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Original Article

Pharmacognostic Evaluations of Leaves and Rootback of *Lophira lanceolata* Tiegh. Ex Keay (False Shea)

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ABSTRACT

Objectives: The present study sought to establish quality control parameters of a locally and ubiquitously occurring medicinal plant, *Lophira lanceolata* which is utilized as folk medicine among the local communities in Northern Nigeria as well as other regions, due to its therapeutic indication mainly as an aphrodisiac, among other uses. **Materials and Methods:** Pharmacognostically, different types of evaluations were carried out that focuses on organoleptic, macroscopic, microscopic, chemical evaluations as well as heavy metal assessments using Atomic Absorption Spectroscopy (AAS). **Results:** Organoleptic and macroscopic studies revealed some features of the leaves as green and oblanceolate, pinnate venation, with an average leaf size length and width of 14-15 and 4-5 cm respectively. The root has a light pale-brown outer surface and a reddish-brown inner surface. The outer surface was soft and dry while the inner surface was moist and smooth. Leaf microscopy indicated the presence of anisocytic or cruciferous type of stomata while both the rootbark and leaves are not devoid of common ergastic cell contents of calcium oxalate, lignin, starch, protein and tannin. Phytochemical evaluations revealed abundance of phytoconstituent that are richly phenolic of the types of saponin, tannin, triterpenoid, flavonoid, glycosides, diterpenoids, alkaloid, steroid, anthraquinones and phenols etc. Physicochemical evaluations showed a good source of mineral content of carbohydrate (35.93 ± 1.9199 for leaves and 33.58 ± 1.6791 for rootbark), Ash (9.33 ± 0.4714 for leaves and 7.17 ± 0.2358 for rootbark) in the plant. Heavy metal analysis of the leaves and rootbark investigated for Cadmium, Copper, Lead and Mercury showed that their concentrations were within the WHO (2002) permissible limits. **Conclusion:** This study provides the scientific data for the proper identification and establishment of standards for the use of the plant, *Lophira lanceolata* (False shea).

Keywords: *Lophira lanceolata*, Stomata, Standardization, Chloral hydrate, Evaluation, Heavy metals.

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INTRODUCTION

Medicinal plants are playing very active role in Traditional Medicines for the treatment of various ailments.[1] The problems often encountered in the developing countries that DE promotes the peoples great use of native medicine are poor documentation or scarcity of records as well as the lack of evidences or a complete absence of stringent quality control measures. There is a need for records of all research findings carried of Traditional Medicines in the form of documentation. With these deficiencies in mind, it has become extremely important to emphasize and ensure the standardization of indigenous plant and parts been used as a medicine. However, the standardization processes, can be achieved using different techniques and methods towards the evaluation of the desired targets. For instance, in a typical pharmacognostic studies of natural products, a whole lot of scientific data can be obtained that would enable the standardization of most Traditional Medicines. The evaluation steps and processes would most often yield results that are helpful in identification and standardization of the plant material. Correct characterization and quality assurance of starting material is an essential step to ensure reproducible quality of herbal medicine, which in turn justify safety and efficacy.[2-3]

Lophira lanceolata belongs to the Ochnaceae family and is originally from Africa.[4] *L. lanceolata* is the most common among the species, specially found in the dry Savannah areas and also in the forest zone of West Africa. African countries hold different names for the plant. In Nigeria, the Igbos calls it *Okopia*; the Yorubas, *Ikponhon* and the Hausas, *Namijin Kadanya*. *L. lanceolata* is commonly known as 'Dwarf Red Ironwood', Beung (False Shea), or *Meni oil tree*.[5-6] The plant can grow 5 to 12 meters tall or up to the size of a big tree with short branches.

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The stem bark is rough in appearance, broken into thin corky patches and of gray colour. The leaves are rounded at the top and elongated in nature. *L. lanceolata* usually starts developing fruits between February and April.[7]

The plant has a long history of use in Traditional Medicine, to which modern research has confirmed the presence of medically active compounds. The leaves contain lanceolatin A and B.[8-9] The meni oil, obtained from the fruit is used to treat dermatosis, toothache and muscular stress.[9] A decoction of the roots is taken orally by women to treat menstrual pain, intestinal ailments and malaria.[10] The bark is also used to treat gastro-intestinal problems and fevers.[8] A decoction of the young, fresh or dried leaves is given to treat pain caused by intestinal worms, dysentery and diarrhea in children.[8] Decoctions of the young red leaves are also employed in the treatment of headache, hypertension and syphilis.[10] A steam bath of the leaves is said to cure general tiredness and rheumatism.[7] The plant is used locally in Sokoto for the treatment of erectile dysfunction in males.[6]

