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PHARMACOGNOSTIC PROFILES AND DNA BARCODING OF THE STEMBARK OF *IRVINGIA GABONENSIS* (AUBRY-LECOMTE EX O'RORKE) BAIL. (IRVINGIACEAE): A FORENSIC PHARMACOGNOSY DATA

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Keywords:

Pharmacognostic fingerprints, *Irvingia gabonensis*, DNA barcoding, Polymerase chain reaction, Adulteration

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ABSTRACT: *Irvingiagabonensis* is a plant popularly used for both medicinal and dietary purposes. The planthave been used to treat various diseases like diabetes, inflammation, alopecia, fever and ulcers. The study was aimed at evaluating some pharmacognostic fingerprints and molecular DNA barcoding of the stembark. The pharmacognostic fingerprint analysis of the stembark was carried out using standard procedures while the molecular DNA barcoding was done using automated DNA extraction and barcoding by the polymerase chain reaction machine. The organoleptic evaluation showed that the greyish brown stembark of *I. gabonensis* has a strong bitter taste, acrid odour and slightly coarse to the touch. Chemomicroscopic evaluations reveal the presence of cellulose, suberin, lignin, mucilage, prismatic calcium oxalate crystals and tannins. The transverse section of the stembark showed bicollateral vascular bundle, beaded hexagonal shaped cork cells, fibre sclereids with wide cell lumen, non-striated eccentric oval shaped aggregate starch grains, and wide pith as observed under the scanning electron microscope. The moisture content was 5.13 ± 0.11 w/w %, total ash was 18.58 ± 0.23 w/w/ %, water soluble and acid insoluble ash were13.96 ± 0.66w/v % and 8.02 ± 0.17 w/v % respectively. All the values were within the acceptable ranges. Molecular studies using ribulose biphosphate carboxylase Large chain (rbcL) primers produced consensus DNA that shared alignment and phylogeny with *I. gabonensis* in the Genbank. Our study showed that *I. gabonensis* contain important pharmacognostic fingerprints that can distintiguish it from closely related species, and help to check adulteration of drug from the stembark.

INTRODUCTION: Traditional medicine system continues to play an essential role in health care, with about 80% of the world population relying mainly on traditional medicine as the primary source of health care [1].

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It has been well documented that higher plants and their extracts have been used in the treatment of several diseases in traditional African medicine as an age-old practice these plants have been useful in the development of new drugs and continue to play an important role in the drug discovery process therefore there is need to standardize these products [2-3]. There is a renewed interest in drugs of natural origin due to their easy availability, accessibility and little or no side effects [4], coupled with a growing interest in correlating the phytochemical

constituents of a medicinal plant with its pharmacological activity [5]. The Family Irvingiaceae comprises ten species of tropical trees found in Africa, Southeast Asia to western Malaysia. These species usually grow in regions with altitudes from 200 to 500 m with annual rainfall from 1200 to 1500 mm and at temperatures ranging from 20 to 38 °C [6].

They are glabrous trees; wood is extremely hard; leaves sometimes papillate underneath mucilage cells in leaf and stem epidermis having secretory canals containing mucilage in leaves and stems. The leaves are alternate, simple, entire, petiolate, pinnately veined, coriaceous; stipules very large, unequal, intrapetiolar, encircling the terminal bud, early caducous and leaving a very distinct scar. Inflorescences are paniculate, axillary or terminal. The flowers are small, hermaphroditic, regular, pedicels articulated; the sepals are five in number, small and imbricate.

The petals are also five in number, free, imbricate, exceeding the sepals, the stamens are ten in number, which are distinct exceeding the petals and inserted below the large intrastaminal nectary disk. The filaments are plicately folded in bud, the anthers are subbasifixed. The ovary is superior, locular with one ovule per locule. The style is terminal and short. The stigma is punctiform. The fruit is a drupe with one or more pyrenes or a broadly winged samara. The seeds have large embryos, while the cotyledons are flattened and cordate, and the endosperm is fatty. The genera include *Desbordesia*, *Klainedoxa* and *Irvingia* [7].

