



Anti-diarrhoeal Effects of Aqueous Extract of *Vitex doniana* Stem Bark in Castor Oil-induced Wistar Rats

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Received 1 July 2020, Revised 19 Sep 2020, Accepted 20 Sep 2020, Published Oct 2020

Abstract

Vitex doniana is a medicinal plant commonly used in traditional medicine for the treatment of diarrhoea-related disorders. In the present study, aqueous extract of *Vitex doniana* stem bark was evaluated for anti-diarrhoeal effects. Thirty albino rats of average weight 124 ± 4.35 g were distributed into five groups. Groups 1, 2, 3, 4 and 5 were orally administered 2 mL of normal saline, 2.5 mg/kg bodyweight (bw) loperamide, 100, 200, 400 mg/kg bodyweight of aqueous extract of *V. doniana* stem bark, respectively. The extract was evaluated using fecal characteristics, gastrointestinal motility and enteropooling test. Results for phytochemical screening revealed the presence of tannins, cardiac glycosides, phenols, steroids and saponins. Aqueous extract of *Vitex doniana* stem bark significantly ($p < 0.05$) reduced total stool frequency (TSF). 100 mg/kg bw of the extract had the least TSF (4.80 ± 1.64) when compared with the reference drug (5.75 ± 2.98). 100 mg/kg bw and 400 mg/kg bw showed higher percentage inhibition of defecation than the standard drug. The extract also exhibited inhibition of wet stooling and intestinal secretion. It possesses anti-diarrhoeal effects and thus could be a potential source of new drug for the treatment of diarrhoea.

Keywords: Diarrhoea, enteropooling gastrointestinal motility, phytochemicals and *Vitex doniana*

Introduction

Diarrhoea remains an important health challenge in developed and developing countries, despite the economic wealth and advancement in public health (Naher et al. 2019). Diarrhoea 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 (Ezeja et al.

2012, Sarin and Bafna 2012, Usman 2020). *Escherichia coli*, *Staphylococcus aureus*, *Shigella flexneri*, and *Salmonella typhi* are the foremost causative agents of diarrhoea in humans (Awe et al. 2011). Diarrhoea could either be acute or chronic depending on longevity of the epidemic. Acute diarrhoea is associated with irregular and repeated release of semisolid faeces or fluid from the bowel lasting for less than fourteen days, while

chronic diarrhoea lasts for more than one month (Mohanta et al. 2010). Acute watery diarrhoea is characterized by significant loss of body fluid and rapid dehydration in an infected person for many hours or days. Acute diarrhoea may be associated with visible blood in the stools resulting from intestinal damage and nutrient loss in an infected person (Mohanta et al. 2010).

Annually, about two and half billion cases of diarrhoea are reported among children of five years and below. This has remained stable for the past two decades and more than half of the cases are in Africa and south Asia (WHO 2010). In developing nations, up to 17% of children infected with diarrhoea die of the disease (Misra et al. 2014). In Nigerian hospitals, the death of children below the age of five is mostly caused by diarrhoea (Azubuike and Nkanginieme 2007).

Nature has bestowed human with many medicinal plants for traditional health care. More than 70% of the habitants of developing countries seek health care from traditional healers using medicinal plants (Ramírez 2005). Herbs are known to contain bioactive metabolites including tannins, phenols, alkaloids, glycosides, volatile essential oils, resins, steroids, which are responsible for their medicinal properties (Garba et al. 2015). Medicinal plants with anti-diarrhoeal properties have been widely used by the traditional medical practitioners (Kola-Mustapha et al. 2019). However, the effectiveness of many of these plants, one of which is stem bark of *V. doniana* have not been scientifically evaluated and proven.

