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PHARMACOGNOSTIC PROFILE AND EVALUATION OF THE ANTIDIARRHEAL ACTIVITY OF *NELSONIA CANESCENS* PLANT (LAM.) ACATHECEAE IN MICE MODEL

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ABSTRACT

Background: This study was designed to evaluate the pharmacognostic standardization and antidiarrhea effect of *Nelsonia canescence* plant. However, its antidiarrhea activity of has not been scientifically evaluated, hence this study. Methods: Fresh leaves was collected, dried, powdered and subjected to macroscopic and microscopic analysis. The plant material was cold-macerated in ethanol, the extract was fractionated using liquid-liquid partitioning to obtain *n*-hexane, ethyl acetate and butanol fractions. Pharmacognostic profile, phytochemical analyses, and acute toxicity were determined using standard procedure. The antidiarrheal activity and gastrointestinal motility study of the ethanol extract and fractions were evaluated using standard procedures. **Results:** Microscopic examination revealed the presence of diacytic stomata, prisms of calcium oxalate crystal, unicellular trichomes. Phytochemical screening revealed the presence of flavonoids, saponins, proteins, tannins and carbohydrates. The leaves were found to be dark green with acute apex, reticulate veination, bitter taste and pungent smell. Proximate analysis *N. canescense* revealed moisture content of 7.3 %, ash value of 19.5 %. The LD_{50} value was found to be greater than 5000mg/kg. *N. canescens* was established to have a significant antidiarrhea effect. At the dose of 50, 100 and 250 mg/kg, the crude extract showed a significant antidiarrhea activity with percentage inhibition of 53.4, 76.4 and 73.2% respectively. The fractions showed a significant antidiarrhea activity with 100 and 250mg/kg ethylacetate showing extremely significant ($P<0.01$) antidiarrhea effect. **Conclusion:** This study demonstrated that the ethanol extract and fractions of *N. canescense* contain bioactive constituents that have antidiarrheal activity. Therefore, this study provides a scientific support for its acclaimed traditional use in the treatment of diarrheal.

KEYWORDS: *Nelsonia canescence*, antidiarrhea, pharmacognostic standardization.

BACKGROUND

Diarrhea is the frequent passage of watery unformed stools. Its causes are many and include irritable bowel syndrome, infectious disorder, thyrotocosis, malabsorption or maldigestion, and laxative abuse. Medications used to treat other disorders also may induce diarrhea. For example. Xanthenes e.g. theophlline preparations cause diarrhea secondary to alteration of mucosal cyclic adenosine monophasphate (cAMP). Antihypertensive drugs, such as reserpine and guanethidine, may induce diarrhea by changing gut neuronal input and reducing noradrenergic mediated relaxation Since the time immemorial, medicinal plants have played an invaluable role in the development of therapeutic agents.[1] Currently, it is estimated that about 80% of people living in developing countries still rely on traditional medicine for their primary health care.[2] There are many medicinal plants that possess antidiarrheal activity with lesser side effects than the conventional drugs. Tannins, alkaloids, flavonoids and terpenoids are the major constituents that are primarily responsible for antidiarrheal activity of these medicinal herbs.[3] A range of medicinal plants have been widely used for the management of diarrhea and related gastrointestinal disorders by traditional healers. However, the safety and therapeutic potentials of some of these medicinal plants have not been validated yet. Among them, *Nelsonia canescence* is one of the medicinal plants being used in the traditional medicine. In Nigeria, diarrheal infection remains the number one killer disease among children under 5 years, while 7 to

12 month old babies remains the most susceptible. In addition, reported cases of diarrhea in many areas, including Kaduna state, still account for more than 30% of admissions to children wards. Despite the effective and simple cheap treatment of oral dehydration therapy, majority of the local populace still rely on herbsto treat diarrhea.[4] The plant *Nelsonia canescense* which belongs to Acanthaceae family is used as an anthelmintic and antidiarrhea agent as well as for common application in folk medicine. *N. canescens* is used in African and Asian traditional medicine. It is found growing in secondary wet evergreen forests, savannah forests and open disturbed habitats, especially in moist areas along roadsides, trails, and as a weed in agricultural land.[5] *N. canescens* is also reported to be used as a cover crop to suppress the growth of weeds in banana plantations. Here this species can invade large areas of the plantation with no visible adverse effects on the banana crop but limiting the possibility of other weeds to invade,[6] *N. canescens* was also reported to have analgesic and anti-inflamatory propreties.[7] *N. canescens* have been used for a long time in diverse contexts, i.e. as an ornamental plant, antioxidant,[8] antibacterial, anti-inflammatory, analgesic and antispasmodic,[9] *Nelsonia canescens* is found growing in secondary wet evergreen forests, savannah forests and open disturbed habitats, especially in moist areas along roadsides, trails, and as a weed in agricultural land,[10] As part of our efforts to screen some ethnomedicinal plants in Nigeria for antidiarrheal activity, *Nelsonia canescens* plant was investigated as well as its pharmacognostic profile and acute toxicity.

METHODS

Drugs and chemicals

Drugs used in the study include; Castor Oil (Bell Sons & Co., England), Loperamide (Janssen, Germany), activated charcoal (Acuro Organics Ltd, New Delhi, India), other chemicals and reagents used for the study were of analytical grade and procured from approved organizations.

Experimental animals

Adult Swiss albino mice (20-30 g) was also used for the study and was obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University. The animals were fed with palletized feed (UAC feed, Nigeria) and had access to water *ad libitum.* Housing of the animals was done in standard cages in the Animal House of the Department of Pharmacology and Toxicology. They were allowed free access to food and water. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (Pub. No. 85 – 23 Revised 2011).

Collection and authentication of plant material

Plant collection and identification

Plant material: *Nelsonia canescences* plant were collected from Agulu in Anaocha local Government area Anambra state Nigeria in November 2019. It was authenticated by a trained taxonomist, Mr Felix Nwafor of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Voucher specimens (No. PCG 894/A/006) were deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University Awka for future reference. The plant materials were subsequently cleaned, the fruit pulp separated from the seeds while the roots were shredded then air-dried at room temperature for 3 weeks and pulverized with a mechanical grinding machine (GX160 Delmar 5.5HP).