Lophira lanceolanta has gained wide acceptance among the local community in the Northern part of Nigeria as well as in other regions, due to its therapeutic indication as an aphrodisiac among other uses. There has been a wide use of its various plant parts traditionally without a corresponding scientific data on authentication, safety, efficacy and other standardization parameters that would ensure an effective quality control. Consequently, this research has set out to evaluate its potential safety through the investigation of heavy metals constituents as well as other Pharmacognostic parameter that would fill in literature gaps leading toward a proper standardization of this plant.

MATERIALS AND METHODS

Plant Collection

The fresh leaves and roots of the plant were collected from its natural habitat in Zuru town of Zuru Local Government Area of Kebbi State, Nigeria. It was authenticated by a botanist in the Biological Sciences Department of Usmanu Danfodiyo University Sokoto (UDUS). The plant sample was labeled and deposited at the Herbarium unit of the Department of Phamarcognosy, UDUS with a voucher identity given as PCG/UDUS/Ocha/0001. The leaves were selected and air dried while the rootbark was separated, cleaned and also air dried to a constant weight. The dried materials were pounded with mortar and pestle to form a dry powder and were safely kept until use.

Organoleptic Evaluations

Organoleptic evaluations were performed according the color, size, odor and taste parameters.

Macroscopic Evaluations

Different macroscopic parameters of the leaves and root were noted. Leaves evaluation include absence or presence of petioles and different characters of lamina shape indentations, base, texture, venations, apex. Root was studied for its size, shape, surface, fracture.

Microscopic Evaluations

Microscopy evaluations were done on both leaves and rootbark sample. All evaluations were performed on student compound microscope.

Qualitative Microscopy

For qualitative microscopic analysis, transverse section of the leaf and root were made by using microtome and free hand sectioning. Staining procedure was performed according to standard procedure. Various identifying characters were studied and images were captured using a digital eyepiece camera.

Powdered Microscopy (Chemomicroscopy)

Shade dried leaves and roots were finely powdered and studied under microscope. Small quantity of different plant parts powder was placed separately on slides and each slide was mounted with the addition of 2-3 drops of chloral hydrate, and each slide was covered with cover slip and examined under microscope. Different cell components were noted and photography was done using digital eyepiece: digital dual camera of android 10 version phone (OnePlus 6 model).[11]

Physicochemical Analysis *L. lanceolata*

Physicochemical analysis of the leaves and rootbark of *L. lanceolata* were carried out to establish parameter of moisture, ash, crude fiber, crude fat, crude protein and carbohydrates compositions. The methods of 'Association of Official Analytic Chemist',[12] were utilized for these determinations.

Phytochemical Evaluation of *L. lanceolata*

The plant samples were phytochemical screened (qualitatively and quantitatively) to determine their constituents using standard procedures as found in various literatures.

Qualitative Analysis

Secondary metabolites were investigated according to various literatures.[13-16]

Quantitative Analysis

The plant samples were quantitatively evaluated of some phenolic phytoconstituents using gravimetric (standard procedure) methods as found in literatures.[17-18] Alkaloids, flavonoids and saponins were evaluated.

Heavy Metal Analysis of *L. lanceolata*

Heavy metal content investigation of *L. lanceolata* leaves and rootbark was performed using Atomic Absorption Spectroscopy (AAS) instrumentation. Measurements were made using a hollow electron discharge lamp (EDL) for cadmium, zinc, chromium, copper, lead, manganese and iron at wavelengths of 228.8, 213.9, 357.9, 324.8, 283.3, 279.5 and 248.3 nanometers (nm) respectively. All samples were run in triplicates to minimize error.

Statistical Analysis

Data were expressed as means + standard deviation of mean. One way analysis of variance (ANOVA) was utilized to analyze result with statistical differences between group means. Significant differences between means were considered at $p<0.05$.

RESULTS

Organoleptic and Macroscopic Evaluations

The organoleptic features of leaves and roots bark of L. *lanceolata* are presented in the Table 1. The leave morphology and rootbark macroscopic features is as shown also in the images of plate 1.

Microscopic Evaluation

Leaf Microscopy

Qualitative microscopic features of the fresh leaves of *L. lanceolata* showed an anisocitic stomata surrounded by guard cell and subsidiary cells, as in Plate 2.

