Phytology

Pharmacognostic studies and anti-trypanosomal properties of *Lindackeria dentata* (Oliv.) Gilg (Flacourtiaceae) leaves for the treatment of sleeping sickness

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Abstract

Introduction: Lindakteria dendata has been known in ethnomedicine to cure back pain, inflammation and toothache.

Aim: The aim of this study was to evaluate the pharmacognostic profile and antitrypanosomal potential of *Lindackeria dentata* (Flacourtiaceae) leaves.

Methodology: The macroscopy, microscopy and physio-chemical studies were carried out to standardize the plant leaf. Five groups of animals were used. To groups A, B and C were administered 200 mg/kg, 400 mg/kg and 600 mg/kg of crude methanol extract respectively. The standard drug Diminazene aceturate (7 mg/kg) was administered to group D while group E received distilled water (negative control). Alterations in the body weight, degree of parasitaemia and hematological parameters were determined.

Results: Pharmacognostic profile revealed tasteless drug with microscopic features of wavy epidermal cell wall of anomocytic stomata, annular xylem and phloem, prism shaped calcium oxalate, phloem parenchyma cells, palisade cells, non-glandular trichome. Phytochemicals analysis showed the presence of flavonoids, alkaloids, tannins, saponins, terpenoids and sterols in varying amounts. The crude methanol extract exhibited significant ($p < 0.05$) anti-trypanosomal activity in the infected rats by reducing the parasitaemia levels, improving weight gain and haematological changes in a dose-dependent manner. Extract at 600 mg/kg gave the second-best activity (after standard drug) for percentage weight gain and parasitaemia levels of 13.31 ± 2.18 % and 74.36 ± 6.24 respectively. Red blood cells (RBC), haemoglobin (HGB) and packed cell volume (PCV) were 1.97 ± 1.99 (5.6 x 10^{12}/l), 11.50± 1.43 g/dl and 35.1 ± 2.72 % respectively.

Conclusion: This study has established the pharmacognostic standards of *Lindackeria dentata* leaves and validated its use in ethnomedicine for the treatment of parasitic infections.

Keywords: *Lindackteria*, *dendata*, pharmacognostic, standards, trypanosomiasis, phytochemical, hematological

1. Introduction

Trypanosomiasis (sleeping sickness) caused by African trypanosomiasis (African sleeping sickness) is a great burden of infectious disease generally experienced worldwide, although their distribution is heavily skewed towards the tropics [1]. Nigeria is one of the endemic areas in sub-Saharan Africa where such tropical diseases and parasitic infections prevail [2]. The problem has persisted despite all therapeutic interventions with no vaccine produced so far [3]. There are limited anti-protozoan drugs available and the few imported ones are quite expensive. Meanwhile, these parasites increasingly defy available therapeutic interventions due to resistance and rapid adaptation [4, 5]. In an effort to combat the relapse of the disease state, higher doses of the anti-protozoan drugs are administered resulting in numerous adverse drug reactions and high toxicity especially on prolonged usage. The increasing global incidence of trypanosomiasis need search for more potent, efficacious, safer, cost effective and readily available alternatives from natural products such as medicinal plants sourced locally [6] reducing long-term reliance on synthetic drugs from major pharmaceutical companies.

1.1 Pharmacognostic profile

Lindackeria dentata (Oliv.) Gilg (Flacourtiaceae) is an evergreen plant where the tree rarely reaches more than 10 m in height with widespread occurrence in distributed cold areas of Africa. It is a plant prevalent in Tropical West Africa and the Sahel [7] It is a tree that grows primarily in the West tropical biome and Angola. The leaves are harvested from farm land in Igede Ekiti, South West of Nigeria. Some important chemical compounds such as cyclopentanoids cyanodendate, hydrin glucoside and Amides have been isolated from the plant [8].

Fig 1: Photograph of *Lindackteria dendata* in its Natural habitat at Diogbe in Igbo-Etiti L.G.A Nsukka

1.1.1 Taxonomy

Kingdom	Plantae
Genus	Lindackteria
Species	dendata
Geographical location	West African Tropical to Angola
Vernacular name	Uagbo (South West Nigeria)
Ethnomedicinal uses	Treatment of back pain, inflammation and toothache
Constituents	Cyclopentanoids, hydrin glucosides and Amides

L. dentata is used as a vermifuge and for the treatment of fever, malaria, cutaneous and subcutaneous parasitic infections. There is an undocumented report that the leaves of *L. dentata* are used to treat trypanosomiasis and other neglected tropical diseases such as filariasis among the indigenous people of Abakaliki. However, there are few research findings to validate this claim as well as other reported ethno-medicinal uses of this plant. Therefore, this present study was carried out to investigate the pharmacognostic standards of *Lindackeria dentata* and its potential anti-trypanosomal activity.