Vitex doniana (Verbenaceae), also known as black-plum, is a perennial shrub widely distributed in tropical West Africa and some East African countries. It is a deciduous evergreen tree, usually 4-8 m high, occasionally up to 15 m with a dense rounded crown. Its bark is light grey with numerous vertical fissures. Branches are not hairy and leaves are long stalked with 5-7 leaflets. Flowers are numerous, white tinged purple, usually borne in short, stout axillary cymes

on a long stalk. Calyx and pedicels are densely hairy. The fruit, which ripens between May and August, is a drupe consisting of a thin exo-carp, the edible mesocarp (pulp) and a thick woody endocarp (Irvine 1961, Etta 1984, Bouquet and Debray 1971, Iwu 1993) normally used for jams and beverages (Egbekun et al. 1996). The savanna species can be found in northern, eastern and western Nigeria where it is commonly known as dinya, galbihi, orinla and uchakoro by the Hausa, Fulani, Yoruba and Ibo speaking of Nigeria (Dluya et al. 2017).

Traditionally, *V. doniana* is used in the treatments of liver disease, stomach ache, hypertension, pains, skin disorders against mycoses, eczema and parasitic diseases (Etuk et al. 2010, Raphael 2011, Ouattara et al. 2013). Ethnopharmacological reports have shown that combinations of *V. doniana* with other plants are useful in the treatment of diarrhoea, dysentery and leprosy (Lagnika et al. 2012). The aerial parts of the plant have been found useful as diuretic, aphrodisiac and bactericide (Agbafor and Nwachukwu 2011), while the leaves are used as antiseptic and antidiabetic (Muanda et al. 2011).

Effective treatment of diarrhoea has been achieved by the use of anti-diarrhoeal drugs such as adsorbents (kaolin, cholestyramine and activated charcoal), antimotility drugs (codeine, camphorated tincture of opium, tincture of opium, loperamide hydrochloride, paregor diphenoxylate with atropine,), racecadotril, bismuth subsalicylate, azithromycin, quinolones, tinidazole etc (Naher et al. 2019). 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 (Sarin et al. 2013). Hence, the need to explore other safe alternative measures in the prevention, management and treatment of diarrhoea. Therefore, this study was carried out to

determine the phytochemical composition of aqueous extract of *Vitex doniana* stem bark and to evaluate its anti-diarrhoeal effects in castor oil-induced diarrhoea wistar rats.

Materials and Methods

Collection of plant samples

Fresh samples of *Vitex doniana* stem barks were collected in June, 2019 from Lapai township in Lapai Local Government Area of Niger State, Nigeria. The plant was identified and authenticated by a Botanist, Dr. Yunusa Audu of the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai.

Sample preparation and extraction

The stem bark samples of *Vitex doniana* collected were washed with tap water cut into pieces, air dried at ambient temperature and finally ground using electric blender, model: CB-8231-L. A 50 g of the powdered stem bark of *Vitex doniana* was extracted with 200 mL of distilled water using cold extraction for 72 hrs. The resulting extract was filtered using muslin cloth followed by Whiteman's filter paper (No 1). The filtrate was evaporated in a rotary evaporator at 40 °C. The paste was poured into a beaker and the resulting extract was freeze dried and stored in an air-tight container for further use. The extract was reconstituted in distilled water to give the required doses of 100, 200 and 400 mg/kg bw used in this study.

Reagents and chemicals

The chemicals used in this study were of analytical grade purchased from Sigma-Aldrich Company (St. Louise, MO, United State).

Phytochemical analysis: The qualitative analysis of the plant constituents was assessed by the methods described by Sofowora (1993), Trease and Evans (1996), El-Olemmy et al. (1994) and Harbone and Baxter (1993). The tests were carried out to determine the presence of active chemical

constituents including; alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides and phenols.

Experimental animals

Thirty healthy adult albino wistar rats of average weight 124 ± 4.35 g were obtained from the Animal Holding Unit of Department of Biochemistry, School of Life Sciences, Federal University of Technology Minna, Niger State. The animals were housed in clean metabolic cages (temperature range between 27 and 32 °C and relative humidity of between 47 and 60%). The rats were acclimatized for two weeks under conducive environmental conditions before the experiment and were allowed free access to standard animal feeds and distilled water during the period. Procedures of the Organization for Economic Co-operation and Development (OECD 1998) guidelines on good laboratory practices were followed.

