARCH PAPER

Anti-Diarrhoeal, Phytochemical and Toxicological Studies of *Vitex doniana* Extracts on *Escherichia coli* Strains Isolated from Diarrhoeal Stool of Some Children (0-5) Years

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ABSTRACT

Diarrhoea is reported to be an infectious disease capable of causing morbidity and mortality in both developing and developed countries. This study was carried out to determine the anti-diarrheal activity, phytochemical and toxicological studies of *Vitex doniana* extracts against *Escherichia coli* strains isolated from diarrhoeal stool of children less than five years. The antibacterial activity was carried out using agar well diffusion method, while phytochemical screening was conducted using Sofowora, Harbone and Hassan's methods. The acute toxicity (LD₅₀) test was also carried out using Lorke and Hassan's methods. The phytochemical screening of *Vitex doniana* leaf extract revealed the presence of alkaloids, steroids, tannins and glycosides, while stem-bark extracts indicated the presence of alkaloids, saponins, steroids, saponins and tannins. Enteraggregative *Escherichia coli* (EAEC), 18.25% (n=13) of the strains were susceptible to acetone leaf fraction, 50% (n=8) were susceptible to acetone stem-bark fraction, 69% (n=11) to hexane leaf fraction, 94% (n=15) to methanol leaf fraction, 75% (n=12) to methanol stem-bark fraction and aqueous leaf fraction, 88.5% (n=14) to aqueous stem-bark fraction and 94% (n=15) to ciprofloxacin. Based on the result of acute toxicity test (LD)₅₀ greater than 5000mg/kg body weight, then the plant extracts are safe for consumption. The result has justified their utilization by the traditional medicine practitioners for the treatment of diarrhoea and other related ailments associated with *Escherichia coli* strains.

Keywords: Diarrhoea, Extract, Phytochemical, Toxicity, *Vitex doniana*

INTRODUCTION

Medically, diarrhoea is defined in terms of frequency, consistency and either weight or volume of faeces (Baba *et al.*, 2016). Liquid stool with frequency of more than three times per day and weighing more than 200 grams in total is referred to as diarrhoea (Forbes, 2003). According to World Health Organization (1991), diarrhoea is defined as decrease in the consistency of the stool from that which is normal for the patient. However, diarrhoea is a symptom of underlying pathology which can impact upon quality of life and also life threatening. It is an infection responsible for considerable morbidity and mortality especially in developing country like Nigeria (Baba *et al.*, 2016).

Medicinal plant is that plant in which one or more of its organ contains substances that can be used for therapeutic purposes or semi-synthesis of drugs (Sofowora, 1982). Medicinal plants have been identified and used

throughout human history and through his inquisitiveness, he has been able to separate the edible plants from the poisonous ones (Lawal *et al.*, 2010). Man discovered that some plant could be used for both preventive and curative purposes of his ailments (Lawal *et al.*, 2012). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances includes tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.*, 2005).

Vitex doniana is a perennial plant belonging to the Family *Verbenaceae* and is commonly called black plum (English), dinchi (Nupe), utakiri' (Ibo), Punnier noir (French), dinya (Hausa), ucha koro (Igbo), oori-nla (Yoruba) and ngarmi (Kanuri) (Mann *et al.*, 2003).

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Vitex doniana is abundantly found in savannah regions and it is a medium-sized deciduous tree, about 8-18m high, with a heavy rounded crown and a clear bole up to 5m (Arokiyaraj *et al.*, 2009).

Vitex doniana has numerous applications in traditional medicine. Leaf sap is used as an eye drop to treat conjunctivitis and other eye complaints. A leaf decoction is applied externally as a galactagogue and against headache, stiffness, measles, body rash, fever, chicken pox and internally as a tonic, anodyne and febrifuge (Arokiyaraj *et al.*, 2009). Pastes of pounded leaves and bark are applied to wounds and burns. A root decoction is administered orally to treat ankylostomiasis, rachitis, gastro-intestinal disorders and jaundice, and well as an anodyne. Powdered bark added to water is taken to treat colitis, and also used to treat stomach complaints and kidney troubles (Dash *et al.*, 2005). The bark is also used against leprosy and liver diseases, and also to control bleeding after childbirth. Dried and fresh fruits are eaten against diarrhea, and as a remedy against lack of vitamin A and B. The blackish extract obtained by boiling leaves, bark, roots and/or fruits are used as ink and as well as a dye for clothes. The flowers serve as source of nectar for honey bees, while Cattle browse the foliage (Padamalatha *et al.*, 2009). The aim of this study is determine the anti-diarrheal, phytochemical and toxicological studies of *Escherichia coli* serotypes isolated from diarrheal stool of children less than five years.