2. Materials and methods

2.1. Plant collection, identification and preparation of plant materials

The leaf samples of *Lindackeria dentata* were collected from its natural habitat in Diogbe in Igbo-Etiti Local Government Area of Enugu State, Nigeria in March 2023. The plant was identified and authenticated by a certified plant taxonomist of Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. A voucher herbarium sample with number (UNH/04/0334A) was prepared and deposited at the University of Nigeria Herbarium (UNH) domiciled in the Department of Plant Science and Biotechnology, Faculty of Biological Sciences, University of Nigeria, Nsukka for reference purposes. The fresh leaves of *Lindackeria dentata* were air-dried at room temperature (28 ± 2 °C.), pulverized and stored air-tight for further use.

2.2. Pharmacognostic standardization

2.2.1. Phytochemical analysis

Chemical tests were performed on the powdered leaves to detect the presence or absence of various major secondary metabolites of pharmacognostic importance using standard methods [9].

2.2.2. Macroscopic examination

The fresh leaves of the plant were virtually examined using the methods described by Evans [9]. The morphological data of the leaf examined include type of margin, venation, size, shape, base, apex, mid-rib, surface character and texture. Also, the organoleptic properties like colour, odour and taste were observed and noted.

2.2.3. Qualitative microscopic examination of powdered leaves

A sample of the leaf powder of *L. dentata* was placed on the slide and two drops of chloral hydrate solution was added to moisten the powder and also act as a clearing agent. The slide was covered with a slip and repeatedly passed over a flame of burner until bubbles occurred. This was later allowed to cool and 2 drops of dilute glycerin were added for clarity of structures and the slide was viewed under the photomicroscope (magnification x 400) where the microscopic characters were observed and noted.

2.2.4. Microscopic examination of transverse section

Anatomical sections of *L. dentata* fresh leaf were prepared for the microscopic studies. The staining was done using standard laboratory methods [9]. The transverse section was done by sectioning of the specimen using a sledge micrometer. It was transferred into a staining jar and stained in safranin for 5 minutes. The section was washed with distilled water, followed with alcohol, and thereafter stained again with 1 % fast green for 5 minutes and washed with absolute alcohol. It was transferred into a jar containing 50/50 alcohol/xylene and washed until they became clear. The section was cleared with chloral hydrate solution and mounted on a slide with dilute glycerin.

These prepared slides were then viewed under the Light phase contrast microscope with camera (Motic B3, Motic Carlsbad, CA, USA) and the microscopic characters were observed and noted. The cleared sample was subjected to various histochemical tests for the presence of starch, calcium oxalate crystals and lignified vessels.

3. Determination of the analytical standards

The qualitative and quantitative analysis of the chief constituents of the crude drugs, determination of various ash values, solvent extractive values and moisture content followed the specification as described in the British Pharmacopoeia [10].

3.1. Extraction

A 4.0 kg of the powdered dried leaves of *Lindackeria dentata* were exhaustively extracted in aliquots (250 g) with 2.5 L of 95 % methanol by cold maceration for 72 h at room temperature (28 ± 2 °C). Constant agitation and filtration were done at every 2 h interval and the whole macerate was filtered through a plug Whatman filter paper. The process was repeated exhaustively until the added solvent remained clear. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator at temperature of 20-30°C to obtain the crude methanol extract (CME, 344.8 g) which was stored in the refrigerator for toxicity, antitrypanosomal and haematological assays.

4. Toxicity test and antitrypanosomal studies

Acute toxicity and lethality (LD_{50}) tests

The acute-toxicity and lethality (LD_{50}) of the crude methanol extract was determined using the method described by Lorke [11] using the oral route.

In vivo assay methods

4.1. Parasite inoculation

The parasites were first inoculated heavily to donor rat. After

establishment of infection (14 days post–inoculation), the donor rats were then subjected to retro-orbital puncture through the median canthus of the eyes and blood was collected and immediately diluted with PBS (phosphate buffer saline) for subsequent *in-vivo* test.

