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Morphological, anatomical and molecular characterisation of the leaves of *Isoberlinia doka* Craib and Stapf and *Isoberlinia tomentosa* (Harms) Craib and Stapf

Authors:
Hadiza Bello[1,2]
Umar A. Katsayal[2]
Aminu A. Ambi[2]
Yusuf B. Abubakar[1]

Affiliations:
[1]Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria

[2]Department of Pharmacognosy, Faculty of Pharmaceutical, Ahmadu Bello University, Zaria, Nigeria

Corresponding author:
Hadiza Bello,
hbello855@gmail.com

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Background: *Isoberlinia* (Craib and Stapf) is a genus with high economic and pharmacological values.

Aim: This study aimed at establishing the morphological, anatomical and molecular characterisation of the leaves of *I. doka* and *I. tomentosa*, which were conducted for proper authentication.

Setting: The leaves of *I. doka* and *I. tomentosa* were obtained from Shika, kaduna State, Nigeria.

Method: Morphological and anatomical characters were determined according to standard procedures, while molecular identifications were performed using ribulose-1,5-bisphosphate carboxylase (rbcl) gene and internal transcribed spacer (ITS) DNA barcode's region.

Result: Morphological studies revealed similar features for both species except for the shiny leaves of *I. doka* and rough abaxial surfaces of *I. tomentosa* because of the presence of trichomes. Variations were observed in their epidermal features, stomatal index, stomata frequency, presence or absence of trichomes, trichomes frequency and their quantitative anatomical features. The quantity and quality of DNA measured at A260/280 ratio using nanodrop spectrophotometer were 29.1 ng/µL and 1.74 ng/µL for *I. doka*, respectively, while the *I. tomentosa* concentration and purity were 71.1 ng/µL and 1.85 ng/µL, respectively. Agarose gel electrophoresis revealed two DNA bands with 700 bp (rbcl) and 600 bp (ITS). The sequence analysis revealed maximum identity with National Centre for Biotechnology Information (NCBI) GeneBank *Isoberlinia* species. Evolutionary analysis supported the monophyletic origin of the genus *Isoberlinia*. The morphological and anatomical characters of *I. doka* and *I. tomentosa* leaves have provided a significant taxonomy tool for proper authentication of this plant.

Conclusion: The findings ascertained that ITS and rbcl served as an improved and efficient tool for species identification of these studied species and could serve as potential DNA barcodes for these taxa.

Contribution: This article suggests that further studies the on screening of these plants, for various pharmacological potentials, might be useful for new drug development.

Keywords DNA barcoding; morphological; GeneBank; polymerase chain reaction; Isoberlinia; anatomical.

Introduction

Isoberlinia Craib and Stapf ex Holland genus in the family Fabaceae (legume family) is a tree native to the hotter parts of tropical Africa (Burkill 1995). The species are significant components of Miombo woodlands and Northern Nigeria guinea Savanah woodlands (Bello & Musa 2016). *Isoberlinia* is a vigorously colonising African tree that often dominates the woodland belt that stretches from Guinea in the west to Uganda in the east.

There are five accepted species of *Isoberlinia*, namely *I. angolensis*, *I. doka*, *I. paradoxa*, *I. scheffleri* and *I. tomentosa* (Burkill 1995). However, the northern Nigeria guinea savanna are dominated with an open woodland of two species, the *I. doka* and *I. tomentosa* (Bello & Musa 2016; Jackson 1970).

Isoberlinia doka is locally called 'doka', while *I. tomentosa* is called 'faradoka' in Hausa. Naturally, these two plants exist as trees or shrubs measuring between 10 m and 20 m tall, with a trunk of about 40 cm – 50 cm diameter, branching from about 5 m upwards. The leaflets are arranged in three or four pairs. The flowers are small and white, forming large open inflorescences that are held conspicuously above the leafy crown (Burkill 1995). The pods are oblong, flat and quite large, about 30 cm long and 10 cm wide. According to Singab and Mostafa (2016), the molecular study of medicinal plants is a promising and prospective scope in the field of pharmacognosy. Deoxyribonucleic acid (DNA) barcoding using ribulose-1,5-bisphosphate carboxylase (rbcl) and ITS genes are an important tool for species identification and standardisation of specific region of the plant genome (Singab & Mostafa 2016; Zabta, Shinwari & Nadia 2014). The molecular approach for the identification of plant species seems to be very effective than morphological markers as it allows direct access to the genome and makes it possible to understand the relationship between individuals (Tripathi et al. 2013). However, DNA markers are based on unique nucleotide sequences and are not affected by environmental factors or physiological conditions (Kim et al. 2016). The leaf epidermal is the most varied organ in providing anatomical features that can be employed as useful taxonomic characters for proper classification, delineation and identification of plant (Aworinde et al. 2009). Leaves can have a wide range of morphological and anatomical studies such as the leaf epidermal studies, stomatal and trichome are considered important in phylogeny and taxonomy (Hameed 2012).