METHODOLOGY

Sample collection and Identification

The fresh leaves and the stem-bark of *Vitex doniana* were collected within Lapai town, Niger state, Nigeria. The plant was identified and authenticated by Dr Jonathan Gana, a botanist from College of Education, Minna, Niger State, Nigeria. A voucher specimen has been deposited in the departmental herbarium.

Sterilization of Materials

All glass wares used in the research were washed with detergent, rinsed with distilled water, air dried and sterilized in a hot oven at 170°C for 60 seconds. Each of the material was wrapped with aluminum foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121°C for 15 minutes. Cork borer and glass rods were

sterilized by dipping them into 70% alcohol prior to flaming in a Bursen burner. The working bench was swabbed with 75% alcohol before and after each experiment.

Preparation of Plant Material

The fresh leaves and stem-bark of *Vitex doniana* were collected and rinsed with distilled water to remove the impurities such as debris and dust particles. The leaves and stem-bark size were reduced to smaller pieces by cutting to small parts, using sterile knife. The leaves and stem-bark were air dried at room temperature for five weeks due to the weather condition. The dried leaves and stem-bark were ground into fine powder using mortar and pestle, then 0.05mm mesh sieve was used to remove particles and fine powdered samples were stored in sterile air tight container for further use.

Extraction of Plant Material

The method of Junaid *et al.* (2006) and Okwori *et al.* (2007) were used for this study. Acetone, aqueous (distilled water), n-hexane and methanol were used as extractants. Distilled water was used to stimulate the condition commonly used by traditional herbalist for the extraction of herbs. Methanol was used because of its broad spectrum, and relatively non-selective property of extracting tannin, while acetone was used because of its property of extracting tannin. However, n-hexane being a non-polar solvent has capacity of extracting lipophilic compounds.

Fifty grams (50g) of each of the *V. doniana* plant parts (leaves and stem-bark) were soaked separately in 400 ml of 95% methanol contained in 500ml capacity conical flask for 72 hours. The same procedure was followed for acetone, hexane and aqueous extraction. The conical flasks were covered with cotton wool, wrapped with aluminum foil in order to avoid contamination of the mixture. Maceration was achieved by constant shaking and solution was filtered using Whatman's No.1 filter paper to obtain a solution free of solids. The samples were then concentrated in a rotary evaporator at 45°C to remove the methanol and acetone, and stored at 2-4°C. The same process was repeated until enough crude extracts was obtained. However, water is a non-volatile solvent; the extracts containing distilled water were evaporated to dryness in a boiling water bath (Russel, 2001).

The filtrates (crude extracts) were successively partitioned using different solvents with the different polarities such as Hexane, ethyl acetate, methanol and aqueous. The fractions obtained includes hexane leaf fraction A, hexane leaf fraction B, methanol stem-bark fraction, methanol leaf fraction, ethyl acetate leaf fraction, ethyl acetate stem-bark fraction, aqueous leaf fraction and aqueous stem-bark fraction. Each fraction obtained was stored for further analysis.

Reconstitution of Plant Extracts

The dried residue was weighed into McCartney bottles. Zero point two grams (0.2g) of the dried residue of the leaf and stem-bark fractions of *V. doniana* were dissolved or dispensed into 10ml of glycerol to make a stock solution of 2 mg/ml respectively.

Sources of Test Organisms

Pure clinical isolates used in this study were *Escherichia coli* strains namely Enteroaggregative *E. coli*, Enteropathogenic *E. coli*, Enteroinvasive *E. coli*, Enterohemorrhagic *E. coli* and Enterotoxigenic *E. coli* isolated from diarrheal stool of children less than five years attending various hospitals in Niger state, Nigeria (Etsu Umaru Sanda General Hospital, Bida; General Hospital, Minna and General Hospital, Kontagora).

Preparation and standardization of inoculums

All test organisms were separately prepared by sub culturing the pure isolates in to nutrient agar and incubated at 37°C for 24 hours. One gram (1g) of Barium chloride was weighed and dissolved in 99ml of sterile distilled water. This was followed by the measurement of 1ml of concentrated sulphuric acid in 99ml of sterile distilled water. To prepare 10ml of McFarland Nephelometer, 0.2ml of 1% barium chloride was added to 9.8ml of concentrated sulphuric acid. Turbidity corresponds to 6- $\cdot 10^8$ ml bacteria cells referred to McFarland standard (McFarland, 1970).