Table 1: Organoleptic/macroscopic features of leaves and roots of *L. lanceolata*.

Parameter	Leaves	Root bark
Average length	14.86 cm	-
Average Width	4.28 cm	-
Shape	Oblanceolate	Curved
Margin	Entire	-
Base	Uneven	-
Apex	Obtuse	-
Venation	Pinnate	-
Colour	Green	Outer surface: Light pale-Brown Inner Surface: Reddish Brown
Odour	Indistinct	Odourless
Taste	Bland	-
Texture	Smooth	Outer surface: soft and dry Inner Surface: Moist and smooth
Leaf presentation on twig	Cluster	-
Secretion	-	Outer surface: No secretions Inner surface: waxy gum

Table 2: Chemomicroscopic features of powdered leaves and rootbark of *L. lanceolata*.

Substance	Test	Observation	Leaves	Root
Starch	Sample+ N50 iodine	Blue black	Present	Present
Calcium Oxalate	Sample+ chloral hydrate +dilute glycerin	Crystal Compounds of calcium oxalate	Present	Present
Lignin	Sample + phloroglucinol +conc. H_2SO_4	Reddish leaves and brownish roots bark	Present	Present
Tannin	Sample+ water +$FeCl_3$	Bluish for both samples	Present	Present
Protein	Test a: Sample +1% Picric acid	Yellowish	Present	Not detected
	Test b: Sample +Millions reagent	Yellowish	Present	Not detected

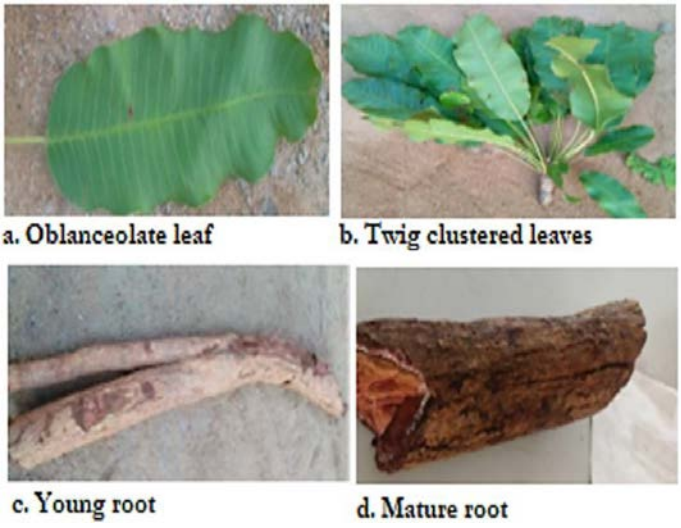

Plate 1: Leaves and rootbark Morphological features of *L. lanceolata*.

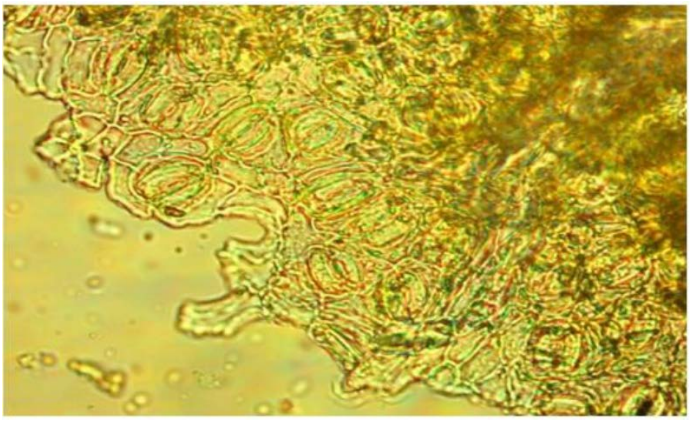

Plate 2: Anisocytic (cruciferous) stomata observed from free hand sectioning of cut leaf.