Isoberlinia doka and *I. tomentosa* are widely overexploited for fuel wood in northern Nigeria, and this makes the plant to exist in their shruby state (Bello & Musa 2016). They are widely used in traditional medicine for the treatment of various kinds of diseases, such as ulcer, stomach pain and arrow poison (Abdulkadir et al. 2011) without much scientific validation in accordance with World Health Organisation standard. Pharmacological activities, such as antioxidant, antibacterial, antifertility, and brine shrimp lethality test have been reported for *I. doka* (Abdu et al. 2016; Abdulkadir et al. 2011; Salka, Abubakar & Hassan 2011).

A systematic standardisation, including morphological, anatomical and molecular studies, that could help in proper identification, prevention of adulteration and quality control of drugs are lacking for *I. doka* and *I. tomentosa* species.

Materials and methods

Plant collection and authentication

The fresh plants of *I. doka* and *I. tomentosa* were collected from the man-made forest at Shika *Isoberlinia* woodland, Giwa local government area, Kaduna State. The plants were authenticated by a taxonomist with Voucher specimen numbers ABU 022478 and ABU 016280 deposited as for *I. doka* and *I. tomentosa*, respectively, at the Herbarium, Department of Botany, Ahmadu Bello University, Zaria, Nigeria.

The morphological and anatomical features of *Isoberlinia doka* and *Isoberlinia tomentosa*

Morphological and anatomical examinations on the fresh *I. doka* and *I. tomentosa* were conducted to assess their anatomical features (Evans 2009; WHO 2011). Macroscopic identity of medicinal plant materials was based on shape, size, colour, surface characteristics, texture, fracture characteristics, odour, and taste of fresh and powdered stem bark. The macroscopic characters of the samples were carried out based on the method described by WHO (2011).

Stomatal number and stomatal index

The Adaxial and abaxial epidermal peels of *I. doka* and *I. tomentosa* leaves were rinsed in distilled water severally, cleared with sodium hypochlorite and mounted on a glass slide with few drops of glycerol covered with cover slip and examined under a light microscope (Hund Wetzlar H600/12, Germany). The number of epidermal cells and stomata was counted. Stomatal index (SI) was calculated using the formula:

> SI = [S \ (S+E)] × 100, where S = No. of stomata in an area of 625 μm² & E = No. of epidermal cells. The experiment was repeated 10 times. Average values were determined, and the results were expressed per square millimeter. (Evans 2009)

Vein-islet and vein termination numbers

Small pieces from the leaves were taken midway from margin to midrib and cleared by boiling in a super saturated choral hydrate solution in a test tube placed in a boiling water bath. Vein islet and vein termination were counted using 10 × 10 magnification. Ten readings from continuous squares were taken for counting the vein islets and vein termination (Evans 2009).

Palisade ratio

The average number of palisade cells present beneath each upper epidermal cell is called palisade cell ratio. Leaf pieces of the two plants were cleared on boiling with chloral hydrate solution. These were viewed under a microscope with 10 × 10 magnifications. Palisade layers were determine under four epidermal cells and the focus were changed until the palisade cells were seen within the epidermal cells. Palisade cells including those, which are more than half covered by the epidermal cells, were counted. Palisade ratio of a group was obtained by dividing the resulted value by 4 (Evans 2009).

Transverse section of the fresh parts *Isoberlinia doka* and *Isoberlinia tomentosa*

Transverse section of the fresh parts of *I. doka* and *I. tomentosa* were cut using surgical blades. The sections were cleared in 10% sodium hypochlorite solution, washed in distilled water, and then stained in safranin and fast green. Sections were then mounted between the slide and coverslip using dilute glycerol, and photomicrographs of the slides were obtained.

Isolation of DNA regions of *Isoberlinia doka* and *Isoberlinia tomentosa*

Genomic DNA were extracted from fresh young leaves of *I. doka* and *I. tomentosa* using a protocol of Zymo quick DNA universal kit.

Determination of the quantity and quality of genomic DNA extracted from *Isoberlinia doka* and *Isoberlinia tomentosa*

The quality and quantity of the genomic DNA extracted from the two plants were determine using Thermo scientific NanoDrop 2000 spectrophotometer, measured at the absorbance ratio of 260 by 280 nm.