Experimental animal; Whistler Albino rats

Young Albino rats (male) with an average body weight 185 – 230g were used for this study. They were obtained from the Animal House Unit of the Department of Biochemistry, Ibrahim Badamasi Babangida University Lapai, Niger state. They were housed in a polypropylene cage and

maintained at 30-37°C for 12 hours' dark/light cycle. The animals were acclimatized to laboratory condition for 7 days prior to experiment. These were fed with standard marsh and water. For hygienic purpose the litter to the cage was renewed twice a week.

Acute toxicity studies (determination of LD₅₀)

The method of Hassan *et al.* (2005) and OECD, (2001) was used to carry out acute toxicity study of the plant extracts. Water (aqueous) extract of *Vitex doniana* leaf extract (1000, 2000, 3000, 4000 and 5000 mg/kg) was administered to six (6) groups, (six wistar albino rat per group) of rats (one after the other at a grace observation of 48 hr) in single oral dose by using a feeding needle. The control group received distilled water. The same procedure was followed with aqueous *Vitex doniana* stem-bark fractions.

Phytochemical screening of leaves and stem-bark of *Vitex doniana*

All test organisms were separately prepared by sub culturing the pure isolates in to nutrient agar and incubated at 37°C for 24 hours. One gram (1g) of Barium chloride was weighed and dissolved in 99ml of sterile distilled water. This was followed by the measurement of 1ml of concentrated sulphuric acid in 99ml of sterile distilled water. To prepare 10ml of McFarland Nephelometer, 0.2ml of 1% barium chloride was added to 9.8ml of concentrated sulphuric acid. Turbidity corresponds to 6- $\cdot 10^8$ ml bacteria cells referred to McFarland standard (McFarland, 1970).

Test for alkaloids

To 3mL of extract introduced into different test tube, 1mL of 1% of hydrochloric acid (HCL) was added. The mixture was later treated with few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids (Ogukwe *et al.*, 2004).

Test for anthroquinone

To 4mL of each extract in a test tube, 4mL of 100% ammonium solution was added. A pink violet or red coloration in the ammoniac layer in each test tube indicated the presence of anthroquinone (Danlami, 2009).

Test for flavonoids

To 1mL of each extract, 3 drops of ammonia solution was added. Half (0.5) mL of concentrated HCL was further added to the

mixture. A pale brown coloration indicated the presence of flavonoids (Odebiyi and Sofowora, 1978).

Test for glycosides

To 1 mL of each extract, 2 mL of acetic acid was added and then cool in an ice bath at 4°C. To the mixture, 1 ml of conc. H₂SO₄ was added drop wise. Oil layer formed on top of the solution indicated the presence of glycosides (Elmahmood and Amey, 2007; Odebiyi and Sofowora, 1987).

Test for resins

To 5mL of each extract, 5mL of copper acetate solution was added and shaken vigorously and then allowed to separate. A reddish brown precipitate indicated the presence of resins (Elmahmood and Doughari, 2008).

Test for saponins

Half milliliter (0.5mL) of each extract in a test tube, 5.0mL of distilled water was added. The mixture was then shaken vigorously for 2 minutes; the froth formation indicated the presence of saponins (Hassan *et al.*, 2005).

Test for steroids

To 1mL of each extract, 1mL of concentrated tetraoxosulphate (IV) acid (H₂SO₄) was added. A reddish coloration indicated the presence of steroid (Hassan *et al.*, 2005).

Test for tannins

To 5mL of each extract, 2 drops of 5% FeCl₃ was added. A dirty green precipitate indicated the presence of tannins (Ogukwe *et al.*, 2004).

Determination of Antibacterial Activity of *V. doniana* Extract

The susceptibilities of the test organisms to the plant fractions were assayed as described by (Aliyu *et al.*, 2009). The standardized suspension was used to inoculate the surface of the nutrient agar, and plates were allowed to set. A standard cork borer of 6mm in diameter was used to punch five wells aseptically on the surface of agar and filled with the desired concentration (2mg/ml,) of the aqueous, ethyl-acetate, hexane, and methanol leaf and stem-bark fractions of *V. doniana*. Commercial antibiotic (Ciprofloxacin) was used as positive control. One of the well was filled with 0.2mL of the control (Ciprofloxacin) at the same concentration (2mg/ml). The same procedure was followed for *V. doniana* leaf fractions, all plates were allowed to stand for 1 hour at room temperature for the extract to diffuse into

the agar and then incubated at 37°C over night (Norrel and Messley 1997). Antibacterial activities were evaluated by measuring zone of inhibition. Zone of inhibition means the zone of clearance around each well and the diameter was measured using transparent metric ruler.