Method of Extraction

Five hundred grams (500 g) of the pulverized *Nelsonia canescense* plant was macerated in two litres of ethanol over a period of 48 hours. The mixture was sieved using porcelain cloth. It was further filtered with no .l Whattman filter paper. The filtrate was concentrated using rotary evaporator. It was further dried in a water bath at a temperature of 40° C to obtain the ethanol extract. The extract was then stored in a refrigerator at 4°C until further use.

Fractionation (Liquid-liquid chromatography)

The methanol extract (100 g) was subjected to liquid-liquid containing water (200 mL in 100 g of extract) were subjected to liquid-liquid partition successively with 1000 ml n-hexane, 1000 ml ethyl acetate and 500 ml n-butanol in increasing order of polarity. The fractions were filtered with Whatman no 1 filter paper and concentrated *in vacuo* using rotary evaporator at 40 °C to obtain the n-hexane fraction (HF), ethylacetate fraction (EF) and butanol fraction (BF). The fractions were stored in refrigerator between 0-4°C until futher use.

Macroscopic examination of leaves

The fresh leaves of *Nelsonia canescences* were visually examined. The organoleptic properties such as colour, odour and taste of the plant material were observed and noted. The macroscopic characters of the leaves which include type of margin, venation, base, shape, size, apex, mid-rib, lamina, presence or absence of petiole were evaluated based on standard protocol.[11,12]

Microscopic examination of leaves[11, 13]

Quantitative microscopy

Foliar epidermis of the adaxial (upper surface) and abaxial (lower surface) surfaces of the leaves were prepared by clearing method. The leaf samples were cleared by soaking in commercial bleach "Hypo" containing 3.5% sodium hypochlorite for 18 hrs. Then, the epidermal strips of the leaf samples were scrapped gently with the aid of a pair of forceps and placed on a clean slide, and then stained with Safranin solution and covered with a cover slip. The slides were viewed under a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) at x 40, x 100 and x 400 magnifications and photomicrographs were taken with a

Moticam 2.0 image system with software (Motic Carlsbad, CA, USA) fitted to the microscope. The following parameters were observed and assessed:

1. Epidermal cells: the type and number of epidermal cells were counted and recorded.
2. Stomata type: the stomatal complex types were observed and recorded
3. Stomata size (length and width): the stomata length and width were measured using Motic microscope software a total of 10 fields of views for each sample.
4. Stomatal density: the stomatal density was determined as the number of stomata per square millimetre.
5. Stomatal index: the stomatal index was determined as follows:

$$SI = \frac{S \times 100}{S + E}$$

Where: S = Number of stomata in a field of view
E = Number of epidermal cells in the same field of view

1. Trichome parameters: the trichome types, size, density and index were determined following the same procedures as the stomata above.
2. Vein islet number, vein islet termination number and palisade ratio.

All parameters were observed on both the adaxial and abaxial surfaces of the leaves.

Chemo-microscopic examination

Chemo-microscopic examination was carried out to determine the presence or absence of starch grain, protein, lignin, fats/oil, calcium carbonate and calcium oxalate crystals using standard techniques.[12,14]

Determination of analytical standards

Analytical standards and physicochemical constants of the leaf were determined to evaluate the quality and purity of the drug,[15,11] The total ash value, water insoluble ash value, acid insoluble ash value, sulphated ash value, extractive values and moisture content.

Total ash values

A tarred nickel crucible was placed in muffle furnace for about 15 minutes at 450^{0}C, cooled in a desiccator for about one hour and the crucible was weighed (W_1). 3.0g of the powered material was placed into the nickel crucible and heated gently until all the moisture has been driven off and the plant material was completely charred (W_2). The heat was slowly increased until the carbon has vaporized and the residue was free from carbon at 650 ^{0}C and the sample turns grey (white ash). The crucible was removed with crucible tong, cooled in a desiccator, and reweighed (W_3). The percentage ash content was determined by the relationship;

% Ash = Final weight of crucible (W_3) – Initial weight of crucible (W_1) x 100

Weight of sample and crucible (W_2) – Initial weight of crucible (W_1)

Water insoluble ash value

The ash contents of the crucible obtained from Total ash were transferred into a beaker; 25 ml of water was added into the beaker and then boiled for 5 minutes. The mixture was filtered through an ashless filter paper, and both the residue and the filter paper were dried in an oven. The ashless filter paper containing the residue was compressed into the crucible and was subjected to heat at 450^{0}C until the ashless paper was eliminated. The crucible was reweighed (W_3) and the differences were noted with formula.

$$\% \text{ Water Insoluble Ash} = \frac{\text{Weight of sample and crucible (W2)} - \text{Initial weight of crucible (W1)}}{\text{Final weight of crucible (W1)}} \times 100$$

% Water Soluble Ash = % Total Ash - % Water Insoluble Ash

Acid insoluble ash

To the crucibles containing total ash, A 25 mL of concentrated hydrochloric acid was added and gently boiled for 5 min while covered with a watch – glass. On cooling, the content was filtered using an ash-less filter paper (Whatman No 1) of known weight (W_1). The residue on the filter paper was washed with hot water until the filtrate became neutral to blue litmus paper. The filter paper with the insoluble matter was dried to a constant weight (W_2) at 105ºC. The percentage acid – insoluble ash was determined by the relationship below;

$$\% \text{ Acid} - \text{Insoluble Acid} = \frac{W_2 - W_1}{W_0} \times 100$$

Sulphated ash value

A nickel crucible was ignited to a constant weight at 450 ^{0}C, cooled and weighed. 3.0 g of the dried material was placed over the bottom of the crucible and then reweighed. The material was moistened with dilute sulphuric acid and then incinerated to 450 ^{0}C by gradually increasing the heat until it was free from carbon. The crucible was cooled in a desiccators and more dilute sulphuric acid was added. The heating was continued to about 800 ^{0}C with occasional cooling and reweighing until a constant weight was obtained. The percentage sulphated ash value was determined by difference of the two weights, thus;

$$\% \text{ Sulphated Ash} = \frac{\text{Final weight of sample} - \text{Initial weight of sample}}{\text{Initial weight of sample}} \times 100$$

Determination of extractive yields

Alcohol soluble extractive value

A 5.0 g of the material was weighed accurately and placed in a stoppard conical flask. A 100 ml of 90 % alcohol was added and the stopper of the conical flask was replaced firmly. The flask and its contents were shaken mechanically for about 6 hours and was allowed to macerate for another 18 hours and then filtered. The filtrate was collected and evaporated to dryness, and then the residue was dried to a constant weight at 105^{0}C.