Ethical approval

Approval for the use of animals was obtained from the Ethical Review Committee of the Department of Biochemistry Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria.

Animal grouping

A total of thirty rats were grouped into five with each group containing six rats. The animals were orally administered as follows:

Group1: Healthy rats administered with normal saline (control);

Group2: Healthy rats administered with 2.5 mg/kg bw reference drug (loperamide);

Group 3: Healthy rats administered with 100 mg/kg bw of the extract;

Group 4: Healthy rats administered with 200 mg/kg of the extract;

Group 5: Healthy rats administered with 400 mg/kg of the extract.

Diarrhoea induction

Diarrhoea was induced into the animals by single oral administration of castor oil according to the method of Capasso et al. (2008). After one hour of oral administration of the extract, 50 mL/kg bw of castor oil was orally administered to the animals. The animals in each group were placed in cages with adsorbent paper on their floors. The episodes of the diarrhoea were observed for 6 hours and the cumulative frequency of wet and formed stools was recorded at the end of the 6th hr. Percentage inhibition of diarrhoea was calculated using the mean stool frequency (MSF) and anti-diarrhoea activity determined in terms of percentage protection (Tijani et al. 2009).

Castor oil-induced enteropooling

The procedure reported by Robert et al. (1976) was used to evaluate the intestinal fluid accumulation of the extract. Another thirty healthy adult wistar rats were used for this experiment and the animal groupings and treatments were the same as in the anti-diarrhoeal experiment. The animals were euthanized two hours after the castor oil administration. The small intestines were removed, the intestinal contents were extracted, and the volumes were measured and recorded using measuring cylinder. The percentage inhibition of enteropooling was computed by calculating the mean volume of intestinal contents and comparing it with values from the control group (Tijani et al. 2009).

Intestinal transit test

The protocol described by Salawu et al. (2007) was used to evaluate the effects of the extract on the gastrointestinal motility. Another thirty healthy adult wistar rats were used for this experiment and the animal groupings and treatments were the same as in the anti-diarrhoeal experiment. A 0.5 mL of marker-charcoal meal (deactivated charcoal suspension in 5% tragacanth) was administered orally 30 minutes after the

extract administration. The animals were sacrificed after 30 min of charcoal meal. The distance travelled by the charcoal was measured and presented as a percentage of the total length of the small intestine (pylorus to caecum).

Statistical analysis: Data obtained were subjected to statistical analysis using SPSS version 21. The results were expressed as the mean \pm SEM (standard error of the mean) of replicate analyses. Results were subjected to analysis of variance (ANOVA) followed by Duncan multiple range test for multiple comparisons. Data were considered significantly different at $p < 0.05$.

Results

Phytochemical composition

The qualitative phytochemical composition of aqueous extract of *Vitex doniana* stem bark is shown in Table 1. The aqueous extract of the plant was found to contain phenols, saponins, steroids, tannins and cardiac glycosides, while alkaloids and flavonoids were not detected.

Table 1: Qualitative phytochemical composition of aqueous extract of *Vitex doniana* stem bark

Phytochemical	Inference
Alkaloids	-
Flavonoids	-
Phenols	+
Saponins	+
Tannins	+
Cardiac glycoside	+
Steroids	+

Key: + = present, - = absent

Total Stool Frequency (TSF)

The effects of aqueous extract of *Vitex doniana* stem bark on faecal characteristics of castor oil induced diarrhoea in albino rat are shown in Table 2. The total stool frequency (TSF) in the diarrhoea untreated rats (normal saline group) were significantly ($p < 0.05$)

higher (8.00 ± 1.73) when compared with loperamide treated group (5.75 ± 2.98). The TSF in groups of rats administered with aqueous extract of *Vitex doniana* stem bark were significantly ($p < 0.05$) reduced when compared with the control. However, rats treated with 100 mg/kg bw of the extract had the least stool frequency (4.80 ± 1.64) showing better anti-diarrhoeal potential than the standard drug (5.75 ± 2.98).