RESULTS AND DISCUSSIONS

The phytochemical screening of *Vitex doniana* leaf extracts revealed the presence of anthroquinones, steroids, saponins, flavonoids and glycosides. This is similar to the findings of Dauda *et al.* (2001) who reported the presence of cardiac glycosides, flavonoids, terpenes, steroids and resins in their studies on phytochemical and in-vitro antibacterial investigation of *Vitex doniana* leaves, stem-bark and root extracts. This is also in conformity with the findings of Agbolafor and Nwuchukwu, (2011) who reported the presence of anthroquinone, saponin, flavonoids, terpenoids, tannins, and alkaloids in their studies of phytochemical analysis and antioxidant properties of leaves extracts of *Vitex doniana* and *Mucuna pruriens*. This study also agrees with the findings of Dawang, (2015), who reported the presences of flavonoids, cardiac glycosides, steroids, terpenes and resin in his studies of constituents and toxicological analysis of *Vitex doniana* leaf extracts. The study is contrary to the findings of Ferguson, (2001) who reported the presence of resins, tannins and saponins in his studies on phytochemical and antimicrobial assessment of hexane leaves extracts of *Vitex doniana*. This could be attributed to hexane used as extractant which is capable of extracting lipophylic compounds.

This study revealed the presence of anthroquinones, tannins, flavonoids, glycosides and saponins in the stem-bark extracts of *Vitex doniana*. This work is similar to the findings of Emmanuel *et al.* (2015) who reported the presence of alkaloids, glycosides, tannins and saponins in their studies on phytochemical and antimicrobial screening of the stem-bark extracts of *Vitex doniana*. This study also agrees with the findings of Ali *et al.* (2017) who reported the presence of anthroquinones, alkaloids, saponins, tannins, phenol, glycosides and alkaloids in their studies on the assessment of antibacterial activity and phytochemical screening of *Vitex doniana* extracts on clinical isolates of *Salmonella typhi*. This is also in conformity

with the findings of Olusola *et al.* (1997) who reported the presence of alkaloids, anthraquinones, saponins, tannins, resins and

glycosides in their studies on the effect of *Vitex doniana* stem-bark extract on blood pressure.

Table 1: Phytochemical screening of acetone, aqueous, hexane and methanolic leaf and stem-bark crude extracts of *Vitex danana*

Extracts	Phytochemical constituents							
	Alkaloids	Anthraquinones	Flavonoids	Glycosides	Resin	Saponin	Steroids	Tannins
Acetone leaf	-	++	-	-	-	++	++	-
Aqueous leaf	-	++	++	-	-	+	++	-
Hexane leaf	+	+	-	-	+	+	++	-
Methanol leaf	-	+	-	+	-	-	++	+
Acetone bark	-	+	+	+	-	+	-	+
Aqueous bark	+	+	+	+	-	+	+	+
Hexane bark	-	+	-	-	-	-	-	+
Methanol bark	+	++	+	++	+	+	-	+

Key: - = Absent + = Moderate concentration ++ = High Concentration

Table 2: The acute toxicity (LD50) screening of crude aqueous leaf and stem-bark extracts of *Vitex doniana* on albino rat

Plant extract	Group	No. of albino rat	Weight of animals(g)	Dosage (mg/kg)	Vol. of extract administered (g/mL)	Mortality
Leaf	A1	1	185	1000	0.2	No mortality
	2	1	190	2000	0.4	"
	3	1	192	3000	0.6	"
	4	1	195	4000	0.8	"
	5	1	202	5000	1.0	"
	6	1	230	00	0.1mL	"
Stem-bark	B 1	1	192	1000	0.2	No mortality
	2	1	195	2000	0.4	"
	3	1	200	3000	0.6	"
	4	1	215	4000	0.9	"
	5	1	220	5000	1.1	"
	6	1	230	00	0.1mL	"

Key: g = Gram; Mg = Milligram; Kg = Kilogram; mL= Mililitre

Table 3: The percentage susceptibility pattern of *E. coli* strains to plant fractions