Water soluble extractive value
A 5.0 g of the material was weighed accurately and placed in a stoppered conical flask. A 100 ml of chloroform-water was added and the stopper of the conical flask was replaced firmly. The flask and its contents were shaken mechanically for 6 hours and were allowed to macerate for another 18 hours and then filtered. The filtrate was collected and evaporated to dryness and then the residue was dried to a constant weight at 105 ^{0}C.

The evaporating dish (W_2) was weighed after cooling. The percentage water-soluble extractive value was calculated using the relationship below;

$$\%\ \text{Water} - \text{soluble Extractive} = \frac{W_2 - W_1}{W_0} \times 100$$

The average of the three water-soluble extractives was calculated

Determination of moisture content
A preheated, tarred porcelain crucible was weighed and its weight with lid recorded (W1). A spatula full of the dried sample was introduced into the crucible and was reweighed, (W2). The sample was heated in an oven at the temperature of 65 ^{0}C for 12 hours, at intervals of 6, 3, 2, 1, hours until a constant weight, followed by cooling in a desiccator before reweighing. The constant weight, W3 was noted. The percentage moisture was calculated from the relationship:

$$\%\ \text{moisture} = \frac{\text{Weight of sample in crucible (W2) - Constant weight (W3)}}{\text{Weight of sample in crucible (W2)- weight of crucible (W1)}} \times 100$$

Where W2 –W1 = weight of sample
W2-W3 = weight of moisture

Phytochemical analysis
Qualitative phytochemical analysis of the crude extract.
Qualitative phytochemical tests to detect the presence of various secondary metabolites in both the crude extract were carried out using standard procedures.[13,14]

Test for carbohydrates (molisch's test)
A 0.1 g of each sample was boiled with 2 ml of distilled water and filtered. Few drops of α-naphthol solution in ethanol (Molisch's reagent) were added to the filtrates. Concentrated sulphuric acid was then gently poured down the side of the test tube to form a lower layer. A purple interfacial ring indicates the presence of carbohydrates.

Test for glycosides (combined reducing sugars)
A 5 ml of dilute sulphuric acid was added to 0.1 g of the powder in a test tube, boiled for 15 minutes on a water bath, and then neutralized with 20 % potassium hydroxide solution. 10 mL of a mixture of equal parts of Fehling solution I and II was added and heated for 5 minutes. A denser brick-red precipitate indicates the presence of glycosides.

Test for alkaloids
A 0.5g of the powdered extracts was stirred in 5 mL of 1% HCl on a steam bath for 5 minutes. The mixture was then filtered using Whatman's no1 filter paper. To the filtrate, two drops of Dragendoff's reagent were added to 1 ml of the filtrate. An orange-red colour was observed indicating the presence of alkaloids

Test for saponin
20 ml of distilled water was added to 2 g of the extract and boiled on a hot water bath for 2 minutes. The mixture was filtered while hot and allowed to cool and the filtrate was used for the following tests:

Frothing test: 5 ml of the filtrate was diluted with 15 ml of distilled water and shaken vigorously. A stable froth (foam) upon standing after two minutes indicates the presence of saponins.

Emulsion test: To the frothing solution was added 2 drops of olive oil and the contents shaken vigorously. The presence of an emulsion confirms that saponins are present.

Test for tannins
1g of the powdered material was boiled with 20 ml of water, filtered and used for the following tests.

Ferric chloride test: 3 ml of the filtrate were added a few drops of ferric chloride. A greenish-black precipitate indicates the presence of tannins.

Test for flavonoids
10 ml of ethyl acetate was added to 0.2 g of the powder and heated on a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate was used for the following tests.

Ammonium hydroxide test: 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. The layers were allowed to separate and yellow colour in the ammoniacal layer confirms that flavonoids are present.

1 % Aluminum chloride solution test: Another 4 ml portion of the filtrate was shaken with 1ml of 1 % aluminum chloride solution. The layers were allowed to separate and yellow colour in the aluminum chloride layer indicates the presence of flavonoids.

Test for steroids
Five mL of acetic anhydride was added to 0.5 g of the powdered sample with 2 mL H2S04. A colour change from violet to blue indicates that steroids are present.

Test for terpenoids (Salkowski test)
Five mL of the crude extract was carefully mixed in 2 mL of chloroform after which concentrated H2S04 (3 mL) was gradually added. The formation of a reddish-brown colour at the interface indicates the presence of terpenoids.

Quantitative phytochemical analysis of the crude extract

Quantitative phytochemical tests to detect the presence of various secondary metabolites in the crude extract were carried out using standard procedures.[16]

Acute toxicity studies

Acute toxicity analysis of the extracts was performed using Lorke's method.[17] This method has two phases (Phase 1 and Phase 2).

Phase 1: Nine adult albino mice were weighed, marked and randomized into three groups of three mice each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of the extracts. The mice were observed for 24 hours for signs of toxicity as well as mortality.

Phase 2: Four mice were weighed, marked and randomized into four groups of one mouse each. Dose selection was based on result obtained in Phase 1. Observation for 24 hours for obvious signs of toxicity and death was recorded accordingly. The LD_{50} was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Anti-Diarrhea Activity

Anti-diarrhea activity of *Nelsonia canescence* was carried out using animal model [18]. A total of 25 mice was used. They were grouped into five groups of five mice per group. The animals were fast for 18hour having access to drinking water only. They were treated as follows:

Group 1 received 10 mg/kg distilled water per oral
Group 2 received 4 mg/kg loperamide per oral
Group 3 received 50 mg/kg of extract per oral
Group 4 received 100 mg/kg of extract per oral
Group 5 received 250mg/kg of extract per oral

Thirty (30) minute post treatment diarrhea was induced by single oral administration of 0.5 ml of castor oil. The animals were observed for onset of diarrhea and the time noted. The number and weight of wet stool was noted 1, 2, 3 and 4 hours post diarrhea induction. The groups and reference group was compared with the control group for significant difference.