Polymerase chain reaction of the DNA extracted from *Isoberlinia doka* and *Isoberlinia tomentosa*

Polymerase chain reactions were performed on the DNA isolated from the two plants according to Prasath, Mohan and Divya (2017). The primers ITS1 (5'CCGTAGGTGAACCTTGCGG 3') and ITS4 (5'TCCTCCGCTTATTGATATGC 3') and rbcl gene with forwarding sequence rbcLaF 5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3' and reverse sequence is rbcLaR 5'-GTA AAA TCA AGT CCA CCR CG-3' were used in Eppendorf Personnel Master Cycler (Germany). The polymerase chain reaction (PCR) total volume of the reaction was 20 µL, which include H_2O (16.0 µL), DNA (2.0 µL) and ITS primers 2.0 µmol/L, and the same reaction was carried out using rbcl primers. The conditions for both ITS and rbcl gene are indicated in Table 1.

Gel electrophoresis of polymerase chain reaction products obtained from *Isoberlinia doka* and *Isoberlinia tomentosa*

The PCR products obtained from the two plants were assessed on a 0.8% agarose gel (in Tris-acetate EDTA buffer) electrophoresis at 100 volts with a 100-bp DNA ladder. The DNA was stained with ethidium bromide visualised on a UV transilluminator. The PCR products were subjected to sequencing by Sanger method in an AB Sequencer.

Nucleotide alignment and phylogenetic analysis

The ITS-rDNA and rbcl region nucleotide sequences of the two plants were performed at the NCBI gene blast. Searches were performed using the BLAST megablast parameter search function, to compare the study sequences with the data from the Genebank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were submitted to the GeneBank by Banklt direct submission, and their accession numbers were generated.

The sequences obtained from the two study plants, and some selected GeneBank sequences were aligned using the multiple alignment Clustal W algorithm (Thompson et al. 1997). The phylogenetic analysis based on the rDNA-ITS and rbcl sequences were constructed by the maximum likelihood bootstrap analysis. A total of 1000 bootstrap replicates were performed using MEGA version 6.06 (Kim et al. 2016; Tamura et al. 2013) software.

TABLE 1: Polymerase chain reaction conditions for internal transcribed spacer and ribulose-1,5-bisphosphate carboxylase gene *Isoberlinia doka* and *Isoberlinia tomentosa*.

Step	ITS	Rbcl
1	Initial denaturation -94 °C for 3 min	Initial denaturation -94 °C for 5 min
2	Denaturation -94 °C for 40 s	Denaturation -94 °C for 30 s
3	Primer annealing -54 °C for 40 s	Primer annealing -54 °C for 30 s
4	Extension -72 °C for 40 s	Extension -72 °C for 1 min
5	Go to step 2 repeat 35 times	Go to step 2 repeat 35 times
6	final extensions -72 °C 10 min	final extensions -72 °C 5 min
7	Hold – 4 °C	Hold – 4 °C

Rbcl, ribulose-1,5-bisphosphate carboxylase; ITS, internal transcribed spacer.

Results and discussions

Macroscopicals studies of *Isoberlinia doka* and *Isoberlinia tomentosa* leaves

The macroscopic studies of *I. doka* and *I. tomentosa* leaves exhibited similar features in their dried state as indicated in Table 2, including rough powdered leaves, the organoleptic characters were also similar for *I. doka* and *I. tomentosa,* which are tasteless with characteristic odour and light green. This study further showed that the plant samples change colour after drying, which is similiar to the findings of Ahmed et al. (2014) that moisture loss could affect organoleptic characters. The only notable macroscopical differences between the two plants samples were seen in their fresh conditions as *I. doka* had smooth shiny leaves at adaxial surface as a result of thick cuticular layer and *I. tomentosa* abaxial leaves surface was rough because of non-glandural trichomes as showed in Table 3.

TABLE 2: Macroscopical studies of fresh and powdered leaves of *I. doka* and *I. tomentosa*.

Features	*I. doka*		*I. tomentosa*	
	Fresh leaves	Powdered Leaves	Fresh leaves	Powdered leaves
Size		-		
Texture/surface Characteristics	Smooth at both surfaces	Rough	Smooth adaxial; rough abaxial	Rough
Fracture	Smooth	-	Smooth	-
Colour	Shiny green	Light green	Green	Light green
Odour	characteristics	Characteristics	Characteristics	Characteristics
Taste	Tasteless	Tasteless	Tasteless	Tasteless

TABLE 3: Qualitative, morphological and anatomical features of *I. doka* and *I. tomentosa* leaves.

Characters	*I. doka*	*I. tomentosa*
Lamina		
Shape	Ovate	Ovate
Venation	Pinnately veined	Pinnately veined
Apices	Acuminate	Acuminate
Margin	Entire	Entire
Base	Acute	Acute
Phyllotaxis	Opposite	Opposite
Petiole	Petiolate	Petiolate
Epidermal		
Stomata	Paracytic	Paracytic
Trichomes	Absent	Non-gladular unicellular
Mesophyll cell types	Circular	Circular
Palisade cell types	Rod shape	Rod shape
Epidermal cell types	Irregular, triangular, quadrilateral	Irregular, triangular, quadrilateral, polygonal

This supports the earlier report of Neinhuis and Barthlott (1997) that trichomes is one of the features that caused roughness in epidermal surfaces of water-repellent plants.