4.2. Administration of crude methanol extract of *Lindackeria dentata* leaves

A total of twenty-five (25) apparently healthy rats (previously acquired from the animal house of the Faculty of Veterinary Medicine, UNN) were randomly grouped (n=5) into five groups (A, B, C, D and E). All experimental groups of rats were then infected intraperitoneally (IP) with 2,000,000 trypanosomes/rat in 0.2 ml of blood as described and used [12]. The animals were left to develop parasitaemia until average parasitaemia became approximately 10^7 parasites/ml. This took a total of one week to be achieved. Groups A, B and C were administered 200, 400 and 600 mg/kg of the *L. dentata* extract respectively whereas groups D and E received Diminazene Aceturate (7 mg/kg) and distilled water respectively. Permission was obtained from the Animal Ethics Committee of the Faculty of Veterinary Sciences, University of Nigeria Nsukka for the care and use of laboratory animals as stipulated by the international declarations on the safety and use of laboratory animals and National Guide on animal Care and Regulations (NCARE). Doses of extracts to be administered were selected based on the results of acute toxicity. The drugs, extract and standard Diminazene aceturate were administered to these groups orally with a gavage syringe every morning for ten days. On day 10 of the experiment, the blood samples were collected for parasitaemia analysis and body weight change and all the animals were sacrificed under chloroform anesthesia and properly disposed.

4.2.1. Determination of parasitaemia level and body weight change

The degree of parasitaemia was estimated using the rapid matching method as described [13]. Briefly, the method involves microscopic counting of parasites per field in blood smears from the peripheral blood obtained from retro-orbital puncture to obtain enough blood from each rat for the determination of parasitaemia. Wet smears were prepared in triplicates from each animal and the mean value of slide counts was taken per sample and examined microscopically. Logarithm values of these counts were obtained by matching with the table given [13]. Parasitaemia was monitored on the first day before treatment (i.e. day 0 or baseline) and on the 10^{th} day. For the assessment of antitrypanosomal effect of the extracts, the level of parasitaemia (expressed as Eq log/mL or ($x10^6$ parasites/mL) in the treated animals was compared to that of the control animals. The body weight of each rat in all groups was also measured on the day treatment commenced (day 0) and on day 10 by a sensitive digital weighing balance.

4.2.3. Determination of Hematological Parameters

The animals were properly restrained. The jugular area was cleaned with 70 % ethanol and 5 ml of blood was collected from the jugular vein using a 5 ml syringe. One ml of the blood was put in a bottle containing anticoagulant (EDTA) to be used for hematology while the remaining 4 ml of blood was put in a plain tube to allow for blood coagulation and the collection of serum for serology. Hematological parameters – packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) count, and Total white blood cell (WBC) counts were assessed using the standard laboratory methods as described [14].

4.3. Statistical analyses

All the data obtained from the experiments were processed on Microsoft Excel Spreadsheet 2010 and statistically analyzed by analysis of variance (ANOVA) using statistical package for social sciences (SPSS) Version 2.10. Significant difference was tested at 95% and 99% levels of probability for both ANOVA and post-hoc analyses.

5. Results and discussion

5.1. Results

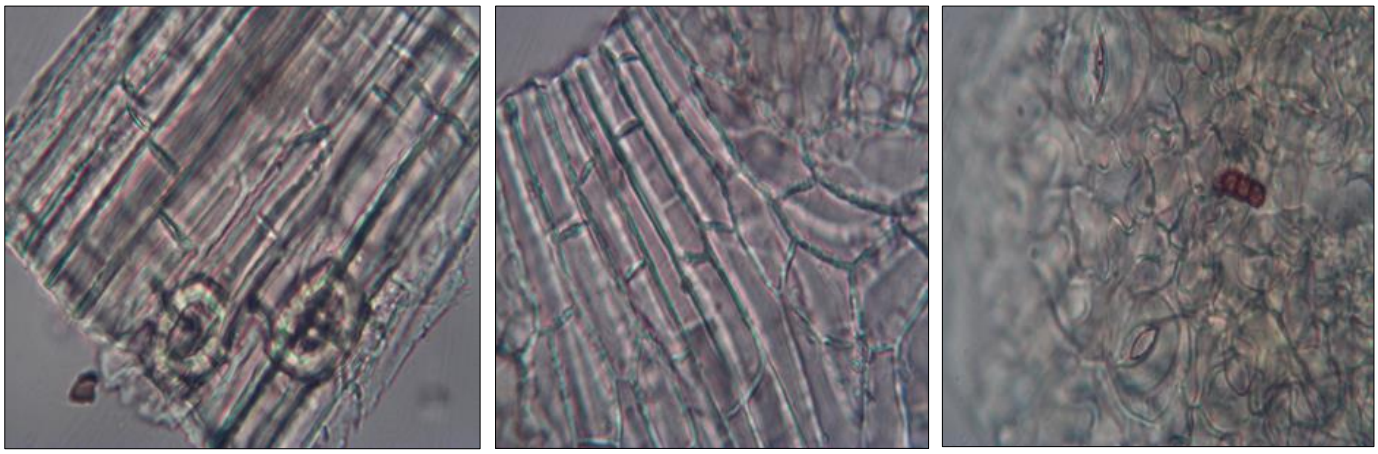

Fig 1: Photomicrograph of epidermal cells of *Lindackteria dendata* showing palisade cells from petiole x 400, **Fig 2:** Photomicrograph of leaf of *Lindackteria dendata* showing epidermal cells from the midrib x 400, **Fig 3:** Photomicrograph of epidermal cells of leaf of *Lindackteria dendata* showing anomocytic stomata x400.