Gastro-Intestinal Motility Study

Gastro-intestinal study was done as was described by Aye-Than *et al* [19] using charcoal meal. Twenty-five (25) adult albino mice of both sex was used, and they were grouped into five (5) groups of mice each. The animal were fasted 18hours prior the experiment. 5% charcoal meal was prepared in mucilage of tragacanth.

Group 1 received 10ml/kg distilled water, and was immediately given 0.5ml of charcoal meal orally.
Group ii received 10mg/kg atropine intra-peritoneal and 0.5ml charcoal per oral
Group iii received 50mg/kg extract and 0.5ml charcoal meal
Group iv received 100mg/kg extract and 0.5ml charcoal meal
Group v received 250mg/kg extract and o.5ml of charcoal meal

Fifteen (15) minute post-treatment, the animals were sacrificed, dissected and their intestine laid aside. The mesentery was cut loosed so that the entire length of the intestine will be free. The distance travelled by the charcoal plug was measured using meter ruler, and was expressed as percentage of the entire length from the pyloric region to the caecum.

Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Dunnett Multiple Comparisons Test. The values are expressed as mean ± standard mean error (SME). $P < 0.05$ was considered statistically significant.

RESULTS

Macroscopic evaluation of the powdered fruit and root of *Nelsonia canescence* (Table 1) revealed useful diagnostic characters. The plant powders seen to be generally rough in texture, darkgreen in colour, bitter in taste with a characteristic odour.

Table 1: Macroscopic examination of *Nelsonia canescence.*

Macroscopic examination	Observations
Colour	Dark green
Margin	Entire margin lamina
Apex	Acute
Venation	Reticulate
Size	Up-right, up to 20cm tall
Texture	Rough
Surface	Glabrous
Odour	Characteristic odour
Taste	Bitter

Microscopic examination of the leaf of *N. canenscens*

The microscopic examination of *N. canenscens* revealed the presence of stomata, trichome, calcium oxalate and different quantitative microscopic character of the plant such as stomatal number, stomatal index, palisade ratio, vein islet and vein termination (Table 2).

Table 2: Result of fresh leaf microscopy of *Nelsonia canescens.*

Parameter	*Nelsonia canescens*
Epidermal cell type	They are polygonal in shape
Leaf type	The leaf is hypostomatic (stomata only occur on the lower surface)
Stomata type	Diacytic (two subsidiary cells positioning adjacent to the guard cells)
Trichome	Present; both covering, multicellular and glandular trichomes
Stomata number (pfv)	10.25 ± 0.48
Stomata density (mm^{-2})	60.30 ± 2.82
Stomata index (%)	24.82 ± 0.88
Stomata length (µm)	27.38 ± 2.16
Stomata width (µm)	15.71 ± 0.26
Stomata size ($µm^2$)	431.32 ± 38.79
Vein islet number	7.25 ± 0.48
Veinlet termination number	10.50 ± 0.65
Palisade ratio	13.50 ± 0.87

Values expressed as mean ± standard error of mean; n = 4

Table 3: Result of qualitative leaf powder microscopy of *Nelsonia canescens* is shown below.

Parameter	*Nelsonia canescens*
Lignin	Present
Starch	Present
Calcium oxalate	Present; Prism shape
Cystoliths	Present
Tannins	Present
Oil body	Absent

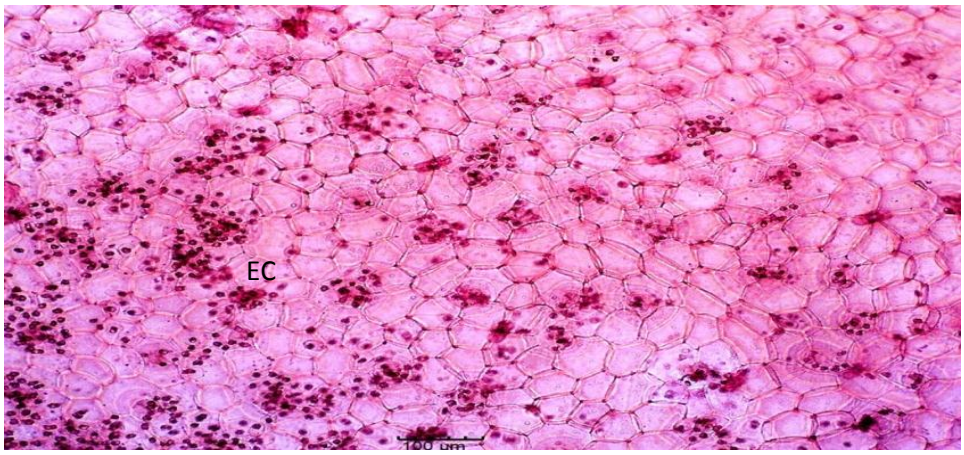


Fig. 1: Adaxial surface of the leaf of *Nelsonia canescens* X 1.

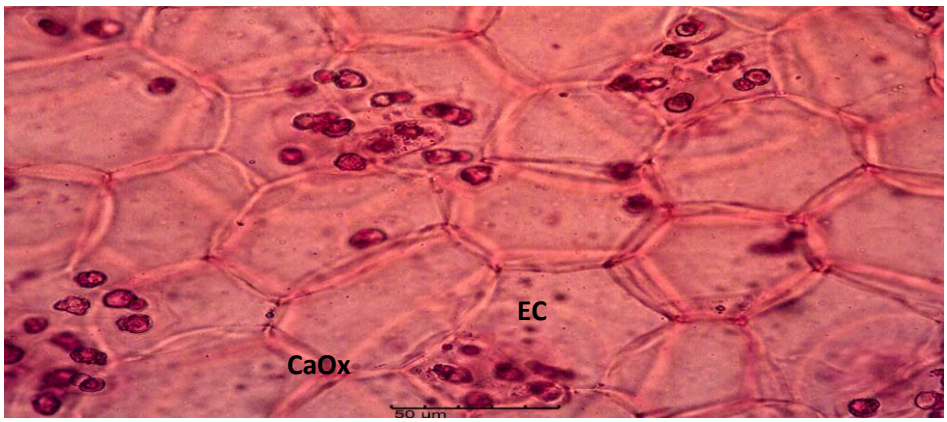


Fig. 2: Abaxial surface of the leaf of *Nelsonia canescens* X 400.