Similarly, the purging index and inhibition of defecation were higher in groups of rats orally administered with different concentrations of aqueous extract of *Vitex doniana* stem bark when compared with the untreated group (control). Group of rats administered with 100 mg/kg bw and 400 mg/kg bw showed significantly ($p < 0.05$) higher percentage inhibition of defecation than the standard drug.

Table 2: Effects of aqueous extract of *Vitex doniana* stem bark on castor oil induced diarrhoea in albino rats

TM (Treatments) mg/kg bw	AW (g)	R (%)	LP (min)	ALP (min)	TSF	PI	ID (%)
Normal saline							
5.0 mL/1.0 kg bw	129.7	50	9–339	278.7	8.00 ± 1.73^c	1.44	0.00
Loperamide							
2.5	122.0	67	11–348	370.0	5.75 ± 2.98^{ab}	1.04	28.13
<i>V. doniana</i>							
100 mg/kg bw	127.8	83	1–353	139.4	4.80 ± 1.64^a	2.90	40.00
200 mg/kg bw	122.0	100	2–345	227.0	6.16 ± 1.94^b	2.71	23.00
400 mg/kg bw	124.9	100	2–227	167.0	5.33 ± 2.16^{ab}	3.19	33.38

Data are means \pm SEM of six determinations. Values with different superscripts were significantly different down the column. TM = Test material, AW = Average weight, R = Respondents, LP = Latent period, ALP = Average latent period, TSF = Total stool frequency, PI = Purging indices, ID = Inhibition of defecation.

Wet stool

The effects of aqueous extract of *Vitex doniana* stem bark on castor oil-induced wet stool in rats are shown in Table 3. There was a significant ($p < 0.05$) reduction in the excretion of wet stool in rats administered with the extract as compared with the control

group and the level of inhibition seemed to be dose dependent. The level of inhibition of wet stool observed in group of rats administered with the extract was significantly ($p < 0.05$) higher than inhibition observed in the group of rats administered with loperamide (standard drug).

Table 3: Effects of aqueous extract of *Vitex doniana* stem bark on castor oil induced wet stool in albino rats

TM (Treatments)	FWS	WC (g)	IWS
5.0 mL/kg bw normal saline	7.00 ± 1.41^a	2.00 ± 1.15^c	0.00
2.5 mg/kg bw loperamide	7.00 ± 7.00^a	0.90 ± 1.06^a	8.74
100 mg/kg bw <i>V. doniana</i>	13.01 ± 1.31^b	1.66 ± 0.91^b	50.45
200 mg/kg bw <i>V. doniana</i>	13.50 ± 10.60^b	1.86 ± 1.81^b	45.24
400 mg/kg bw <i>V. doniana</i>	11.00 ± 7.07^b	0.91 ± 0.47^a	58.27

Data are means \pm SEM of six determinations. Values with different superscripts were significantly different down the column. TM = Test material, FWS = Frequency of wet stool, WC = Water content and IWS = Inhibition of wet stool.

Intestinal transit test

Effects of aqueous extract of *Vitex doniana* stem bark on intestinal transit in rats are shown in Table 4. Administration of aqueous extract of *Vitex doniana* stem bark before diarrhoea induction significantly ($p < 0.05$) reduced the intestinal transit time in a dose independent manner. The average intestinal transit in rats administered with standard drug was not significantly ($p > 0.05$) different from the untreated group of rats (control). The groups of the rats administered with the extract exhibited significantly ($p < 0.05$) lowered intestinal transit time and produced higher percentage inhibition. However, 200 mg/kg bw of the extract seemed to be the most potent.