Qualitative morphological and anatomical characters of *Isoberlinia doka* and *Isoberlinia tomentosa* leaves

The qualitative, morphological and anatomical characters of *I. doka* and *I. tomentosa*, as shown in Table 3, exhibited similar lamina and epidermal features. The lamina features included ovate shaped, pinnately veined, acuminate apex, entire margin and acute base. Paracytic types of stomata were observed on both epidermal surfaces of the two plants. The main diagnostic taxonomical features to differentiate the two plants were revealed in the presence of trichomes in the abaxial surfaces and additional polygonal types of epidermal cells of *I. tomentosa*, whereas trichomes and polygonal epidermal cells were absent in *I. doka* as shown in Table 3.

Quantitative morphological and anatomical characters of *Isoberlinia doka* and *Isoberlinia tomentosa* leaves

The quantitative, morphological and anatomical characters, as listed in Table 4, showed more variable features amongst *I. doka* and *I. tomentosa.* The petiole length ranges from 0.9 cm to 0.96 cm for the two study species. The lamina length of *I. tomentosa* (16.5 ± 0.12 cm) was larger than *I. doka*. High epidermal cell numbers were observed at the adaxial surfaces of both studied species but their stomata number were found to be higher at the lower epidermis than the upper surfaces. Amphistomata nature exhibited by these plants serve as an adaptative features to help withstand harsh environmental conditions. This finding was different from the report of Abubakar et al. (2018), which revealed a higher stomata number at upper epidermal surfaces of *Cassia* and *Senna* studied species. The study had shown that the types, abundance and nature of stomata distributions in plants are essential for unique physiological responds to changes in the environment (Aguoru, Ahemen & Olasan 2015). The leaf surface constants varied between the two species. However, stomatal index (0.23), vein islets (40.6 ± 0.87 mm^2) and vein termination number (35.6 ± 3.36 mm^2) of *I. tomentosa* were higher than the values obtained for *I. doka,* as shown in Table 4 and Figure 1. Quantitative measurement of transverse

TABLE 4: Quantitative studies of *I. doka* and *I. tomentosa* leaves and stembark.

Features	*I. doka*	*I. tomentosa*
Leaf		
Petiole length Width	0.96 ± 0.02 cm 0.24 ± 0.02 cm	0.9 ± 0.02 cm 0.25 ± 0.02 cm
Lamina length Width	11.8 ± 0.75 cm 6.0 ± 0.28 cm	16.5 ± 0.12 cm 8.21 ± 0.24 cm
Epidermal cell numbers (Ad, Ab)	546 ± 11.15 mm^2 378 ± 13.77 mm^2	627 ± 8.6 mm^2 282 ± 16.5 mm^2
Epidermal cell length (Ad) Width	20 µm – 60 µm 15 µm – 30 µm	30 µm – 50 µm 20 µm – 30 µm
Guard cell length Ad Width	12.5 µm 5 µm	15 µm 7.5 µm
Stomata number (Ad Ab)	14.8 ± 1.34 mm^2 76 ± .6.36 mm^2	13.8.5 ± 0.56 mm^2 60.2 ± 4.0 mm^2
Stomata index Ad Ab	21.7 ± 2.2 mm^2 9.70 ± 0.54 mm^2	17.2 ± 1.45 mm^2 8.3 ± 1.32 mm^2
Stomata ratio	0.19	0.23
Vein islet number	20.6 ± 1.08 mm^2	40.6 ± 0.87 mm^2
Vein termination number	35.2 ± 1.02 mm^2	35.6 ± 3.36 mm^2
Palisade ratio	5.25 mm^2 – 6.25 mm^2	4 mm^2 – 5.25 mm^2
Trichome numbers	Nill	23 mm^2 – 38 mm^2
Midrib (T.S.)		
Palisade mesophyll layer	150 µm	160 µm
Spongy mesophyll layers	300 µm	440 µm
Vascular bundles region	300 µm diameter	400 µm
Xylem vessels	40 µm	40 µm
Phloem	10 µm	10 µm

Ad, adaxial epidermal surface; Ab, abaxial epidermal surfaces.
Results are expressed as mean ± standard error of mean.