EC = epidermal cell; CaOx = calcium oxalate crystal

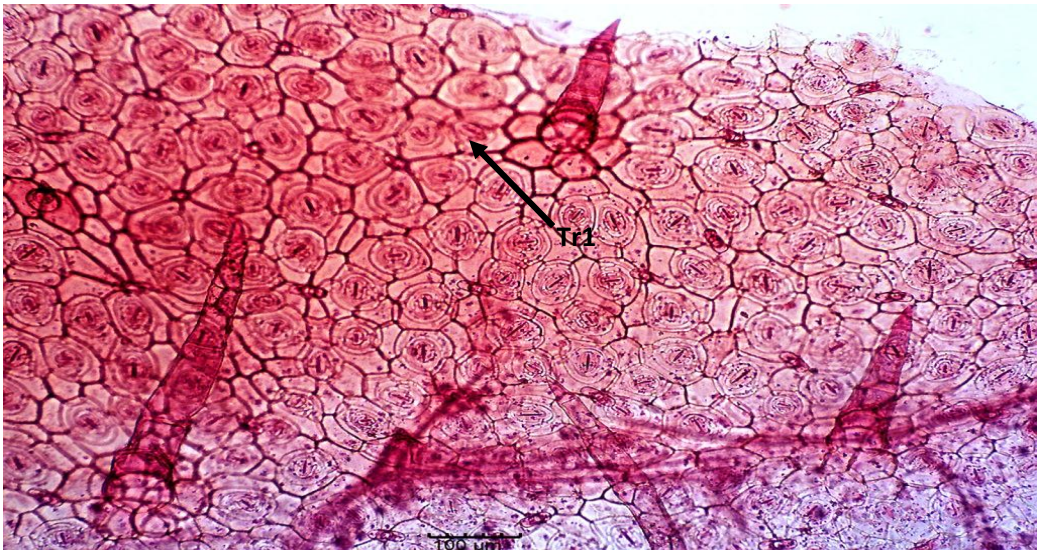


Fig. 3: Abaxial surface of the leaf of *Nelsonia canescens*X 100.

Tri = Trichome

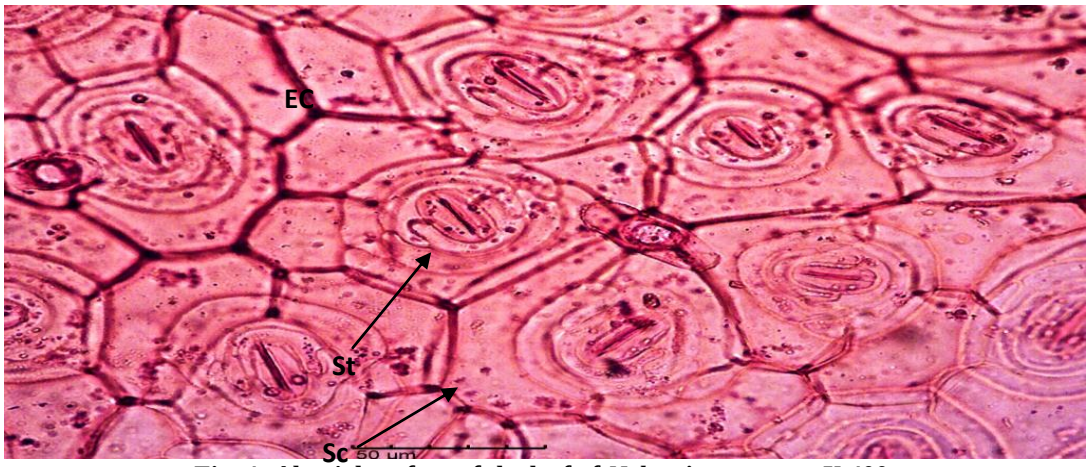
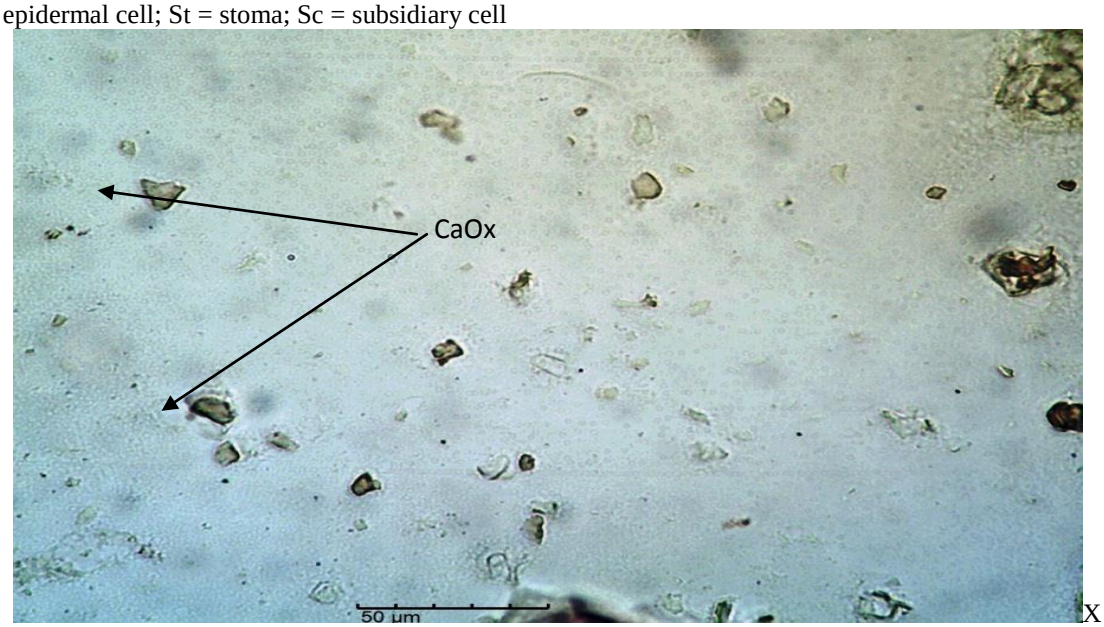