Irvingia is a genus of African and Southeast Asian trees of the Irvingiaceae family, sometimes known as wild African mango or bush mango. They produce edible mango-like fruits and are prized for their fat and protein-rich nuts. The species are vastly distributed with *Irvingia smithii* (central Africa), *Irvingia gabonensis* (west and central Africa), *Irvingiagrandifolia* (central Africa), *Irvingia excels* (central Africa), *Irvingia malayana* (Southeast Asia), *Irvingia robur* (west and central Africa), *Irvingiatenuinucleata* (west and central Africa) and *Irvingia tenuinucleata* (west and central Africa) being the seven (7) species [8-9]. *Irvingia gabonensis* is widely cultivated in West African countries including southwest and southeast Nigeria, southern Cameroon, Côte d'Ivoire, Ghana, Togo, and Benin, to produce its edible fruit whose seed is used in the preparation of local delicious viscous soup for swallowing yam and cassava puddings Oladunjoye and Awani-Aguma [10]. Fat extracted from its seeds is commonly known as dika fat and majorly consists of C_{12} and C_{14} fatty acids, alongside smaller quantities of C_{10}, C_{16}, and C_{18}, glycerides and proteins.

Irvingia gabonensis seeds are also a good source of nutrients including a variety of vitamins and minerals such as sodium, calcium, magnesium, phosphorus, and iron. It is also a rich source of flavonoids (quercetin and kaempferol), ellagic acid, mono-, di-, and tri-O-methyl-ellagic acids, and their glycosides which are potent antioxidants [11].

Phytochemical analysis of its seeds showed that it contains tannins, alkaloids, flavonoids, cardiac glycosides, steroids, carbohydrates, volatile oils, and terpenoids and its proximate moisture 1.4 ± 0.11%, ash 6.8 ± 0.12%, crude lipid 7.9 ± 0.01%, crude fibre 21.6 ± 0.45%, and crude protein 5.6 ± 0.20% (Mahunu *et al.*, 2019; Olorundare *et al*., 2020). Pure compounds already isolated from the seed extract include methyl 2-[2-formyl-5-hydroxymethyl)-1 H-pyrrol1yl]-propanoate, kaempferol-3-0-*β*-D-6″ (p-coumaroyl) glucopyranoside and lupeol (3*β*-lup-20(29)-en-3-ol). Meanwhile, the antioxidant property of *Irvingia gabonensis* seed extract has been largely attributed to its high lupeol content [12].

Plant molecular barcoding is a technique used in identifying and classifying plant species based on their DNA sequences. It involves the use of short, standardized DNA sequences, usually from specific genes, as "barcodes" to uniquely identify plant species. The most commonly used DNA sequences for plant molecular barcoding are those from the chloroplast genome, specifically the regions known as ribulose bisphosphate carboxylate large gene (*rbcL*) and Maturase K gene (*mat K).* These regions are highly conserved, meaning they are similar across plant species, but also contain enough variation to differentiate between different plant species [13]. The process of plant molecular barcoding typically involves several steps. First, plant tissue is collected and DNA is extracted from

the sample. The rbcL and mat K regions of the chloroplast genome are then amplified using polymerase chain reaction (PCR), a process that selectively amplifies specific DNA sequences. The PCR products are then sequenced, and the resulting DNA sequences are compared to a reference database of known plant barcodes to identify the plant species [14].

Plant molecular barcoding has several advantages over traditional methods of plant identification, such as morphology-based identification. DNA sequences are less subject to variation due to environmental factors, making molecular barcoding more reliable and accurate. Additionally, DNA sequences can be easily stored and shared in databases, facilitating the creation of a comprehensive library of plant barcodes for future use [15].

Irvingia gabonensis is known to contain a variety of bioactive compounds with potential health benefits. However, there are concerns about the sustainability of harvesting the bark of this tree for medicinal use, as well as the potential for misidentification and adulteration of different samples of bark. By using DNA barcoding techniques, researchers can accurately identify different samples of *Irvingia gabonensis* stembark and distinguish them from other plant species. This can help to ensure that the bark is being harvested sustainably and that consumers are getting the genuine product. It can also help to prevent the sale of adulterated or counterfeit products [16].

Several methods can be used for DNA barcoding of *Irvingia gabonensis* stembark, including PCR amplification of specific regions of the DNA and sequencing of the amplified DNA. Once the DNA sequences have been obtained, they can be compared to a reference database of known sequences to identify the species.

DNA barcoding of *Irvingia gabonensis* stembark can provide a valuable tool for ensuring the authenticity and sustainability of this important medicinal resource. It has the potential to improve the quality and safety of traditional medicines and commercial products, as well as aid in forensic investigations [17]. However, to the best of our knowledge, there is no report on the pharmacognostic fingerprints and DNA barcoding of the stembark of *I. gabonensis*. Therefore, the present study was aimed at identifying some pharmacognostic profiles of stembark and providing its DNA barcoding for easy taxonomic identification from closely related species which will help in assessing adulteration of medicinal and economic products from the stembark of the plant.