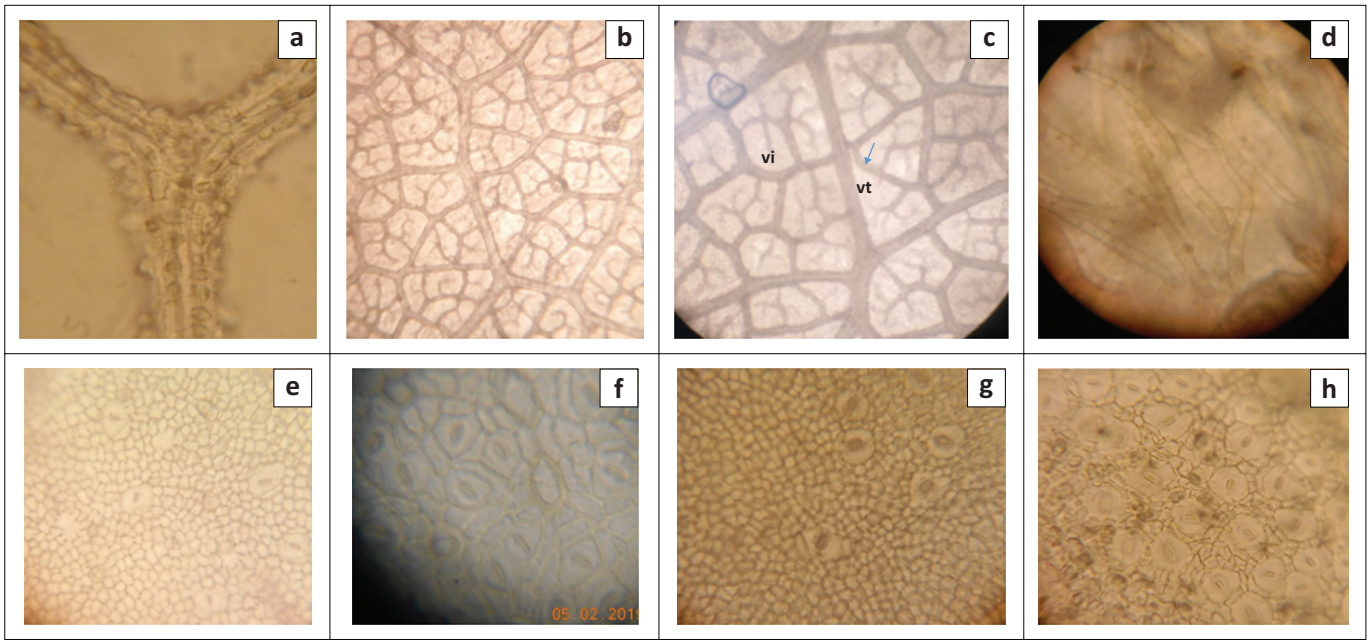


FIGURE 1: Epidermal leaf microscopy of *Isoberlinia doka* and *Isoberlinia tomentosa*. (a). Calcium oxalates crystals of *I. tomentosa*, mag ×400. (b) Vein islets (vi), vt: vein termination of *I. tomentosa* mag x100. (c). Vein islets (vi), vt: vein termination *I. doka* mag x100. (d). Trichomes of *I. tomentosa* mag x100. (e). Adaxial epidermis *I. tomentosa* of mag x400. (f). Abaxial epidermis *I. tomentosa* mag x400. (g). Stomata of *I. doka* at adaxial epidermis mag x400. (h). Stomata of *I. doka* at abaxial epidermis mag x400.

section (TS) across the midrib of leave (Table 4) revealed more variable taxonomical features of *I. doka* and *I. tomentosa*, in contrast with Hohn (1999) that *Isoberlinia* types are weakly delimited, even though they possessed similiar qualitative features.

The transverse section of the leaves of *Isoberlinia doka* and *Isoberlinia tomentosa*

The TS of the leaves of *I. doka* and *I. tomentosa* is dorsoventral in nature. The palisade parenchyma cells of both species appeared in double layers, while the spongy mesophyll cells varied between the two plants. The spongy mesophyll cells of *I. doka* were between 4 and 6 layered, while that of *I. tomentosa* ranged between 6 and 10 layered. The vascular tissue system of both species were well defined with prominent bundle sheath. There were double vascular bundle distributions in both plants, one of the vascular bundle was concentric, amphivasal (phloem towards the inner and xylem at the pheriphery) and the other appeared concave with similar tissue distributions. Trichomes were absent in *I. doka*, while non-glandular trichomes were present at the lower epidermal region of *I. tomentosa* as shown in Figure 2 and Figure 3.

Molecular characterisation of *Isoberlinia doka* and *Isoberlinia tomentosa*

Total genomic DNA were isolated from the collected *I. doka* and *I. tomentosa* fresh leaves and there concentration and purity using nanodrop spectrophotometer were 29.1 ng/uL and 1.74 ng/uL for *I. doka*, respectively, while *I. tomentosa* concentration and purity were 71.1 ng/uL and 1.85 ng/uL, respectively, as shown in Table 5.