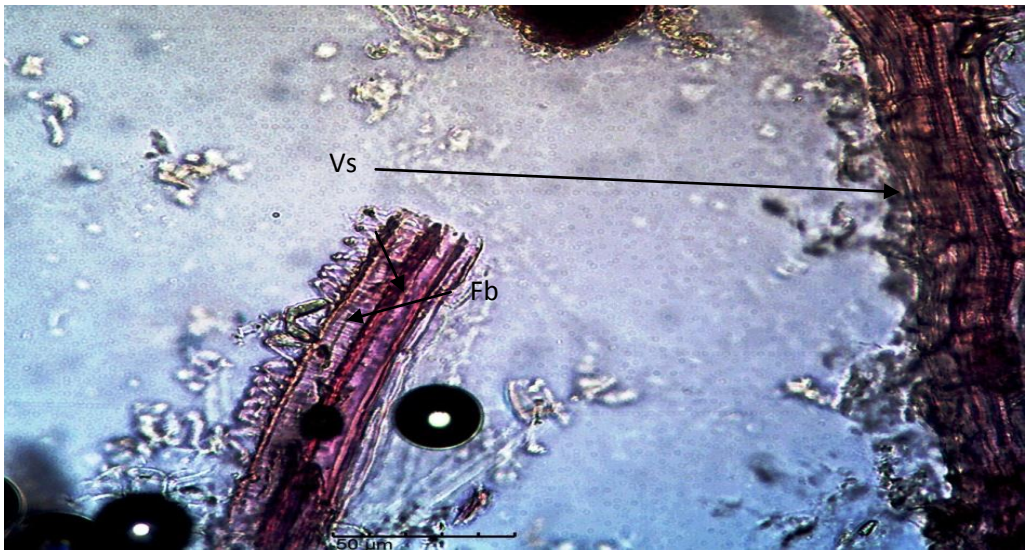
Fig. 4: Abaxial surface of the leaf of *Nelsonia canescens*X 400.

EC = epidermal cell; St = stoma; Sc = subsidiary cell



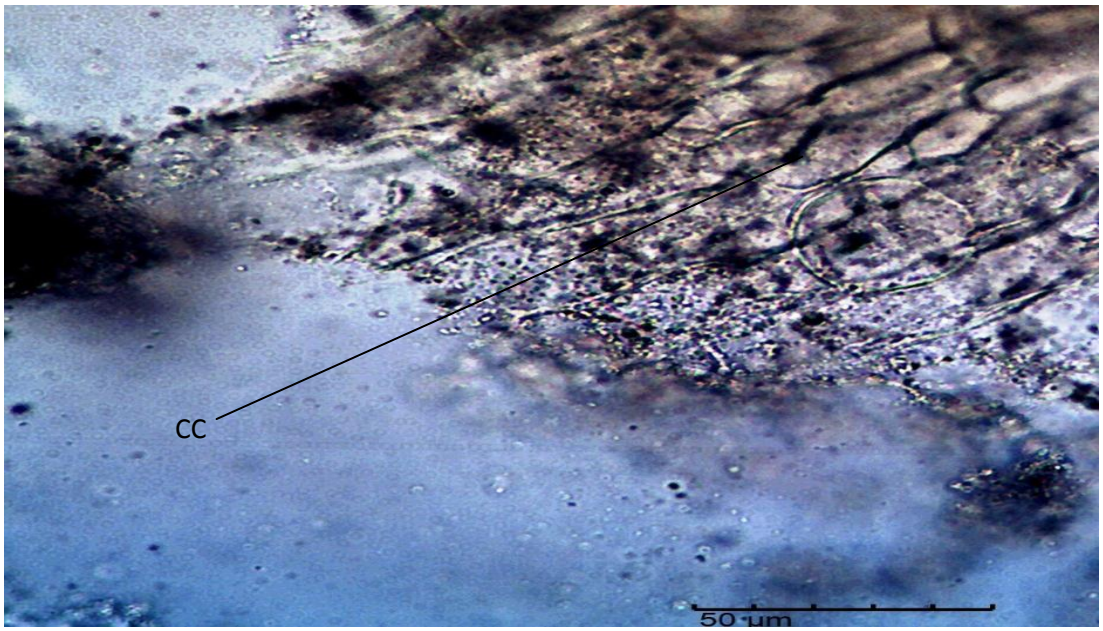
X 400

Fig 5: Chemomicrograph of the leaf powder showing prism shaped calcium oxalate crystals (CaOx).



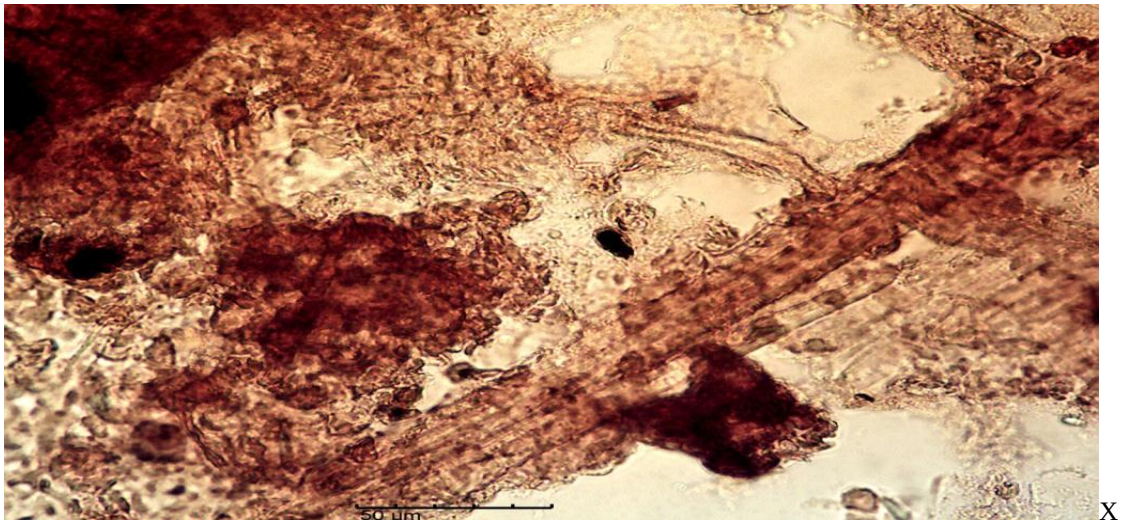
X 400

Fig. 6: Chemomicrograph of the leaf powder showing lignified scalariform vessels (vs) and fibre elements (fb).