MATERIALS AND METHODS:

Chemicals, Reagents, and Equipment: Molecular and analytical tools such as cetyltri-methylammonium bromide buffer (CTAB), Proteinase- K, R-Nase A, 95% ethanol, isopropanol, DNA polymerase (Taq polymerase), deoxyribonucleoside triphosphates (dNTPs), PCR primers (rbcL II), DNA polymerase (e.g., Taq polymerase), sequencing primers (specific to the barcoding region of interest), fluorescent dyes, PCR machine (Eppendorf master-cycler nexus X2 thermal cycler), UV-vis spectrophotometer r(NANODROP 2000c Thermo-Scientific, UK), gel electrophoresis system (Maxi-Broad-H), centrifuge (Eppendorf 5702), refrigerator and freezer (Haier Thermocool Refrigerator HR-130 AS –Silver/Haier Thermocool Chest Freezer HTF 150HAS R6 Silver), Vortex mixer (LABOID L 20), UV trans-illuminator Visualizer (Enduro-temp V1) and DNA extraction kits **Table 1.**

TABLE 1: DNA EXTRACTION KIT SPECIFICATIONS USED

Component	Product procured (mL)	Volume used (mL)
Lysis buffer L	1 x 30, 2 x 60	30
Binding buffer I	1 x 7, 1 x 25	7
Solution WN	1 x 18, 1 x 55	18
Wash solution A	2 x 38	38
Elution buffer B	1 x 30	15
RNase A	5 vials (5 x 80 µL)	1 vial (80 µL)
Spin columns	1 x 250	50
Filter columns	1 x 250	50
Collection tubes	1 x500	100
Elution tubes	1 x 250	50
Product insert	1 x 1	1

Collection, Identification, and Preparation of Plant Materials: Fresh stembarks of *I. gabonensis* were collected from a forest in the Iragbiji community, Osun State, Nigeria, in October 2018. The plant was identified by a taxonomist Mr Namadi Sunusi of the Department of Botany, Ahmadu Bello University, Zaria.

A voucher specimen number ABU06926 was deposited for the plant at the herbarium of the Department of Botany for referencing. The stembarks were allowed to dry under shade for two weeks and prepared accordingly following standard procedures.

Macroscopic examination of *I. gabonensis* Stembark: Macroscopic observations were carried out on the whole dried stembark of the plant, and these entail colour, odour, taste, texture, shape and fracture [18].

Microscopic analysis of *I. gabonensis* Stembark: To remove obscuring materials, the powdered stembark of *I. gabonensis* was cleared with a 70% chloralhydrate solution and boiled in a water bath for 30 min. The cleared sample was mounted on a microscopic slide with dilute glycerol and observed under the microscope (40x), for the presence of pharmacognostic fingerprints of the powdered stembarks. Suitable photographs were taken and recorded [19].

Scanning Electron Microscopy of the Leaf of *I. gabonensis*: The detailed internal structure (transverse section) of the leaf was observed using a Phenom desktop scanning electron microscope (PhenomWorld, USA) at 8000x magnification to reveal some pharmacognostic features.

Chemo-microscopic Analysis on *I. gabonensis* Stembark: This was carried out on powdered stembark *of I. gabonensis*. Briefly, the finely crushed stembark was cleared using 70% chloral hydrate solutionto remove obscuring materials. It was then heated for 30 minin a water bath. Using two drops of dilute glycerol, the cleared sample was then placed on a microscope slide, and the presence of several cell inclusions and cell wall components was observed using various detecting reagents [19-20].

Determination of Physicochemical Parameters of *I. gabonensis* Powdered Stembark: The physicochemical parameters such as moisture content, ash value, total ash, extractive values, acid insoluble, water-soluble ash values, swelling and foaming indices where determined following standard procedures. For each parameter, a total of ten replicate readings were taken, and results were recorded as mean ± SE.

DNA Barcoding of *I. gabonensis* Stembark: DNA barcoding of *I. gabonensis* was carried out according to the methods by Kress *et al.*, [15] and de Oliveira*et al.*, [21]. Various steps were involved following protocols previously described.DNA extraction was carried out using the DNA kit (NORGEN BIOTEK© Plant DNA isolation kit, USA).

DNA Extraction Procedure:

Lysate Preparation: Less than 100 mg of plant tissue was placed into a mortar and ground into a powder. The ground material was then transferred into a DNase-free 1.7 mL- micro-centrifuge tube with 500 µL of lysis buffer L, and 1µL of RNAse A, was added. This was incubated at 65^0 C for 10 min with occasional mixing of the lysate 3 times during incubation by inverting the tube. 100µL of binding buffer 1 was then added, mixed thoroughly and incubated for 5 min on ice.