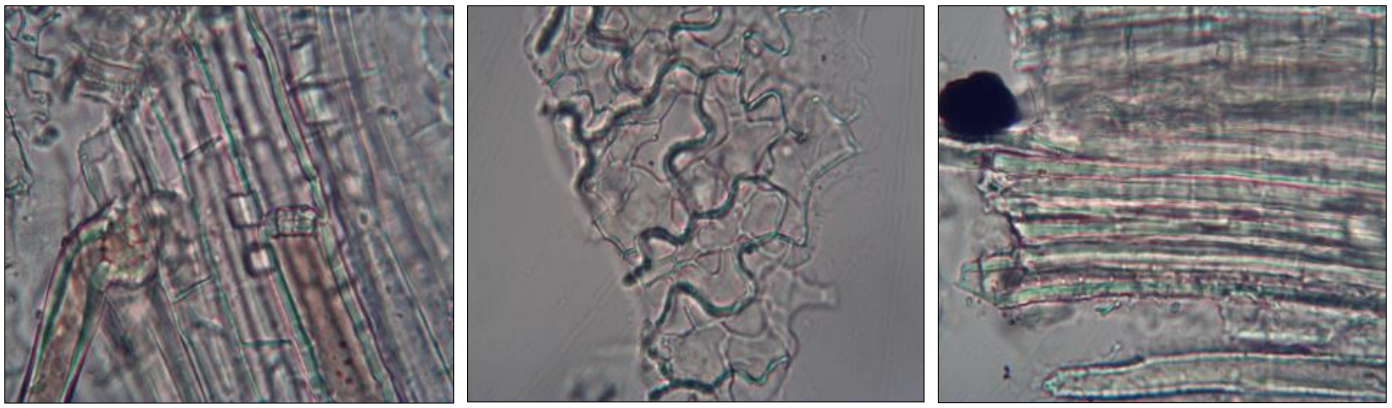

Fig 4: Photomicrograph of epidermal cells showing prisms of calcium oxalate crystals, **Fig 5:** Photomicrograph of epidermal cells with wavy cell wall x 400, **Fig 6:** Photomicrograph of epidermal cells showing group of fibres from cell wall. X 400

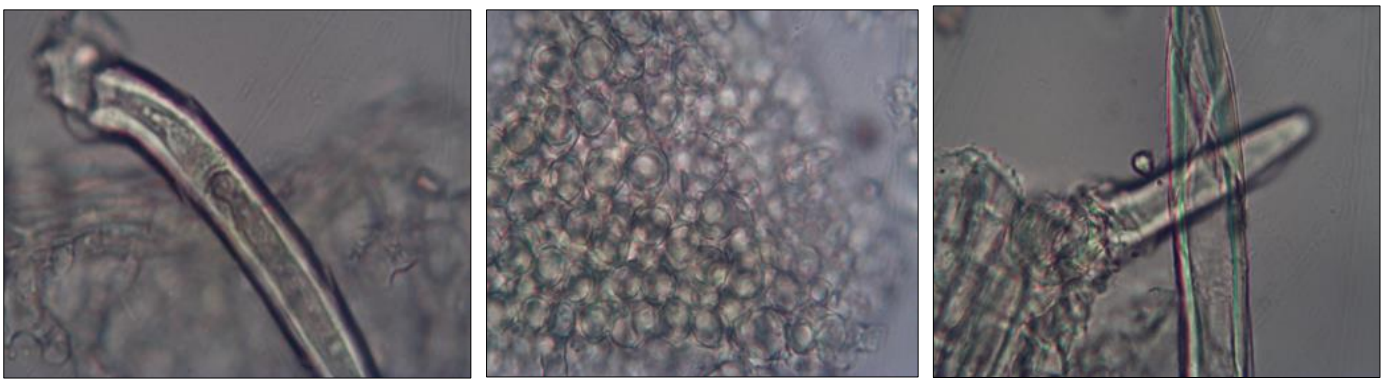

Fig 7: Photomicrograph of epidermal cells showing non-glandular trichome x 400, **Fig 8:** Photomicrograph of epidermal cell showing numerous palisade cells x 400, **Fig 9:** Photomicrograph of epidermal cell showing a pair of unicellular clothing trichome x 400