Amplicons obtained after PCR were of 700 bp and 600 bp for rbcl and ITS, respectively, and visualised on a 0.8% agarose gel as shown in Figure 4 and Figure 5).

Phylogenetic analysis of *Isoberlinia doka* and *Isoberlinia*

The phylogenetic tree consisted of two main groups ITS and rbcl. The first ITS group consist of two clusters, the first cluster had four series of two cluster with 91%, 98%, 71% and 69% DNA of a genetic similiarities with the following species and their accession numbers : MG949328.1 *I. schefferi*, AF513691.1 *I. doka*, KX057884.1. *I. doka*, KX057885.1 *I. tomentosa*, in NCBI Blast.

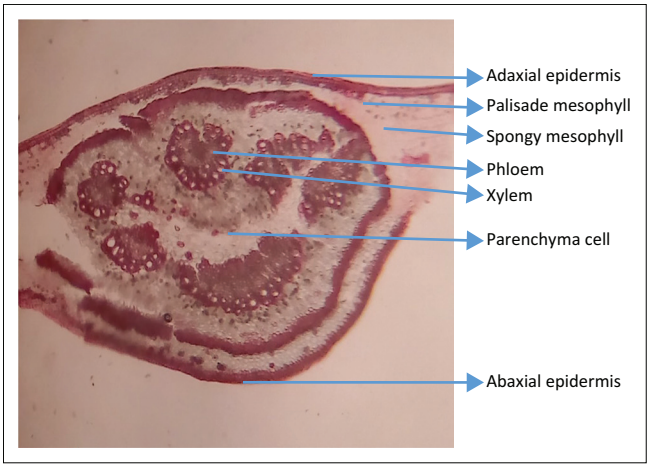
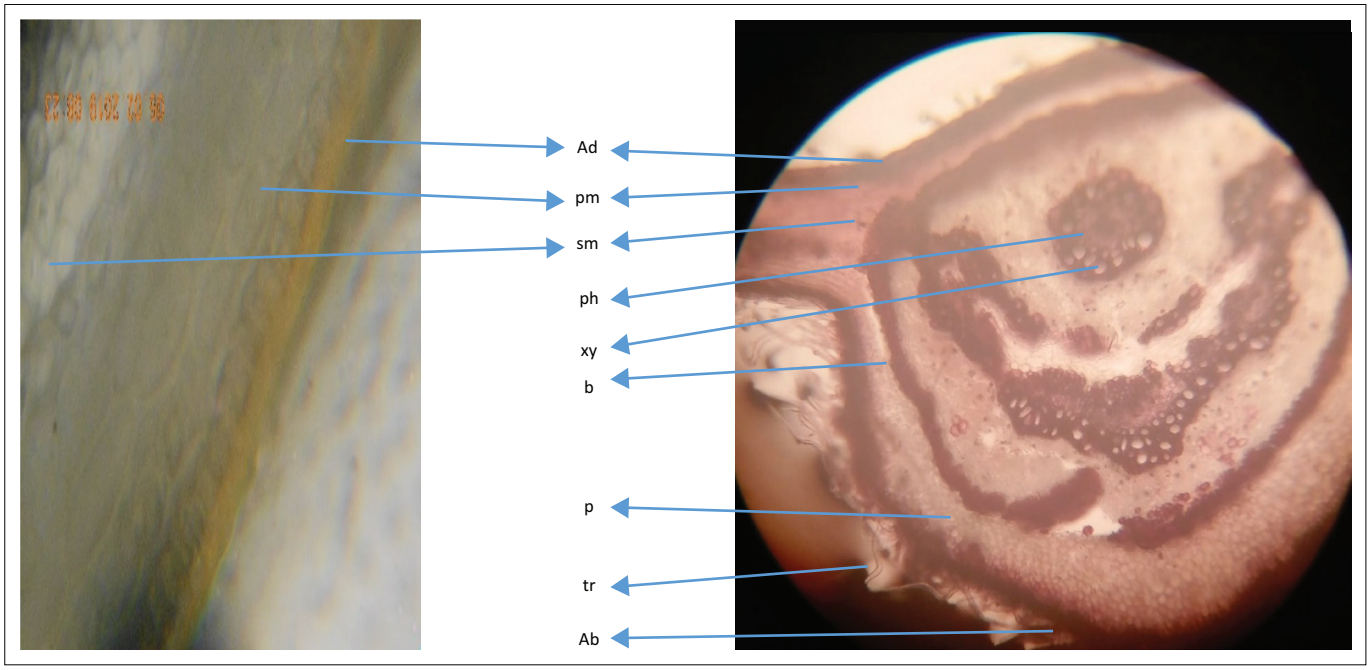


FIGURE 2: Transverse section of *Isoberlinia doka* with magnification × 40.