The filter column (clear O-ring) with one of the provided collection tubes was then assembled, and the lysate was pipetted into the filter column and spun for 2 min at 20,000 x g (~14,000 RPM). The clear supernatant from the flow-through was then transferred into a DNAase-free micro-centrifuge tube using a pipette. An equal volume of 70% ethanol was added to the lysate collected (100µL of ethanol was added to every 100µL of lysate), and vortex to mixed [21].

Binding to Column: A spin column (grey O-ring) with a collection tube was assembled, and then up to 650µL of the clarified lysate with ethanol onto the spin column and centrifuge for 1 min at 10,000 x g (~10,000 RPM). The flow-through was discarded, and then the spin column with the collection tube was reassembled.

Column Wash: 500µL of solution WN was added to the column and centrifuge for 1 min at 20,000 x g (~14,000 RPM). The flow-through was discarded and the spin column with its collection tube was reassembled. 500µL of Wash solution A was added to the column and centrifuge for 1 min at 20,000 x g (~14,000 RPM).

The flow-through was then discarded and the spin column with its collection tube was reassembled, and the process repeated. The column was spun for

2 min at 20,000 x g (~14,000 RPM) to thoroughly dry the resin, and the collection tube was discarded.

DNA Elution: The column was placed into a fresh 1.7 mL elution tube, and 100 µL of elution buffer B was added to the column and incubated for 1 min at room temperature, then centrifuged for 1-2 min at 10,000 x g (~10,000 RPM).

DNA Quantification: The extracted DNA was then analysed using a UV-vis spectrophotometer (NANODROP 2000c), and the concentration of DNA was then obtained.

DNA Storage: The purified genomic DNA extracted was then stored in a DNA sample bottle at 2-8 ^{0} C in a refrigerator.

DNA Amplification: The polymerase chain reaction (PCR) master mix containing the *I. gabonensis* DNA, the forward primer, reverse primer, deoxynucleotide triphosphates (dNTPs) mix, taq polymerase, buffer solution with DNA polymerase and magnesium chloride ($MgCl_2$) solution was placed in a PCR tube and vortex gently to ensure homogeneity.

The rbcL II primer was used in this mix. The PCR tubes were then placed into the thermal cycler, ensuring they were securely sealed; then the appropriate heating block was set on the PCR machine. After the thermal cycling process was completed, the tubes were removed and run on agarose gel with a DNA ladder to estimate the size of the amplified product [21].

Agarose Gel Electrophoresis: This procedure was used to separate and analyze the DNA obtained from thermocycler [15].

Briefly, 2 g of agarose powder was weighed and added to a beaker containing 200 mL volume of TAE/TBE buffer, to make (1% w/v) the mix was then gently heated on a hot plate until the agarose was completely dissolved. 0.5 µg/mL ethidium bromide was the added to the warm agarose solution.

The solution was mixed well and poured into the gel casting tray, a space was left for the comb and was allowed to cool and solidify. The DNA sample and DNA ladder were then mixed with bromophenol blue which is the loading dye and then kept ready. The comb was carefully removed from the solidified gel and placed into the gel electrophoresis apparatus filled with TAE/TBE buffer, ensuring the wells were on the cathode side. Using a micropipette, the DNA sample and ladder were carefully placed into separate wells.

The electrophoresis was run at 90V for 45 min. After this, the gel was removed and placed on a UV transilluminator where the separated DNA fragments were visualized and recorded [21].

DNA Sequencing and Analysis: The purified DNA fragment obtained was then sequenced; where the sequence obtained was trimmed and cleaned to remove any low-quality or ambiguous bases. The consensus sequences were then subjected to sequence analysis using bioinformatics tools; comparing with reference sequences available in public DNA barcode databases GenBank and BOLD (Barcode of Life Data Systems).

Statistical Analysis: Data obtained from the physicochemical analysis were expressed as mean± SE (n = 10).

RESULTS:

Macroscopic Parameters of *I. gabonensis* Powdered Stembark: The organoleptic evaluation of *I. gabonensis* stembark showed a brown to greyish colour powder with a slightly acrid odour and strong bitter taste as well as coarse texture **Table 2**.

TABLE 2: ORGANOLEPTIC PARAMETERS OF *I. GABONENSIS* POWDERED STEMBARK

Test	Observation
Colour	Greyish-brown
Odour	Acrid
Taste	Strong bitter
Texture	Coarse

Microscopic Examination of *I. gabonensis* Stembark: The scanning electron microscopy of the leaf revealed the presence of a few numbers of paracytic stomata on the adaxial surface (upper) than on the abaxial surface (lower) which is typical of most terrestrial plants among the angiosperms **Fig. 1**.