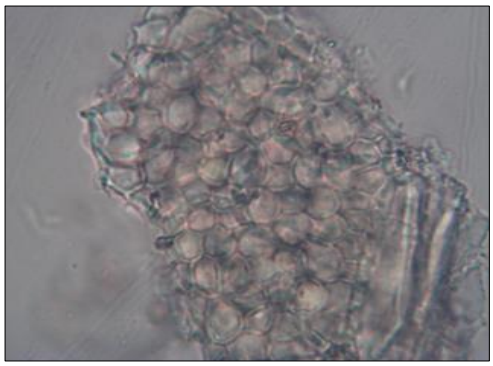

Fig 10: Photomicrograph of the leaf of *Lindakteria dendata* showing palisade cells

Table 1: Result of chemo-microscopic evaluation of *Lindacteria dendata* leaf

Chemical	Reagent	Observation
Starch	Iodine solution	*
Lignins	Phloroglucinol; Conc. HCl	***
Cellulose	Zinc chloride; Conc. H_2SO_4	**
Calcium oxalate	Iodine solution; Conc. H_2SO_4	**
Protein bodies	Biuret reagent; Nihydrin	-
Fats and oil	Sudan IV reagent	*
Gum and mucilage	Ruthenium red	*

- Absent *Present in trace amount, **Moderately present, ***Abundantly present

Table 2: Analytical standards of *Lindackteria dendata*

S/N	Parameter	Mean Values (% w/w)
1.	Moisture content	11.17±0.16
	Ash values	
2.	Total ash	7.9±0.03
3.	Acid insoluble ash	1.26±0.02
4.	Water soluble ash	1.86±0.03
	Extractive values	
5.	Water soluble extractive value	8.05±0.03
6.	Alcohol (methanol) soluble extractive value	5.84±0.05

Values expressed in mean ± SEM of 3 replicate data.

Table 3: Result of phytochemical components of the methanol extract of *L. dentata* leaf

S/N	Component	Qualitative test	Quantitative (mg/g)
1.	Saponins	+	11.99 ± 0.03
2.	Tannins	+	2.33+0.01
3.	Alkaloids	+	0.33±0.01
4.	Terpenoids	+	35.09±0.16
5.	Steroids	+	20.50±0.08
6.	Flavonoids	+	1.04±0.02

Key: + Present values expressed as mean± standard error of mean (SEM)

Table 4: Effect of methanol extract of *L. dentata* on weight gain and parasitaemia level of *T. brucei* infected rats

Formulation	Initial weight (g)	Final weight (g)	% Weight gain/loss	Initial parasitaemia	Final parasitaemia	% Change in parasitaemia
(200 mg/kg)	115.92 ± 4.21	103.38 ± 5.32	-10.81 ± 2.35	20.82 ± 3.26	27.50 ± 6.83	-32.08 ± 8.25
(400 mg/kg)	119.94 ± 7.53	124.91 ± 4.45	4.14 ± 2.32*	24.81 ± 8.48	13.50 ± 4.39	45.59 ± 2.74*
(600 mg/kg)	114.31 ± 5.45	129.53 ± 8.67	13.31 ± 2.18**	23.40 ± 4.36	6.00 ± 2.48	74.36 ± 6.24**
Standard Diminazene Aceturate (7mg/kg)	113.59 ± 3.52	135.37 ± 5.98	19.17 ± 3.14**	23.11 ± 12.63	4.18 ± 3.01	81.91 ± 5.91**
Distilled water (5 ml/kg)	109.86 ± 2.69	91.64 ± 4.81	-16.58 ± 1.69	22.95 ± 4.59	43.0 ± 11.25	-87.36 ± 27.28

Values expressed as mean and standard error of 5 replicate data, *Significant at $p \leq 0.05$; ** significant at $p \leq 0.001$

Table 5: Result of crude methanol extract of *L. dentata* on haematological changes

Parameters	Group A (200 mg/kg)	Group B (400 mg/kg)	Group C (600 mg/kg)	Group D (Diminazene Aceturate, 7mg/kg)	Group E (Distilled Water)
RBC ($5.6x10^{12}$/l)	1.47 ± 5.60^{c}	1.65 ± 4.82^{c}	1.97 ± 1.99^{b}	2.05 ± 20.58^{a}	1.42 ± 3.47^{d}
HGB (g/dl)	9.90 ± 1.20^{c}	10.10 ± 4.31^{c}	11.50 ± 1.43^{b}	10.30 ± 4.74^{a}	9.50 ± 2.52^{d}
PCV (%)	30.03 ± 5.92^{b}	31.50 ± 6.45^{b}	35.1 ± 2.72^{b}	31.50 ± 13.14^{a}	28.00 ± 4.94^{c}
Total WBC (10^{9}/l)	14.15 ± 25.06^{b}	13.73 ± 15.93^{a}	12.37 ± 12.91^{d}	6.60 ± 18.78^{c}	31.15 ± 48.61^{a}