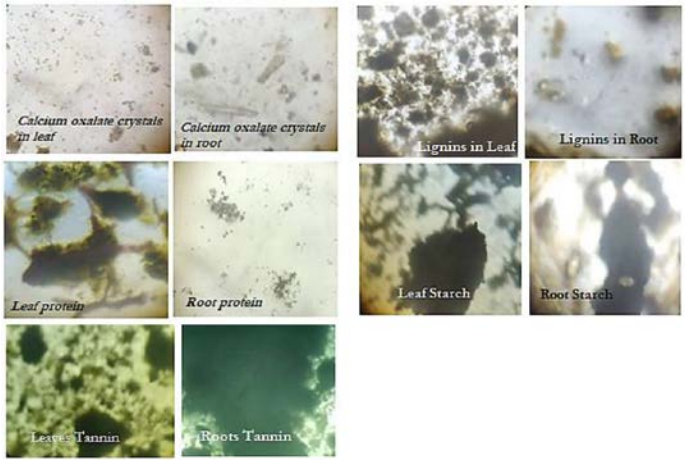

Plate 3: Chemomicroscopical features of ergastic cell content of leaves and rootbark samples of *L. lanceolata*.

Chemomicroscopy

Stained powdered sections of both leaves and root samples under the microscope revealed features such as starch, Calcium oxalate, Lignin, tannin and protein. These are presented in Table 2. These features of cell ergastic contents are also shown in plate 3.

Physicochemical Evaluation

Physicochemical analyses of *L. lanceolata* leaves and roots bark (Table 3) showed the presence of moisture, crude protein, ash, fibre lipid, nitrogen and carbohydrate. Both leaves and rootbark have high concentrations of carbohydrate (35.93 ± 1.9199% and 33.58 ± 1.6791% respectively) while Lipid was found to be high in root 17.83 ± 1.0274% comperes to 9.00 ± 0.8165% lower concentrations in the leaves.

Table 3: Physicochemical compositions of the leaves and rootbark samples of *L. lanceolata*.

Composition	Leaves	Roots bark
	%	%
Moisture	8.83 ± 0.2358[a]	8.83 ± 0.2358[b]
Ash	9.33 ± 0.4714[a]	7.17 ± 0.2358[a]
Lipid	9.00 ± 0.8165[a]	17.83 ± 1.0274[b]
Fiber	24.33 ± 0.6236[a]	23.67 ± 0.6236[b]
Nitrogen	2.0113±0.0283[a]	1.428 ± 0.0300[a]
Crude protein	12.57 ± 0.1806[a]	8.92 ± 0.1897[a]
Carbohydrate	35.93 ± 1.9199[a]	33.58 ± 1.6791[b]

- Values presented as mean ± standard deviation.
- Values with the same letter as superscript on the same column are not significantly different at $p \leq 0.05$ ($n = 3$)

Table 4: Qualitative Phytochemical of test of leaves and rootbark of *L. lanceolata*.

2[0] Metabolite	Qualitative Test	Observation	Leaves	Root
Saponin	Froth test	Formation of Froth 1cm height	+	+++
Flavonoid	Ferric chloride	Greenish black	++	++
	Alkaline	Orange color	+	+
	Shinoda's	Orange Color	+	++
Tannins	Lead acetate	Milky ppt.	++	+
	Ferric Chloride	Blue-black ppt	++	++
Phenols	Ferric chloride	Blue- black ppt	++	++
Anthraquinone	Bontrager's test	Rose Pink	-	+
Glycosides	Fehling's test		+	++
Triterpenoids	Liberman Burchard	Reddish violet	++	+
	Salkowski's test		++	+
Diterpenoids	Copper acetate	Emerald green	++	-
Alkaloids	Hager's	Yellow ppt	++	+
	Dragendorff's	Reddish ppt.	++	+
	Mayer	Creamy-white ppt.	+	+
Steroids	Liberman Burchard	Yellowish green fluorescence	++	+
	Salkowski's Test		++	+

Phytochemical Analysis

Qualitative

Qualitative phytochemical screening of *L. lanceolata* leaves and rootbark showed the presences of secondary metabolites of alkaloids, steroids, diterpenoids, phenols tannins and triterpenes, etc. these are presented in Table 4.

Quantitative

Table 5 shows quantitatively, the phytochemical composition of *L. lanceolata* leaves and rootbark. Flavonoids were found to be in highest amount for both roots and leaves (254.0 ± 10.6145 mg/g and 264.0 ± 9.2015 mg/g) respectively compared to other phytochemicals. Alkaloids (detected moderately in leaves earlier) were found in concentration ranges of 81.3±2.4944 mg/g compare to lower concentration in root bark of 39.3±0.9428 mg/g. The concentrations of saponins were found in moderately in the rootbark (116.8 ± 0.1513 mg/g) and lower in the leaf (21.0 ± 0.00 mg/g).