Plant fractions	EAEC n=16	EHEC n=13	EIEC n=8	EPEC n=27	ETEC n=17
ALF	13(81.25)	12(92)	06(75)	23(85)	15(88)
ASF	08(50)	05(38.5)	03(38)	16(59)	09(53)
HLF	11(69)	10(77)	05(63)	26(96)	15(88)
MLF	15(94)	13(100)	04(50)	27(100)	17(100)
MSF	12(75)	13(100)	05(63)	25(93)	14(82)
QLF	12(75)	12(92)	04(50)	24(89)	14(82)
QSF	14(88)	09(69)	03(38)	22(81.5)	15(88)
CPX	15(94)	13(100)	07(88)	27(100)	17(100)

Key: EAEC=Enteroaggregative *E. coli*; EHEC=Enterohemorrhagic *E. coli*. EIEC=Enteroinvasive *E. coli*; EPEC=Enteropathogenic *E. coli*; ETEC=Enterotoxigenic *E. coli*; ALF=Acetone leaf fraction; ASF=Acetone stem-bark fraction; HLF=Hexane leaf isolate; MLF=Methanolic leaf fraction; MSF=Methanolic stem-bark fraction; QLF=Aqueous leaf fraction; QSF=Aqueous stem-bark fraction; CPX=Ciprofloxacin.

The percentage susceptibility of Enteroaggregative *E. coli* to Methanol leaf fraction was 95%. This clearly indicates the efficacy of the fraction against the bacteria strain. Ugwuoke *et al.* (2017) reported 100% of susceptibility of *E. coli* isolates from diarrhoeal stool to butanol leaf fraction, which support the result of this study. This finding is similar to the report of Abdulrahman *et al.* (2010) where *E. coli* strains isolated from diarrhoea were susceptible to methanol leaf fraction by 94%, similar to what was reported by Adisa *et al.* (2015). However, another plant fraction that was found to be active against the Enteroaggregative *E. coli* in this study was aqueous stem-bark fraction, which showed 88% potency towards EAEC. Ugwikwe *et al.* (2015) earlier reported 100% potency towards the *E. coli* strain.

The percentage susceptibility of EHEC to methanol leaf fraction and methanol stem-bark fraction were 100% respectively. Muhammad *et al.* (2005) reported 100% susceptibility of *E. coli* isolates from diarrhoeal stool to methanol leaf fraction which is in support of this study. This finding is similar to the report of Sani *et al.* (2015) where *E. coli* strains were susceptible to methanol leaf fraction by 92%, which is also similar to what was reported by Musa *et al.* (2016).

Several research findings have also reported the susceptibility of EIEC to *Vitex doniana* fraction. In support of the finding in this study, Ogukwe *et al.* (2012) and Abdulrahman *et al.*

(2012) reported 75% susceptibility of EIEC isolates from diarrhoeal stool to acetone leaf fraction, methanol stem-bark fraction and hexane leaf fraction respectively. Contrary to the report of this study is the report of Kure *et al.* (2014) that EIEC from diarrhoeal stool were 38% susceptible to methanol stem-bark fraction and methanol leaf fraction respectively. EIEC strain isolated from diarrhoea in this study showed moderate susceptibility of 50% to methanol leaf fraction and aqueous leaf fraction. To support this finding, Sani *et al.* (2012) reported 39% and 40% of EIEC to methanol leaf fraction and aqueous leaf fraction respectively.

The EPEC were found to be the most dominant *E. coli* strain isolated from diarrhoeal stool in the study area. The susceptibility of EPEC isolates from diarrhoeal stool to methanol leaf fraction and methanol stem-bark fraction in this study is supported by the reports of Muhammad *et al.* (2015) that EPEC from diarrhoeal stool with 94 and 98% susceptibility to methanol leaf and stem-bark fraction respectively. Another fractions of high activity against EPEC isolates in the present study are methanol stem-bark fraction and hexane leaf fraction with 93 and 96% respectively. To support this assertion, Abdullahi *et al.* (2012) also reported that methanol stem-bark fraction and hexane leaf fraction have high activity of 83 and 80% respectively against EPEC isolates. In support of this, Abdulkadir *et al.* (2013) reported that methanol leaf fraction and hexane fraction had

activity of 82 and 83% respectively against the isolates.

As reported by the findings in this study, aqueous leaf fraction and acetone leaf fraction also proved to be effective against EPEC isolates of diarrhoeal stool with 89 and 85%. However, this finding is corroborated by Hassan *et al.* (2011) documented that 84% of EPEC isolates were susceptible to aqueous leaf fraction and acetone leaf fraction respectively. The susceptibility of EPEC isolates in this study to both aqueous stem-bark fraction and acetone stem-bark fraction has been reported. To support this finding, Hassan *et al.* (2011) and Yushau *et al.* (2013) reported the susceptibility of EPEC to aqueous stem-bark fraction and acetone stem-bark fraction by as much as 90%.