Values expressed in mean ± SEM of 5 replicate data, means with different letters across a row are significantly different at $p \leq 0.05$

5.2. Discussion

Standardization of drugs is the process of establishing or prescribing a set of peculiar identities, specific characteristics which are generally unique and of unshared qualities [15]. Pharmacognostic standardization of *Lindackeria dentata* revealed and assembled a set of inherent peculiar characteristics, such as, constant parameters, definite qualitative and quantitative value or specific and unique features on the bases of which similar herbal medicines, claimed to be the same, can be compared for the purpose of authenticity, efficacy, genuineness, purity, reproducibility, overall quality assurance and inclusion in the monograph of the official books or Pharmacopoea [16, 17].

Macroscopic and Microscopic features. The macroscopic features showed that the plant is herbaceous and grows in the Sahel or Tropical biome. The stem outer bark was smooth and grey in colour. The inner slash was milky at first, then pinkish yellow after a while with visible gums). The sample powder was smooth, light brown in colour, slightly bitter and had sharp uncharacteristic odour.

The microscopy and chemo-microscopy showed presence of lignins, cellulose, and calcium oxalate in moderate to high quantities while starch, fats and oil and gums and mucilage are scarcely present (Table 1 and Plate 2). The transverse sections of the leaves of *Lindackeria dentata* at a magnification of x100 reveals an external wall of thick layer of cells called the periderm (replacing the epidermis at maturity). The periderm is made up of the phellogen (a lateral meristem), phellen (true cork) and phelloderm (secondary cortex). There is a layer of polygonal shaped cells known as the cortex, composed of collenchyma and parenchyma. The vascular bundles are continuous, collateral and arranged ring of parenchymatous pith at the center. Between the xylem and the phloem is a thin layer of actively dividing cell called the cambium. The xylem vessel was diffuse-porous in aggregates with banded axial parenchyma.

The analytical standards of the leaves of *L. dentata* showed the composition of the total ash, water soluble ash, sulphated ash, acid insoluble ash, alcohol soluble extractive value, water soluble extractive value and moisture content (Table 2). The analytical standardization of *L. dentata* leaves were within pharmacopoeia standards and developed numerical standards which could be used as reference guides for the identification and assessment of their quality and purity. The percentage total ash value which is the total amount of material remaining after ignition at 450 ^{0}C was calculated to be 7.900 %, this represents the ash from the plant tissue and other extraneous matter adhering to the surface of the plant, it sets a standard for the level of soil and mineral matter present. The percentage water soluble ash which detects water exhausted drugs was gotten to be 1.260 % while the percentage acid insoluble ash indicating the amount of silica present, especially as sand and siliceous earth was calculated to be 1.860 %. Water soluble ash is relevant in quantifying the extraneous material that has been exhausted by water. It is an important indication of the presence of exhausted material [15-17]. Therefore an *L. dentata* sample with percentage total ash, percentage water soluble ash and percentage acid insoluble ash more than 7.900, 1.260 and 1.860% respectively indicates the possible presence of adulterants. The percentage moisture content of the sample drug was obtained as 12.170%. High moisture content of a drug sample results to a higher risk of deterioration of the drug sample by microorganisms, fungi, molds, due to this; lower percentage moisture contents are preferred for drug samples [15].

Extractive values determine the number of active constituents extracted with solvents from a given amount of medicinal plant material. The alcohol soluble (methanol) extractives and water-soluble extractives were gotten as 5.84 and 8.05% respectively. These extractive values indicate that there are more polar (hydrophilic) compounds than non-polar (hydrophobic) compounds in the leaves of *L. dentata*. The water-soluble extractive has the greatest value, which indicates that the constituents of the leaves of *L. dentata* will be most soluble and extracted in water. Therefore, water seems to be the best solvent for the extraction of the leaves of *L. dentata* [15].

The phytochemical analysis carried out on *L. dentata* revealed the presence of various secondary metabolites including alkaloids which possesses a lot of physiological effects on the human body like analgesic, antimalarial, antispasmodic, psychostimulant. Steroids (hormonal and some effects on fertility), terpenoids, flavonoids (polyphenolic compounds known to possess antioxidant, anticarcinogenic, antimicrobial properties too many effects to mention) [7, 8]. Saponins (known for their hemolytic, inhibiting tumor growth, cholesterol lowering, cancer risk lowering, and blood glucose lowering properties), and the presence of trace amount of tannins, flavonoids and glycosides (Table 3). Flavonoids, tannins and saponins are collectively known as phenolic compounds. The presence of phytochemicals in plants is known to be responsible for the pharmacological activities exhibited by plants [7, 8, 15, 17].