Table 5: Phytochemicals compositions (mg/g) of leaves and roots bark of *L. lanceolata*.

Composition	Leaves	Rootbark
Saponin	21.0 ± 0.00[a]	116.8 ± 0.1513[b]
Flavonoid	264.0 ± 9.2015[a]	254.0 ± 10.6145[b]
Alkaloid	81.3 ± 2.4944[a]	39.3 ± 0.9428[a]

Table 6: Heavy Metals Concentration (ppm) in leaves and rootbark of *L. lanceolata*, compares to WHO permissible limits.

Composition			WHO *PL (ppm)
	Leaves	Rootbark	
Cd	0.0137	0.0172	0.30
Zn	0.8753	0.0510	50.0
Cr	0.0043	0.0043	2.00
Pb	-0.0675	-0.9028	10.00
Cu	0.1260	0.0751	20.00
Mn	0.2759	0.2161	2.00
Fe	2.1653	2.7782	

*Permissible limits of WHO in part per million.

Heavy Metal Analysis

Seven metals were assayed in both the leaves and rootbark of *L. lanceolata*. Their compositions apparently revealed relatively high concentration of iron (Table 6). The metals analyzed indicate that the leaves contained some high level of zinc and manganese (0.8753 and 0.2759ppm respectively) compared to the rootbark for same metals with 0.0510 and 0.2161 ppm respectively. Very low concentrations of lead were seen for both leaves and rootbark, with comparable values of -0.0675 ppm and -0.9028 ppm respectively.

DISCUSSION

The very need for standardization of herbal drugs, especially of ones ubiquitously used in unlettered societies has become necessarily important due to issues of safety and the gains that can be obtained from maximum beneficial use of natural products. Thus, to fill in gap of information lacking concerning the traditional use of a particular crude drug, the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that offers guaranty for the assurance of quality, efficacy, safety and reproducibility has become a necessary and an indispensable task to be achieved. The immediate gains would be the elimination of the danger of drug substitution or counterfeit of herbal materials which are often found in the markets.

The various parameters studied such as organoleptic/macroscopic and microscopic analysis (quantitative microscopy was not carried out due to some constrains), are fairly one of the cheapest methods to correctly

identify a particular drug and to establish authenticity of raw material. The Morphological and microscopical studies of the stem bark and rootbark of *L. lanceolata* shows that the data obtained are unique for this specie and can be reproducibly studied further, which is a measures of a good quality assurance indicators.

Phytochemically, the results revealed the plant to be a good source of phenolic (Table 4). Hence, consumption of it leaves and roots as drugs may serve as a good source of antioxidant. The quantitative analysis evaluated for total flavonoid, saponin and alkaloid contents in the leaves and rootbark revealed a significant difference in the flavonoid and saponin contents between the leaf and rootbark samples. The results of alkaloid contents between the leaves and rootbark showed no significant difference (Table 5). Flavonoid is a natural antioxidant; they play a role in plant defense system. Alkaloids are nitrogenous compounds that play a role in protection of plant against pathogens and herbivores and are widely used as stimulants, pharmaceuticals, poisons and narcotics. These findings are thus, in line with those obtained and reported in literatures[8-9,19-21] for the leaves, seeds, rootbark and stem bark of *L. lanceolata.*

Table 6, show the concentrations of heavy metals in leaves and rootbark of *L. lanceolata*. Heavy metals affect the nutritive contents of agricultural products and also have a harmful effect on humans. The World Health Organization (WHO) sets the maximum permissible limit of toxic metals in human herbs and other food items; hence an essential aspect of herbs and food quality demands the regulation of the concentrations of heavy metals in plants, especially crude drugs.[22-23]