X 400

Fig. 7: Chemomicrograph of the leaf powder showing collechyma cells (CC) of the midrib



X 400

Fig. 8: Chemomicrograph of the leaf powder showing lignified tissues

Results of Physiochemical parameters and extractive yield of *Nelsonia canescens*

The Physiochemical parametersof *Nelsonia canescens*. showed the percentage composition of the total ash, water soluble ash, sulphated ash, acid-insoluble ash, alcohol soluble extractive value, water soluble extractive value and moisture content as presented in Table 7. The total ash obtained was 19.50% and it's used as a measure of purity. Moisture content was 7.30 % and it's used as a measure of stability.

Table 4: Physiochemical parameters of *Nelsonia canescens.*

Analytical standards	Percentage Composition (%)
Total ash	19.50
Water soluble ash	9.50
Acid insoluble ash	8.41
Water soluble extractive value	5.12
Sulphated ash	3.82
Alcohol soluble extractive value	6.27
Moisture content	7.30

All experiment was performed in triplicate. Values are expressed as mean ± standard deviation.

Phytochemical Analysis

From the result of the phytochemical test (see table 3) the extract of *Nelsonia canescense* plant contains Saponin, flavonoids, terpenoid, cardiac glycoside, carbohydrate, tannin and proteins. Every other phytochemical test run on the sample was negative. The phytochemical study was based on the qualitative and quantitative analysis as listed below

Table 5: The result of the phytochemical screening of *Nelsonia canescense* powder is presented on table below.

S/N	Phytochemical constituents	Relative abundance
1	Alkaloids	_
2	Saponins	+
3	Tannins	+
4	Flavoniods	+
5	Steroids	-
6	Terpeniods	+
7	Cardiac glucosides	+
8	Carbohydrates	+

Key: - =absent; +=present

Quantitative Analysis Result

Table 6: Quantitative analysis of *N. canescense.*

Phytochemical	Composition (%)
Alkaloids	1.2
Saponins	9.6
Flavonoids	9.8
Tannins	5.8

Result of Acute toxicity test

In the acute toxicity and lethality test, results (Table 12) indicated no physical and behavioural change such as diarrhea, sleepiness, loss of appetite, coma or death. The LD_{50} was thus calculated to be ≥ 5000 mg/kg.

Table 7: Results of acute toxicity (LD_{50}) test.

Phase one	DOSE (Mg/kg)	Mortality
	10	0/3
	100	0/3
	1000	0/3
Phase two		
	1600	0/1
	2900	0/1
	5000	0/1

Anti-diarrhea test result

The extract and fractions of *N canescense*. significantly ($p<0.05$) protected the mice against Castor oil-induced diarrhea when compared with the control. This was comparable to that of loperamide (5mg/kg), the standard agents.

Table 8: The result of the anti-diarrhea effect of the crude extract and the fractions.

Group	Time of onset of diarrhea (min)	Total number of wet stool (g)	Total weight of wet stool (g)	Percentage inhibition
10mg/kg distilled water.	0.36± 0.26	7.65± 0.26	9.72± 0.61	0
4ml/kg loperamide	0.98± 0.82**	1.67± 0.82**	1.38± 0.69**	85.80
50mg/kg extract	0.50± 0.32	5.16± 0.32	4.51± 0.49*	53.41
100mg/kg extract	0.82± 0.16**	3.11± 0.16*	2.31± 0.05**	76.40
250mg/kg extract	0.77 ± 0.97*	3.83 ± 0.97*	2.60 ± 0.82**	73.20
100mg/kg ethyl acetate	1.05 ± 0.61**	4.11± 0.61*	3.30 ± 0.67*	66.01
250mg/kg ethyl acetate	0.99 ± 0.69*	1.83 ± 0.69**	1.81 ± 0.13**	81.31
100mg/kg N-hexane	0.36±0.04	6.16±0.04	5.88± 0.91	39.05
250mg/kg N-hexane	0.46 ± 0.50	4.19 ± 0.50	4.65± 0.67*	52.11
100mg/kg Butanol	0.75 ± 0.25*	5.09 ± 0.25	5.71± 0.22	41.13
250mg/kg Butanol	0.69± 0.47*	4.27± 0.47*	3.79± 0.07*	61.13

All values are expressed as mean ±standard error of mean (SEM)

n=5

*= significant (p<0.05), **= extremely significant (p<0.01) as compared with Control group (one way ANOVA followed by Dunnett t-test, 2 sided).

Table 9: Table showing git motility test result.

Group	Distance Travelled BY Charcoal	Total Length of The Intestine	% Distance Travelled
10ml/kg in distilled water	19.5 ± 1.35	35.50 ± 0.66	56.00 ± 3.00
10mg/kg atropine	9.35 ± 0.49	42.00 ± 0.66	22.40 ± 0.47**
50ml/kg extract	15.15 ± 1.76	35.20 ± 1.44	42.68 ± 1.37*
100mg/kg extract	12.40 ± 0.06	44.90 ± 1.96	27.80 ± 1.33**
250mg/kg extract	16.18 ± 0.14	47.20 ± 2.25	34.30 ± 2.89*
100mg/kg ethyl acetate	9.22± 1.71	28.72 ± 0.74	32.12 ± 4.69*
250mg/kg ethyl acetate	9.73 ± 0.77	32.37 ± 0.54	28.96 ± 1.76**
100mg/kg N-hexane	21.97 ± 0.89	29.53± 0.66	74.42 ± 3.21
250mg/kg N-hexane	14.69± 1.30	30.01 ± 0.58	48.83 ± 4.73*
100mg/kg Butanol	19.57 ± 0.56	31.40 ± 1.23	62.79 ± 2.29
250mg/kg Butanol	13,02± 1.07	30.30 ± 0.78	42.99 ± 5.69*

All values are expressed as mean ±standard error of mean (SEM)

n=5

*= significant (p<0.05), **= extremely significant (p<0.01) as compared with Control group (one way ANOVA followed by Dunnett t-test, 2 sided).