The acute toxicity (LD_{50}) of *L. dentata* leaves in swiss mice was found to be >5000 $mgkg^{-1}$. The plant crude methanol extract was characterized by a very low degree of toxicity which implies that the leaves of *L. dentata* is non-toxic and relatively safe for consumption [11].

The results of the present research have shown that the crude methanol extract of *L. dentata* exhibited significant (p<0.05) antitrypanosomal activity in experimentally infected rats by reducing the parasitaemia levels and improving weight gain, all in a dose-dependent manner. Extract at 600 mg/kg gave the second highest values (after standard drug) for percentage weight gain and second lowest percentage change in parasitaemia level of 13.31 ± 2.18 and 74.36 ± 6.24 % respectively (Table 4). This plant has been traditionally used in the management of cutaneous and subcutaneous parasitic infections (Nwafor, F. I. personal communication, June 24, 2021). The observed reduction in parasitic blood level showed marked inhibition suggesting the possible effectiveness of *L. dentata* methanol leaf extract as an anti-trypanosomal drug. This observation showed that when the parasitaemia was treated with the extract of *L. dentata* leaf, there was a modification of the disease condition. The decrease in the blood parasite level across the days was an indication that extended treatment with the extract would result in the elimination of the parasites from the blood. The capability of the extract to inhibit parasitic virulence or infestation could be said to be due to the presence of some active secondary metabolites found in the leaf of the plant such as flavonoids, saponins tannins, alkaloids which had been reported in it [18, 19].

In trypanosomiasis, several conditions may be contributory to the significant deficits in blood parameters observed in infected animals and these may include haemolysis [20]. In this study, the crude methanol extract of the leaf of *Lindacteria dendata showed* remarkable changes alterations in hematological parameters of *T. brucei*-infected rats by enhancing the blood parameters in a dose-dependent manner. Extract at 600 mg/kg gave the second highest values for RBC, HGB and PCV of 1.97 ± 1.99 (5.6 x 10^{12}/l), 11.50± 1.43 g/dl and 35.1 ± 2.72 % respectively when compared to both the standard and the untreated group with 1.42 ± 3.47 (5.6 x 10^{12}/l), 9.50 ± 2.52 g/dl and 28.00 ± 4.94 % respectively**.** The decrease in heamoglobin and RBC levels in the negative control group is an indication of anaemia caused by the infection. This is in agreement with previous studies which have shown that infection with trypanosomes resulted in increased susceptibility of red blood cell membrane to oxidative damage probably due to depletion or reduction of glutathione on the surface of the red blood cell [21, 22].

The severity of anaemia has been related to the intensity and length of parasitaemia, and proliferating parasites [23]. Other possible cause of anaemia which before now has been disregarded is malnutrition which often arise sequel to anorexia. Anorexia is a common clinical finding in infected animals and over time compromises the availability of essential elements necessary for haematopoesis which are obtainable from food. Anorexia plays a significant role in anaemia especially in chronic conditions which enhance depletion of nutrient body reserves [22].

The significant reduction of PCV of the untreated groups was in agreement with the report of a number of studies who reported a reduction in PCV [24] in Vevert monkeys. Treatment with methanol extract of *L. dentata* leaf caused improvement in PCV values of the infected rats. The result of this investigation shows that *L. dentata* leaf is as potent as Diminazene Aceturate (7 mg/kg) (the standard drug) in the elimination of trypanosomes parasites from an infected blood.

5.3. Conclusion

The study evaluated the pharmacognostic profile of the leaves of *L. dentata* which can be included in the monograph for future reference. The study also revealed the presence of possible usefulness of *L. dentata* leaf extract as an anti-trypanosomal agent through the reduced concentration of parasitaemia in the blood of the test animals as seen in the elimination of trypanosome parasites from an infected blood.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

1. Fletcher SM, Stark D, Harkness J, Ellis J. Enteric Protozoa in the Developed World: A Public Health Perspective. Clinical Microbiology Reviews. 2012;25(3):420-449.
2. Hotez PJ, Bottazzi ME, Dumonteil E, Valenzuela JG, Kamhawi S, Ortega J. Texas and Mexico: Sharing a Legacy of Poverty and Neglected Tropical Diseases. PLoS Neglected Tropical Diseases. 2012b;6(3):e1497.
3. Hanboonkunupakarn H, White NJ. The Threat of Antimalaria Drug Resistance. Tropical Diseases, Travel Medicine and Vaccines. 2016;2:10.
4. Blasco B, Leroy D, Fidock DA. Antimalaria Drug