Cadmium is a non-essential element in plants and primarily it accumulates in the kidneys and liver.[24] In all the samples analyzed, the concentration was observed to be below the permissible limit of 0.3 ppm as reported by WHO[25] which might mean safe for human consumption. It was highest in rootbark with a value of 0.0172 ppm and lowest in the leaves with a value of 0.0137 ppm. Zinc is one of the most essential metals for normal growth and development in humans.[24] Zinc deficiency is of growing concern in developing countries. Excess Zinc can also be harmful, and cause Zinc toxicity. The concentration of Zinc in all the samples analyzed was observed to be below the WHO permissible limit of 50.0 ppm. Zinc (Zn) accumulation in high amount can cause eminent health problems, such as stomach cramp, skin irritation, vomiting, nausea and anemia.[26] The concentrations of lead (Pb) in the samples were found to be below WHO established permissible limit of 10.0 ppm. Thus, Pb was higher in the leaves with -0.0675 ppm. than the rootbark. Lead being a harmful body poison can enter into the human system through air, water and food and cannot be eliminated through plant washing.[24] The traces of lead found in the plant could be linked to its concentration in the plant's habitat resulting from pollution consequence of road traffic lead emission from petrol engines. Similarly attest to this in their findings. Copper is the third most used metal in the world.[27] Copper is an essential micronutrient required in the growth of both plants and animals. The concentration of Cu in all the samples analyzed was lower than the WHO permissible limit of 20.0 ppm. The highest levels of Cu were observed in leaves (0.1260 ppm). This result obtained tends to be lower compared to others from literature. Manganese (Mn) is essential for normal bone structures and reproduction. Mn plays a very essential role in the functioning of the central nervous system. Mn deficiency will lead to reproductivity failure in both male and female.[28] The Mn content in the plant samples evaluated were in agreement with earlier reported findings.[29-30] Since the level of Mn in both samples analyzed were lower than the standard, it is likely that continuous consumption of these parts of the plant would not have any health effects. Iron (Fe) is an important element in humans and plays a significant role in the formation of hemoglobin, oxygen and electron transfer in the human body. In all the samples analyzed, Fe concentration was observed to be below the standard limit of 2.0 ppm stipulated by WHO and FAO.[31] The concentration of Chromium (Cr) in the plant samples were found to be below the WHO[25] standard. Chromium is not essential for plant growth; it was not detected in high concentration for both the leaves and roots bark of *L. lanceolata* due to the fact that uptake of Cr by plant shoot is generally low.[10]

CONCLUSION

In Nigeria herbal medicines are sold by the road sides in markets and some herbal medicines stores. People, mostly of unlettered societies believe that herbal medicines are often safe and therefore use same without requisite knowledge on safety and authenticity. The present study thus, offers some findings on pharmacognostic parameters of leaves and rootbark samples of *L. lanceolata*. These can be employed as suitable quality and safety measures as well as standards for the validation and authentication of folklore information. It could as well serve as safety indicators of crude/herbal drug of *L. lanceolata*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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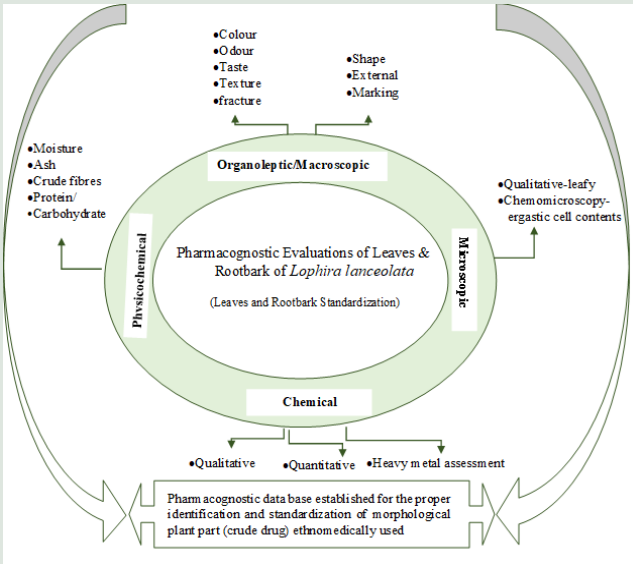
GRAPHICAL ABSTRACT



SUMMARY

This study revealed some pharmacognostic data of organoleptic, macroscopic, microscopic, physicochemical and chemical constituent analysis of *Lophira lanceolata* leaves and rootbark, which has gained a wide acceptance and folklore use among the local communities in Northern Nigeria. Cruciferous type of stomata was present in leaves while in both leaves and roots, common ergastic cell contents of calcium oxalate, lignin, starch, protein and tannin were identified. The studied plant parts had revealed a rich phytoconstituents of phenolic metabolites while physicochemical evaluations showed a good source of mineral content of carbohydrate (35.93 ± 1.9199 for leaves and 33.58 ± 1.6791 for rootbark), Ash (9.33 ± 0.4714 for leaves and 7.17 ± 0.2358 for rootbark) in the plant. Heavy metal analyzed for both leaves and rootbark for Cadmium, Copper, Lead and Mercury showed that their concentrations were within the WHO (2002) permissible limits.

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