Enterotoxigenic *E. coli* (ETEC) isolates were also found to be present in children diarrhoeal stool sampled across the hospitals in the study area. ETEC was reported on the basis of their susceptibility to various fractions. All ETEC isolates were susceptible to plant isolates. This finding is in accordance with the report of Abdulkadir *et al.* (2016). The level of ETEC susceptibility pattern is in order; methanol leaf fraction, acetone leaf fraction, hexane leaf fraction, aqueous stem-bark fraction, methanol stem-bark fraction and aqueous leaf fraction.

Methanol leaf fraction has high activity against ETEC as reported in the present study, with a potency of 100%. A research study conducted on the isolation of ETEC strains from diarrhoeal stool showcasing the susceptibility of these strains to various plant isolates revealed that the strains were all above 80% susceptibility to fractions (Umaru *et al.*, 2014), to corroborate the findings in this study. The outcome of the research study conducted by Mahmood *et al.* (2015) on the antimicrobial activity of *Vitex doniana* extracts against ETEC strains corroborated the findings of this study, since ETEC isolates showed 100% susceptibility to methanol leaf fraction.

In a similar report, ETEC strain isolated from diarrhoeal stool of patient were found to be 100% susceptible to methanol leaf fraction (Yushau *et al.*, 2013). Acetone leaf fraction, hexane leaf fraction and aqueous stem-bark fraction were found to be active against ETEC isolates in the present study. The percentage susceptibility of ETEC to acetone leaf fraction,

hexane leaf fraction and aqueous stem-bark fraction respectively were 80%. Similarly, a research on the evaluation of *Vitex doniana* extracts on *E. coli* strains revealed that ETEC isolates showed 75% to acetone leaf fraction, hexane leaf fraction and aqueous stem-bark fraction (Hassan *et al.*, 2011), which agrees with finding in this study. The efficacy of methanol stem-bark fraction and aqueous leaf fraction against ETEC were found to be 82% respectively. To support the finding in this study (Hassan *et al.*, 2011) reported that methanol stem-bark fraction and aqueous leaf fraction to have 78% and 81% efficacy against ETEC, in other word the isolates were confirmed to be susceptible to plant fractions.

To justify the findings in this study, Ugwukwe *et al.* (2017) did report that fraction and pure isolates were more active than crude extracts on *E. coli* strains. The percentage of *E. coli* strains susceptible to crude extract was low compared to partially purified plant isolates. Also the report of Abdullahi *et al.* (2017) on *E. coli* strain also corroborated the outcome of the finding in this study. However still, the outcome of the activity of the fractions compared to control (ciprofloxacin) showed no difference especially the methanol leaf fraction.

The result of acute toxicity test (LD₅₀) of the *Vitex doniana* extracts on the Albino rats in the present study indicated that all albino rats test survived at 5000mg/kg body weight. No mortality was recorded and one of the toxicological indices accepted for the determination of the safety of drug/substances/extract as lethal dose 50% (LD₅₀) which is the amount of acute dose required to kill half of the test population (Dawang, 2015). However, since there was no death recorded during acute toxicity experiment by oral administration, then it is an evident that the median lethal dose (LD₅₀) is greater than 5000mg/kg body weight (Assao *et al.*, 2011).

This mean that *Vitex doniana* extracts is assumed to be safe (non-toxic) as suggested by Lorke (1983). The result in this study agrees with the finding of Dawang, (2015) who reported that all albino tests against *Vitex doniana* ethanolic leaf extract survived at 5000mg/kg. This is in conformity with findings of Abdulrahman *et al.* (2016),

reported that *Vitex doniana* methanolic leaf extract had (LD₅₀) of greater 5000mg/kg body weight. The result also agrees with the findings of Abdulrahman *et al.*, (2007) and Onyeyili *et al.* (1998).

Conclusion

The phytochemical constituents detected in the plants contributed immensely to antibacterial activity of the plants fractions, while methanol leaf fraction of *Vitex doniana* had highest efficacy against all *E. coli* strains except Enteroinvasive *E. coli* with 50%. The acute toxicity test (LD₅₀) of the plant extracts on albino rats was greater than 5000mg/kg, meaning that the plant is safe for consumption.

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