Resistance: Linking *Plasmodium falciparium*. Parasite Biology to the Clinic. Nature Medicine. 2017;23(8):917-928.
5. De Koning HP. Drug Resistance in Protozoan Parasites. Emerging Tropics in Life Sciences. 2017;1:627-632.
6. Simoben CV, Ntie-Kang F, Akone SH. Compounds from African medicinal plants with activities against selected parasitic diseases: schistosomiasis, trypanosomiasis and leishmaniasis. Nat. Prod Bioprospecting. 2018;8:151-169.
7. https://powo.science.Kew.org/taxon/urn:/Sid:ipn.org:names:365668.
8. IBID.
9. Evans WC. Trease and Evans Pharmacognosy. 15th Edition. London: W.B. Saunders and Co, 2002, 583-596.
10. British Pharmacopoeia London: Her Royal Majesty Stationary Press, 2001, A108-A110.
11. Lorke D. A new approach to practice acute toxicity testing. Arch Toxicol. 1983;53:275-289.
12. Ene AC, Atawodi SE, Ameh DA, Kwanshie HO, Agomo PU. Experimental induction of chloroquin resistance in Plasmodium berghei NK 65. Trends Med. Res. 2008;3(1):16-23.
13. Herbert WJ, Lumsden WH. *Trypanosoma brucei*: a rapid 'matching' method for estimating the host's parasitaemia. Exp Parasitol. 1976;40:427-431.
14. Cheesbrough M. Haematological tests. In: District laboratory practice in tropical countries. Part 2. Second edition. Cambridge, UK: Cambridge University Press, 2006, 268-347.
15. Onyekere PF, Odoh UE, Peculiar-Onyekere CO, Nwafor FI, Ezugwu CO. Phytochemical analysis and anti-diabetic activity of leaf extract of *Psydrax horizontalis* Schum. & Thonn (Rubiaceae). Pharmacog J. 2020;12(1):1699-1706.
16. Kokila K, Priyadharshini SD, Sujatha V. Phytopharmacological properties of *Albizia* species: A review. Int J Pharm Pharma. 2013;5(3):70-73.
17. Udodeme HO, Odoh UE, Ugwu PN, Diovu EO, Okonta EO, Onyekere PF, *et al*. Pharmacognostic studies of the leaves of *Stachytarpheta jamaicensis* Linn. (Vahl) (Verbenaceae). Int J Pharmacog Phytochem Res. 2016;8(9):1503-1508.
18. Adamu M, Nwosu CO, Igbokwe IO. Toxicity and phytochemical constituents of aqueous extract of *Ocimum gratissimum* leaf. Nig Vet J. 2008;29(3):48-57.
19. Adamu M, Nwosu CO, Agbede RIS. Anti-trypanosomal effects of aqueous extract of *Ocimum gratissimum* (Lamiaceae) leaf in rats infected with *Trypanosome brucei.* Afr J Trad Compl Alt Med. 2009;6(3):262-267.
20. Nwoha RIO, Omamegbe JO. Comparative haematological changes in rats experimentally infected with *Trypanosoma brucei brucei* and treated with Imidocarb Dipropionate and Diminazene Aceturate. Res J Vet Sci. 2015;8:36-41.
21. Taiwo VO, Olaniyi MO, Ogunsanmi AO. Comparative plasma biochemical changes and susceptibility of erythrocytes to *in vitro* peroxidation during experimental *Trypanosome congolense* and *T. brucei* infections in sheep. Israel J Vet Med. 2003;58:4.
22. Abdullahi AM, Iliyasu D, Galadima HB, Ibrahim UI, Mbaya AW, Wiam I. Effects of trypanosomosis on hemogram and some biochemical parameters of guinea pigs experimentally infected with *Trypanosome Brucei Brucei* in Maiduguri, Nigeria. Dairy Vet Sci J. 2018;8(3):555-737.
23. Ekanem JT, Kolawole OM, Abbah OC. Trypanocidal potential of methanolic extract of *Bridelia ferruginea* Benth bark in *Rattus novergicus*. Afr J Biochem Res. 2008;2(2):045-050.
24. Kagira JM, Thuita JK, Ngotho M, Mdachi R, Mwangangi DM. Haematology of experimental *Trypanosoma brucei* rhodesiense infection in vervet monkeys. Afr J Health Sci. 2006;13:59-65.