PHYTOCHEMICAL AND INVITRO ANTIDIARRHEAL POTENTIALS OF ETHANOL LEAF EXTRACT OF *CLERODENDRUM VOLUBILE* P. BEAUV

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Abstract: Diarrhea is a common gastrointestinal disease that affects several people all over the world. Due to its escalating rate in developing countries, the need arises to discover more anti-diarrheal drugs of natural origin with little or no side effects. This research evaluated the phytochemical and anti-diarrheal properties of the ethanol extract of *Clerodendrum volubile*. *Clerodendrum volubile* was harvested within the area of Computer Village, Ago-Iwoye and Ijebu- Ode, Ogun State, Nigeria. Phytochemical screening was carried out using standard protocols while the antimicrobial assay was done using agar well diffusion method with different concentrations (2.5mg/ml, 12.5mg/ml, 50mg/ml, 100mg/ml) of *Clerodendrum volubile* extract administered on the test organisms (*Bacillus cereus*, *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*). The zone of inhibition was checked and data were subjected to statistical analysis. Values were expressed as mean ± standard deviation using one-way analysis of variance (ANOVA) with Post Hoc test. Phytochemical screening revealed the presence of flavonoids and tannins in appreciable amount (+++), alkaloids, saponins and steroids were moderate while terpenoids were in trace amount. The minimum inhibitory concentration of the antidiarrheal activity of *Clerodendrum volubile* revealed higher inhibition in *Bacillus cereus* (6.25mg/ml) compared to *Salmonella typhi*, *Escherichia coli*, and *Staphylococcus aureus*. While the minimum bactericidal concentration in all tested organisms were less than 100mg/ml, only *Bacillus cereus* was100mg/ml. These phytochemicals might have contributed to the considerable in-vitro antidiarrheal potential of *Clerodendrum volubile*. Hence, further study should be done to unravel its mechanism of action and management, to ensure better health and safety.

Keywords: Antidiarrheal drugs, *Clerodendrum volubile*, Diarrhea, phytochemicals

Introduction

Diarrhea is a common gastrointestinal ailment that affects millions of individuals worldwide, especially in underdeveloped nations (Fitzwater *et al*., 2019; Thapar & Sanderson, 2004). Constant bowel motions, loose, watery stools, and cramping in the abdomen are its hallmarks. Diarrhea can have serious effects on someone's well-being, including mortality. This is particularly true for young children and older people. Thus,to lessen the impact of this condition, proper control and therapy options are essential (Vos *et al*., 2015). The study of natural products as possible avenues for therapeutic substances towards many disorders, especially diarrhea, has drawn increasing attention in recent years (Mensah *et al*., 2019; Singh *et al*., 2024).

The native tropical herb, *Clerodendrum volubile* p. beauv., a member of the family Verbenaceae was considered in this study, it typically has leaves that are oblong and measure 0.6 to 6 cm width and 1.5 to 15.5 cm length. Its stem can be terete or spherical. Additionally, it is distinguished by its dense terminal inflorescence or subcorymbose with delicate pubescence (Erukainure *et al*., 2011a). *Clerodendrum volubile* is one of the underutilized vegetables in Nigeria, particularly in the south, with wide report of being useful in folkloric therapies to manage illnesses such as pain dysentery, arthritis, rheumatism, nodules, and pulmonary edema and anaemia, used as anti-abortifacients, anti-plasmodic and sleeping pills. It is also used as a dehydrating, expectorant, anthelminthic, anti-dysenteric, and analgesic (Senjobi *et al*., 2017), in addition, to encouraging menstruation, inducing labor, decreasing high blood pressureand high blood sugar, baby asthenia, appetite resuscitation, toothache relief with bark macerate, and pulmonary problems from decoction (Erukainure *et al*., 2011a, b; Odebiyi *et al*., 2019). It can be also be used to treat sores and wounds, as well as a cough suppressant. Other biological activities exhibited by *Clerodendrum volubile* are anti-fungal, antibacterial, antiviral, antidiabetic, hepatoprotective, antihypertensive, anti-inflammatory and antioxidant (Ajao *et al*., 2018; Ibrahim *et al*., 2020; Ali *et al*., 2019; Telagari and Hullatti, 2015; Adefegha and Oboh, 2013; Amole *et al*., 2018, Erukainure *et al*., 2019).

Due to the prevailing increase in diarrhea related issues in some parts of Nigeria, this study was carried out to screen *Clerodendrum volubile* for its metabolite composition and antidiarrheal activity using standard protocols.

Materials and Method

Plant Collection and Identification of Plants Material

Clerodendrum volubile was harvested between December 2022 and January 2023 within the area of Computer Village, Ago-Iwoye and Ijebu- Ode, Ogun State. The plant was authenticated at the Forest Research Institute of Nigeria (FRIN) with FHI number 108884

Plant preparation and extraction

Leaves of *Clerodendrum volubile* were air-dried for two weeks to prevent the decomposition of active ingredients, continuously rotate and finely ground using mortar and pestle. The powdered sample was measured and stored in an air-tight jar before extraction. Two hundred grams (200g) of finely ground *Clerodendrum volubile* leaves were extracted in 2.5L ethanol using rotary evaporator and a total of 42 grams of the crude extract was obtained.