TABLE 5: Quality and quantity of DNA sample from *Isoberlinia doka* and *Isoberlinia tomentosa*.

Species	Concentration/quantity	Purity (quality)
I. doka	29.1 ng/µL	1.74 ng/µL
I. tomentosa	71.1 ng/µL	1.85 ng/µL

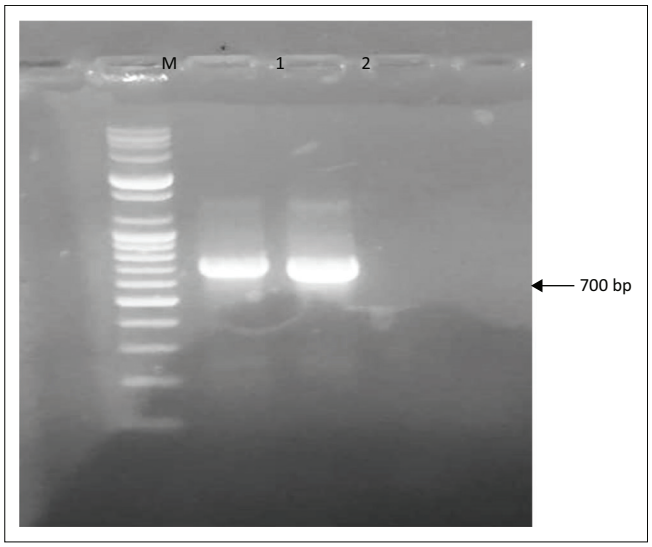


Ad, adaxial epidermis; pm, palisade mesophyll; sm, spongyll mesophyll; ph, phloem; xy, xylem; b, bundle sheath; p, parenchyma cell; tr, trichomes; Ab, abaxial epidermis.

FIGURE 3: Transverse section of *Isoberlinia tomentosa* with enlarge palisade parenchyma cells and a whole section. Magnification × 10.

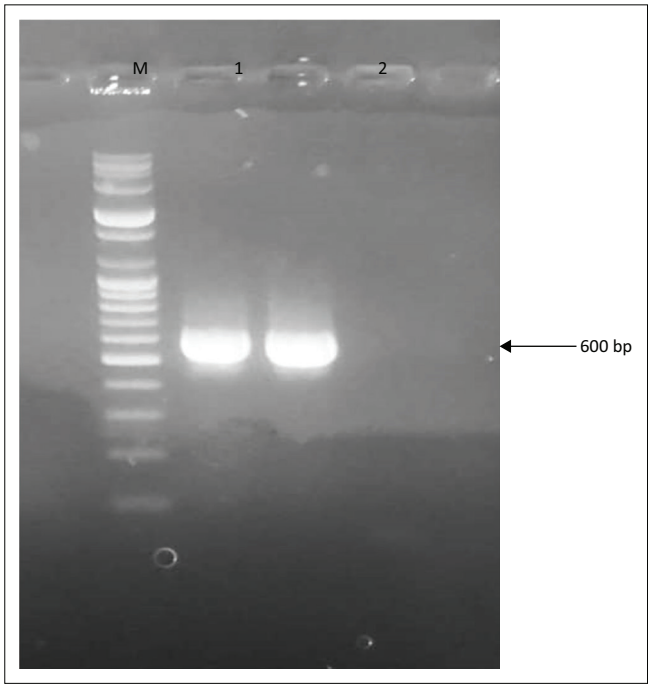
The second cluster had two series with 99% and 100% genetic similiarities with HM041839 *Berlinia orientalis*, KY306506 *Berlinia confusa* and AF513669 *Berlinia confuse* with GeneBank NCBI Blast.

The second group rbcl consists of two cluster, the first cluster shared 67% similiarities between KX119305 *I. doka*, KX119306 *I. tomentosa* and ID rbcl, IT rbcl (study species), respectively. The second cluster shared 88% DNA similiarities with three *Tamarindus indica* AB378732, AB378731, AB378730, as showed in (Figure 6) and sequences were submitted to NCBI GeneBank using Banklt direct submission. The accession number generated for rbcl gene for *I. tomentosa was* MN 879280 and *I. doka* (MN885536). The accession number for ITS rDNA generated for *I. tomentosa* and *I. doka* were MN857896 and MN857895, respectively.



M, molecular weight; 1, *I. doka*; 2, *I. tomentosa*; bp, base pair.

FIGURE 4: Agarose gel electrophosesis of ribulose-1,5-bisphosphate carboxylase gene of *I. doka* and *I. tomentosa*.