Table 4: Effects of aqueous stem bark extract of *Vitex doniana* on intestinal transit in rats

TM (Treatments)	ABW (g)	IT (cm)	Inhibition (%)
0.5 mL/10 g bw normal saline	143.0 ± 38.53 ^b	44.33	
2.5 mg/kg bw loperamide	135.4 ± 30.27 ^a	43.07	2.84
100 mg/kg bw <i>V. doniana</i>	145.8 ± 43.73 ^b	18.90	57.36
200 mg/kg bw <i>V. doniana</i>	133.1 ± 26.95 ^a	12.45	71.99
400 mg/kg bw <i>V. doniana</i>	150.9 ± 0.78 ^c	22.77	48.63

Data are means ± SEM of six determinations. Values with different superscripts were significantly different down the column. TM = Test material, ABW = Average body weight, and IT = Intestinal transit enteropooling.

Effects of aqueous extract of stem bark of *Vitex doniana* on castor oil-induced enteropooling in rats are shown in Table 5. The extract of aqueous stem bark of *Vitex doniana* exhibited significant ($p < 0.05$) difference and dose dependent inhibition of intestinal secretions. Rats administered with 200 and 400 mg/kg bw of the extract exhibited higher inhibition of intestinal

secretion than the standard drug (loperamide).

Table 5: Effects of aqueous stem bark extract of *Vitex doniana* on castor oil-induced enteropooling in rats

TM (Treatments)	VIF (cm ³)	I (%)
0.5 mL/10 g bw normal saline	0.33 ± 0.17 ^a	0.00
2.5 mg/kg bw loperamide	1.10 ± 0.85 ^c	72.73
100 mg/kg bw <i>V. doniana</i>	0.56 ± 0.05 ^b	46.43
200 mg/kg bw <i>V. doniana</i>	1.35 ± 0.57 ^{cd}	77.44
400 mg/kg bw <i>V. doniana</i>	1.66 ± 0.30 ^d	81.93

Data are means ± SEM of six determinations. Values with different superscripts were significantly different down the column. TM = Test material, VIF = Volume of intestinal fluid, and I = Inhibition.

Discussion

At present, the most common form of alternative medicine on which about 80% of the world's population rely is the use of medicinal plants for health care needs (Latha et al. 2016). This is due to the presence of phytochemicals that are of health importance in such plants. In the present study, aqueous stem bark extract of *V. doniana* was found to contain phenols, saponins, steroids, tannins and cardiac glycosides, while alkaloids and flavonoids were not detected. The presence of these bioactive compounds which have been found useful as therapeutic agents may be responsible for the traditional uses of aqueous extract of *Vitex doniana* stem bark by the locals. This is in tandem with the findings of Dauda et al. (2011) who reported the presence of alkaloids, flavonoids, cardiac glycosides and steroids in aqueous extract of stem bark of *V. doniana*. Dawang (2015) also reported the presence of flavonoids, alkaloids, cardiac glycosides, steroids and terpenes but absence of saponins, tannins, phenol and balsam in the leaf extract of *V.*

doniana. The variations in the phytoconstituents of the stem bark of *V. doniana* in this study in comparison with previous studies could be attributable to the plant parts used, solvent of extraction and geographical location where the plants were harvested. The findings agree with earlier findings which reported that not all plants contain all phytochemicals and that those present vary according to the parts of the plant used, location and extraction solvents (Lawal et al. 2014).