Phytochemical screening of Clerodendrum volubile leaf extract

Phytochemical screening was conducted on the leaf extract of *Clerodendrum volubile* for the presence or absence of alkaloids,

terpenes, tannins, glycosides, flavonoids, phenols, saponins, steroids and carbohydrates using standard methods proposed by Ekaete *et al*. (2013).

Test for Alkaloids

Extract of *Clerodendrum volubile* placed in water bath for 5 minutes with 10ml of 10% hydrochloric acid (Hcl) and was allowed to cool after filtering. Ammonia (NH3) was used to alter the pH to around 6-7. In separate test tubes, 0.5mL of each filtrate was placed, and 2-3 drops of Dragendoff, Mayer, and Wagner's reagent were added and mixed together. A turbid appearance indicated the presence of alkaloids.

Test for anthraquinone glycoside (The Borntrager's test)

Extract of *Clerodendrum volubile* was placed in a dry test tube and 5ml of chloroform was added and shaken for 5mins. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. The presence of a rose-pink color in the aqueous layer indicated the presence of anthraquinone.

Cardiac glycosides

Keller-kelani's test: 2 ml of glacial acetic acid and water was added to the extract of *Clerodendrum volubile* including a drop of the ferric chloride solution, Concentrated tetraoxosulphate (vi) acid (H_2SO_4) (1 ml) was used to generate an underlayer. A dark, purple, or reddish-brown circle which developed at the junction and the green-blue colouration of the acetic acid layers were indicators of the presence of cardiac glycoside.

Test for Flavonoids

i. The extract of *Clerodendrum volubile* was examined with 10ml of ethanol and 3 drops of ferric chloride ($Fecl_3$)solution. A dark green colouration indicated the presence of flavonoid.

ii. Ethyl acetate was used to examine the plant extract for three minutes over heat. 10% ammonia was used to clean up the leftovers (NH_3). A yellow colouration indicated the presence of flavonoids.

Test for Tannins (The Braemer's test)

extract of *Clerodendrum volubile* was heated for ten minutes, filtered despite being warm, and then evaluated with 10ml of purified water. The filtrate received 0.1% ferric chloride solution addition. A blue-black, green, or blue-green precipitate indicated the presence of tannins.

Test for saponin

i. Frothing

Leaf extract of *Clerodendrum volubile* was transferred into a test tube with 10ml of distilled water, boiled for 5 minutes and filtered. The filtrate was briskly shaken, the appearance of froth indicated the presence of saponins.

ii. Three drops of olive oil were added to the froth and stirred; the appearance of emulsions indicated the presence of saponins

Test for Phenols

Extract of *Clerodendrum volubile* was mixed with 10ml of ethanol and 3 drops of phenol solution was added. A dark green tint indicated the presence of phenols.

Test for Steriods

Acetic anhydride (2ml) was added to 0.5g of the extract of *Clerodendrum volubile* with 2ml H_2SO_4. A color shift from violet to blue orgreen indicated the presence of steroids.

Test for Terpenoids (Salkowski test)

Extract of *Clerodendrum volubile* was examined with 5ml of ethanol which was mixed with 2ml of chloroform and 3ml Concentrated H_2SO_4. The inter-phase created had a reddish-brown coloring, which showed the presence of terpenoid Test for Phyloba-tannins

Extract of *Clerodendrum volubile* was transferred into a test tube with 10ml of distilled water, boiled for 5 minutes and filtered. Five milliliters (5ml) of 1% HCL was added to the filtrate and heated for 5 minutes. The appearance of yellow precipitate showed the presence of phylobatannins.

Antimicrobial activities of Clerodendrum volubile leaf extract against selected pathogen

Eighteen (18) plates of prepared Mueller Hinton agar having 24hours growth of the test organisms (*Bacillus cereus, Salmonella typhi, Escherichia coli*, and *Staphylococcus aureus*) was utilized while different concentrations (2.5mg/ml, 12.5mg/ml, 50mg/ml, 100mg/ml) of *Clerodendrum volubile* ethanol extract were administered after 48 hours. The test organisms were collected after 24 hours, dissolved in saline water, and contrasted to 0.5 MacFarland benchmark prior being streaked over the outermost layer of the Mueller Hinton agar plates that had been previously madewith a swab-stick immersed in the solution. With the help of a cork borer, holes were drilled into the prepared plates. Different concentrations of plant extracts were subsequently inserted into the holes, allowed to spread for 15 minutes and incubated at 37^0C for 24 hours. The plates were examined for regions of inhibitions, using meter rule, the antibacterial efficacy of various concentrations of plant extract were tested and results documented.

Statistical Analysis

Data were analyzed for descriptive statistics using one-way analysis of variance (ANOVA) with Post Hoc test also conducted.

Results

Phytochemical screening of the ethanol extract of the leaves of *Clerodendrum volubile* as shown in Table 1 revealed the abundant presence of flavonoids and tannins; saponins, alkaloids, and steroids were moderately present, terpenoids were present in trace while cardiac glycosides, anthraquinone glycosides, phylobatanins and phenols were absent.