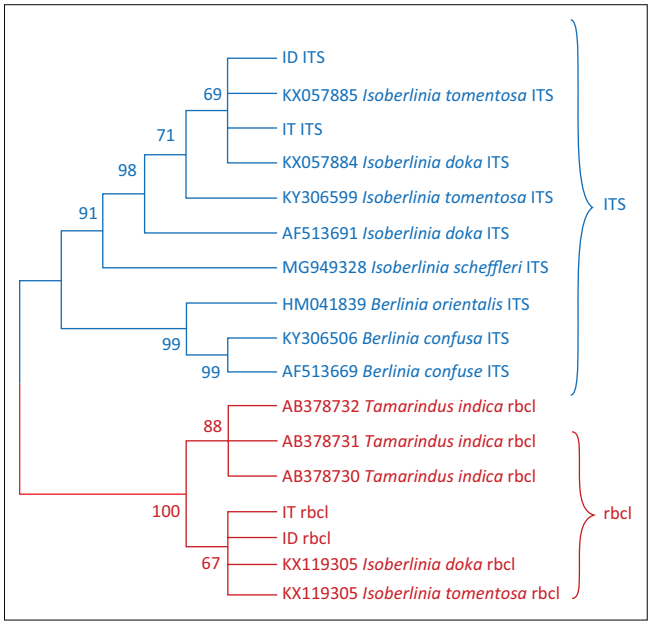
M, molecular weight; 1, *I. doka*; 2, *I. tomentosa*; bp, base pair.

FIGURE 5: Agarose gel electrophoresis of internal transcribed spacer gene of *Isoberlinia doka* and *Isoberlinia tomentosa*.

The ITS and rbcl DNA barcoding marker revealed considerable genetic similarities and identification among the two studied species, which strongly agrees with the morphological similarities observed in this study. A similar report was revealed by Zabta et al. (2014 that the rbcl region was more effective to identify and discriminate the species of *Acacia* and *Abizia*. DNA barcoding could be considered as a good approach for distinguishing and identifying the mint plants (Hameed 2018).

The subfamily detarioideae are depicted by clustering of distinct species within its clade similar to the findings of Oshingboye (2017) that the relationship among the cesalpinoideae also depicted distinct clusters in their tribes. Phylogenetic analysis of this study presents a monophyletic origin of the genus *Isoberlinia*.

The percentage BLAST identity of the sequences obtained for rbcl and ITS gene from the two studied plants when compared with the *Isoberlinia* species at NCBI GeneBank was above 99% to *I. doka* and *I. tomentosa* (Table 6).



ITS, internal transcribed spacer; ID, *I. doka*; IT, *I. tomentosa*.

FIGURE 6: Phylogenetic tree of *Isoberlinia tomentosa* and *Isoberlinia doka*.

TABLE 6: Percentage BLAST identity of *Isoberlinia doka* and *Isoberlinia tomentosa* with the GeneBank sequence data.

Species	Gene region	% Identity in GeneBank
I. tomentosa	rbcl	99.6% to *I. tomentosa,* 99% to multiple species and other genera of Fabaceae
I. doka	rbcl	100% to an *I. doka* voucher and 99% to *I. tomentosa, I. scheferi* and other genera of Fabaceae
I. tomentosa	ITS	99.5% to *I. tomentosa,* 99% to *I. doka* and other genera of Fabaceae
I. doka	ITS	99.2% to an *I. doka* voucher and 99% to *I. tomentosa, I. scheferi* and other genera of Fabaceae

BLAST, Basic local alignment search tool; rbcl, ribulose-1,5-bisphosphate carboxylase; ITS, internal transcribed spacer.

Conclusion

The macroscopic studies of *I. doka* and *I. tomentosa* showed similar features in their fresh leaves with smooth surface characteristics, hard and fibrous fracture, grey in colour, tasteless and characteristics odour. The qualitative, morphological and anatomical characters of *I. doka* and *I. tomentosa* leaves revealed similar lamina features, such as ovate shaped, pinnately veined, acuminate apex, entired margin and acute base. The presence of trichomes in the abaxial surfaces and additional polygonal types of epidermal cells of *I. tomentosa* can serve as a diagnostic tool for proper authentication of this plant. The blast percentage identity of the sequences obtained for the two plants revealed above 99% similarities with the same species in the GeneBank. This finding also ascertained that ITS and rbcl are an efficient tool for species identification of these medicinal plants. Further studies on screening, these plants for various pharmacological potentials might be useful for new drug development.

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Competing interests

The authors declare that they have no conflict of interests.

Authors' contributions

H.B. wrote the first draft of the manuscript; carried out the research, and A.U.K., A.A.A. and B.Y.A. supervised the work and made all the necessary contribution towards the success of the work. All authors read and approved the final manuscript submission.

Ethical considerations

This study followed all ethical standards for research without direct contact with human or animal subjects.

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Data availability

The accession number generated were submitted at the NCBI GeneBank.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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