Diarrhoea is a frequent passage of liquid stools. Castor oil has been widely used to induce diarrhoea in animal models (Rang et al. 2003). In the present study, castor oil at the dose of 0.5 mL /10 g bw induced diarrhoea in all the animal groups as the control untreated rats excreted significantly ($p < 0.05$) higher total stool frequency of 8.00 ± 1.73 than other experimental groups that were treated with the aqueous extract of *V. doniana*. The ability of the castor oil to cause diarrhoea in animals as observed in this study supported the claim that chemical agents could also cause diarrhoea apart from microorganism. This finding is in agreement with the reported mechanism employed by castor oil in inducing diarrhoea which increases intestinal volume through the prevention of water reabsorption from intraluminal space and thus stimulates secretion by mucosal glands (Pierce et al. 1971, Gaginella et al. 1975). Administration of aqueous extract of *Vitex doniana* stem bark at 100, 200 and 400 mg/kg bw significantly reduced the total stool frequency when compared with the untreated control, thus indicating the anti-diarrhoeal potential of the plant extract. The anti-diarrhoeal activity demonstrated by *V. doniana* in this study could be attributed to the presence of phenols, saponins, tannins and cardiac glycosides. This assertion is in line with the earlier findings which reported that tannins and cardiac glycosides have anti-diarrhoeal, antifungal, anti-inflammatory and cytotoxic activities (Nkomo 2010). This study showed

a reduction in the intestinal transit time of rats administered with aqueous extract of *Vitex doniana* stem bark at a dose independent manner. However, our finding is in contrast with the work of Suleiman and Yusuf (2008) which reported that aqueous extract of *Vitex doniana* leaves inhibit gastric peristalsis and significantly protected mice against castor oil-induced diarrhoea in a dose-dependent manner. Loperamide is a well-known anti-diarrhoeal agent that increases colonic phasic segmenting effect via the inhibition of presynaptic cholinergic nerves in the myenteric and submucosal plexuses. These effects result in increased colonic transit time and faecal water absorption thus decreasing the defecation frequency (Camilleri 2004). This study also revealed that administration of rats with 100 mg/kg bw of the extract of *Vitex doniana* stem bark exhibited a considerable anti-diarrhoea activity as shown by the least stool frequency (4.80 ± 1.64), indicating a better performance than the standard drug (5.75 ± 2.98). Similarly, rats administered with 100 mg/kg bw and 400 mg/kg bw showed higher purging index and percentage inhibition of defecation than the standard drug. This is an indication that aqueous extract of *Vitex doniana* stem bark could be a potential source of new drug for prevention or management of diarrhoea.

Inhibition of gastrointestinal tract motility is one mechanism of action of anti-diarrhoeal drugs (Akah et al. 1999). The present study indicated that rats administered with extract of *Vitex doniana* stem bark significantly inhibited the intestinal transit time with high percentage inhibition, while administration of 2 mg/kg bw loperamide had no effect on intestinal transit in rats. This is an indication that aqueous extract of *Vitex doniana* stem bark extract could be a better anti-diarrhoea agent than a well-known standard drug (loperamide). This also agrees with the work of Agunu et al. (2005) who reported that the leaves extracts of *Vitex doniana* have anti-diarrhoea activity.

The possible mechanism of action for the anti-diarrhoeal effects of aqueous extract of *Vitex doniana* stem bark might include; inhibition of gastric peristalsis, inhibition of gastrointestinal motility, inhibition of gastrointestinal secretion and the inhibition of presynaptic cholinergic nerves in the myenteric and submucosal plexuses. It is recommended that safety evaluation of aqueous extract of *Vitex doniana* stem bark should be carried out and the possible active principle(s) in the extract responsible for its anti-diarrhoeal effects should be identified and isolated.

Conclusion

In conclusion, the study indicated that aqueous extract of *Vitex doniana* stem bark has inhibitory effects on castor oil-induced diarrhoea. The outcome of this study supports the traditional use of *Vitex doniana* as oral remedy for diarrhoea and thus could be a potential source of new drug for prevention or treatment of diarrhoea.

Conflict of Interest

Authors hereby declare that no conflicting interests exist in publishing this work

Acknowledgements

The contributions of Abubakar Hauwa, Muhammad Hassan Badeggi, Mustapha Mariya Abubakar and Muhammad Umar Emtsu are hereby acknowledged and appreciated.

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