Table 1: Phytochemical screening of the leaves of *Clerodendrum volubile*

Phytochemicals	Results
Alkaloids	
Wagner	++
Dragendorff	++
Anthraquinone glycosides	–
Cardiac glycosides	–
Saponins	
Frothing	++
Emulsification	++
Phenols	–
Flavonoids	
Water	+++
Ethanol	+++
Ethylacetate	+++
Tannins	+++
Phlobatannin	–
Terpenoids	+

Steroids	++

Absent (-), trace (+), moderately present (++), abundantly present (+++)

The antimicrobial properties of ethanol extract of *Clerodendrum volubile* at different concentration towards *Staphylococcus* aureus, *Salmonella* typhi, *Bacillus cereus*, and *Escherichia coli* is revealed in Table 2. *Bacillus cereus* has the highest inhibitory zone but low inhibitory concentration while Escherichia coli has the lowest zone but a better inhibitory concentration.

Table 2: Antimicrobial properties of ethanol extract of *Clerodendrum volubile*

S/N	Test Organisms	Concentration(mg/mL) / Zones of inhibition (mm)			
		12.5	25	50	100
1	Bacillus cereus	9.3 ± 0.58	11.3± 1.15	13.3±1.73	15.3±3.21
2	Staphylococcus aureus	16.7±5.03	12.6±0.58	13.6±2.31	14.3 ± 2.89
3	Escherichia coli	12 ± 1.73	14.7 ± 3.52	16.7 ± 3.52	23.3 ± 14.00
4	Salmonella typhi	10.6 ± 0.58	11.6 ± 0.58	14 ± 1.00	17.3 ± 2.52

n = 3, Mean ± Standard deviation, Size cork = 6mm, p= 0.05 ** significant borer

Table 3: Minimum Inhibitory concentration reading for standard antibiotics

S/N	Antibiotics	*Bacillus cereus*	*Staphylococcus Aureus*	*Salmonella Typhi*	*Escherichia coli*
1	CPX	20	20	16	21
2	AM	19	12	11	19
3	AU	-	-	20	15
4	GM	18	19	20	17
5	PEF	18	18	21	18
6	OFX	-	-	19	20
7	S	17	13	11	16
8	SXT	18	10	10	10
9	CH	-	-	10	13
10	SP	-	-	21	15
11	ERY	20	18	-	-
12	APX	18	16	-	-
13	RA	15	16	-	-
14	AMP	18	18	-	-
15	Z	10	14	-	-

Keys: Ciprofloxacin (CPX), Amoxicillin (AM), Augumentin (AU), Gentamycin (GM), Pefloxacin (PEF), Ofloxacin (OFX), Streptomycin (S), Trimethoprim/sulfamethazole (SXT), Cephalothin (CH), Spiramycin (SP), Erythromycin (ERY), Ampicillin/cloxacillin (APX), Rifampicin (RA), Ampicillin (AMP), Azithromycin (Z).

Table 4: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methanolic extract of *Clerodendrum volubile*

S/N	Bacterial strain	MIC (mg/mL)	MBC (mg/mL)
1	Bacillus cereus	6.25	100
2	Staphylococcus aureus	1.56	>100
3	Escherichia coli	0.75	>100
4	Salmonella typhi	1.56	>100

Keyword: minimum bactericidal concentration (MBC)

Discussion

Phytochemical analysis of *Clerodendrum volubile* (Table1) revealed the presence of terpenoids, alkaloids, flavonoids, tannins, steroids, and saponins. This correlates with the report of Senjobi *et al*. (2017), on investigation of the phytochemical composition of the leaf extracts (Aqueous, diethyl ether and methanol) of *Clerodendrum volubile*.

Clerodendrum volubile as shown in Tables 2, 3 and 4 exhibited potent antimicrobial activity at different concentrations. There is reduction in the frequency of defecation and severity of diarrhea when administered. Ajayi *et al.* (2015) noted the anti-diarrheal efficacy of the aqueous leaf extract of *Clerodendrum volubile* in-vivo while Izevbuwa *et al.* (2015) confirmed such anti-diarrheal activity using methanol extract in-vitro and in-vivo. Ethanol extract was observed to be more effective when compared with extracts from other solvents; *Clerodendrum volubile* ethanol extract gave the greatest zone of inhibition (6.25 mg/ml). This report according to Chang *et al.* (2018) showed that solvent extraction had an impact on the anti-bacterial activities exhibited by *Clerodendrum volubile* and that phytochemicals, most importantly alkaloids and flavonoids are believed to decrease intestinal mobility by improving fluid absorption, decreasing fluid and electrolyte output.

Conclusion

The presence of some phytochemicals may have contributed to the considerable in-vitro anti-diarrheal potential of the ethanol extract of the leaves of *Clerodendrum volubile*. Our observations are consistent with the historical usage of *Clerodendrum volubile* in diarrhea treatment. Further research would be necessary to isolate and characterize the major active ingredients responsible for the anti-diarrheal activity.

Conflict of interest

The author clearly declares that there is no conflict of interest with the co-authors.

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