DISCUSSION

The macroscopic examination of the whole leaves of *N. canenscens* showed that it has an entire margin lamina, acute apex, reticulate venation and the glabrous surface. The organoleptic property reveals that the front surface of the leaf is dark green in colour and it has a bitter taste. Macroscopic techniques may be useful to discriminate morphologically similar plant to distinguish between the desired plant species and plant part in the field during the plant sampling. According to World Health Organization, the macroscopic determination of plants is the first step towards establishing its identity and purity hence, should be carried out before any test is undertaken [20]. This microscopic and macroscopic evaluation of *N. canenscens a* leaves will serve as diagnostic tools for its differentiation from other plants and will also help in detection of adulteration with related species. It revealed the type of stomata and trichome present and presence of calcium oxalate, epidermal cells, scalariform vessels and fibre element. The transverse section of the leaf showed the presence of the outermost covering tissues - the upper and the lower epidermises, which are uniseriate and lack chloroplasts. There was presence of closely packed palisade mesophyll cells with numerous chloroplasts (the main photosynthetic organ) and scattered spongy mesophyll cells that are loosely fitted to leave air spaces. The midrib bears the vascular bundle which comprises the phloem (exteriorly located) and the xylem (interior located) - the main conducting organs. Some mass of parenchymatous cells formed the pith at the centre. The vascular bundles are in circular shapes and arranged in a concentric manner within the ground tissue**.**

Proximate analysis is done for the purpose of correct identification and quantification such that any other sample of the plant at any other time can be compared with and related to the original sample when subjected to the recommended monograph.[21] The determination of water-soluble or alcohol-soluble extractive value is used as means of evaluating drugs, the constituents of which

are not readily estimated by other means and these extractive values are highly required as pharmacopoeial standards.[21] The moisture content is very low and will therefore not favor microbial growth. Its moisture contain was established to be 7.8%. The percentage yield of the extraction process of *Nelsonia Canescense* was 2.5%. The extractive values are relevant when the chemical natures of the medicinal components are not known and the values help in extraction procedures.[22]

The oral median lethal dose (LD50) was estimated to be ≥5000mg/kg body weight the absence of death following the oral administration of the crude extract of *Nelsonia canescense* at 5000mg/kg body weight observed in the rats suggest that the extract is practically non-toxic. The result of the phytochemical analysis of *Nelsonia Canescens* proves that it contains saponins, tannins, flavoniods, terpenoids, cardiac glycosides, carbohydrates and proteins. To further ascertain the preliminary result, a quantitative estimation of some phytochemicals was carried out, and the result showed that it contains more of flavonoids and lesser in alkaloids. These active phytochemicals are known for their medicinal activity as well as physiological actions; as such they confer the therapeutic potentials of all medicinal plants. Earlier studies showed that anti-dysenteric and anti-diarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids and/or terpenoids are reducing sugars.[23] Also, certain flavonoids, tannins and terpenoids revealed antidiarrheal activities via a multitude of mechanisms.[24] Most of the aforementioned secondary metabolites were screened from the leaves of this plant so far.

Diarrhea occurs when there is an imbalance between the secretary and absorptive processes of gastrointestinal tract and/or an alteration of motility of intestinal smooth muscles.[25] The use of castor oil as diarrhea inducer has been well documented.[26] *N canensces* significantly delayed the initiation of diarrhea and reduced the number and weight of both wet and total fecal outputs. Substances exhibiting significant (P>0.01) antidiarrheal activity may have a potential to retard the onset of diarrhea as observed at 50, 100 and 250 mg/kg ethanol crude extract. The highest effect on both volume and weight of intestinal content was achieved at 100 mg/kg of the extract with percentage inhibition of 76.4% when compared to the loperamide, the positive control with a percentage inhibition of 85%. The negative control group (10ml/kg distilled water) has the highest number and weight of wet stool, compared to the other groups. Coming to the fractions, ehtylacetate (at 100 and 250 mg/kg) and n-hexane at 250 mg/kg fractions produced significant effects with the dose 250mg/kg ethylacetate showing the highest activity. This might be attributed to qualitative and quantitative differences in bioactive constituents of these fractions. On the contrary, the butanol fraction was devoid of any significant effect at all tested doses. Loperamide reduces the daily fecal volume, and decreases intestinal fluid and electrolyte loss. Loperamide produces a rapid and sustained inhibition of the peristaltic reflux through depression of longitudinal and circular muscle activity, presumably through an effect on intestinal opiate receptors. Loperamide is effective against a wide range of secretory stimuli and can be utilized in the control and symptomatic relieve of acute diarrhea that is not secondary to bacterial infection.[23] This plant extract exhibited antidiarrheal activity. The effect was comparable to loperamide which is presently one of the most widely used anti-diarrheal drugs. Castor oil induced diarrheal model was designed to assess the potential of a test substance in its overall antidiarrheal activities. The onset of defecation, the frequency and weight of fecal outputs were determined as the main parameters. Determination of the percentage inhibition has been based on the reduction of frequency of wet fecal outputs as a good marker of antidiarrheal activity. Diarrhea is also presented with an increase in weight of defecation.[27] Accordingly, the plant displayed a dose-dependent reduction in weight of fecal outputs indicating the antidiarrheal potential of the *N canensces* in this model.

In addition, the administration of the extract and fractions of *N. Canescense* in rats caused a significant reduction in the progression of charcoal meal and in the intestinal transit time wth ethylacetate fraction giving the least transit time. This activity is comparable to that of atropine used here as reference drug and which is known to reduce intestinal motility.[28] The antidiarrheal effects of *N. Canescense* could thus result from a reduction of intestinal motility and an increase in the intestinal absorption of water and electrolytes. In fact, many previous studies have shown that drugs and natural products as well, can induce their antidiarrheal effect through antispasmodic activity.[29]

CONLUSION

The results of this investigation suggest that the leaf extract of *Nelsonia Canescens* possesses antidiarrheal activity and justify the ethnomedicinal use of the plant in the treatment of diarrhea in Nigeria. The presence of some of these phytochemical constituents in *Nelsonia Canescens* may be responsible for the antidiarrheal effect. The plant seems safe based on the results of acute toxicity testing.

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CONFLICT OF INTERESTS

Declared none.

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