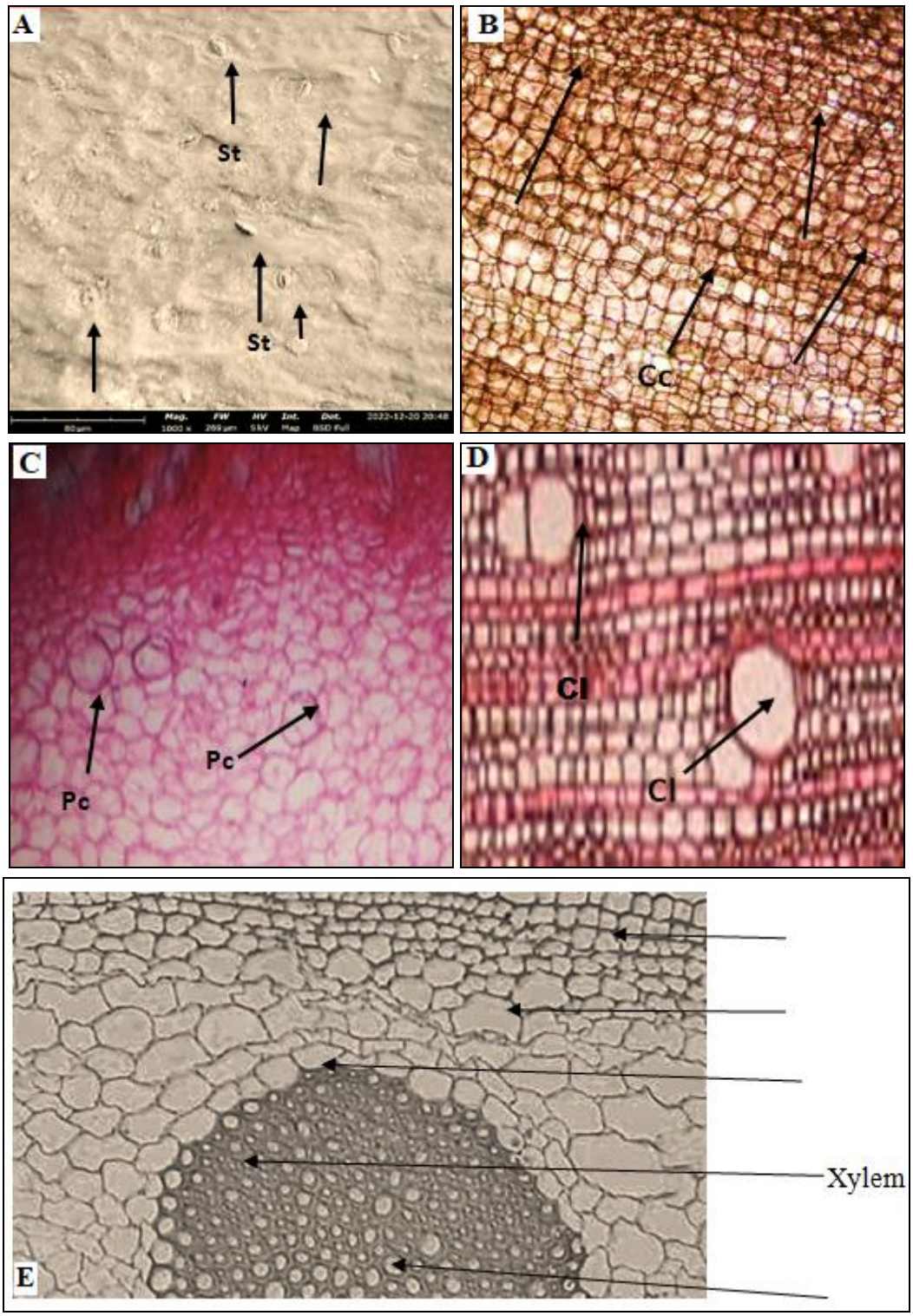


FIG. 1: SCANNING ELECTRON MICROSCOPY OFA LONGITUDINAL SECTION THROUGH *I. GABONENSIS* SHOWING PARACYTIC STOMATA (ARROWS); 8000X (A), BEADED CORK CELLS (B), PARENCHYMATOUS CELLS; PC STAINED WITH SAFRANIN (C), FIBRE SCLEREIDS WITH WIDE CELL LUMEN; CL (D), AND TRANSVERSE SECTION OF STEMBARK WITH WIDE PITH (E) AS VIEWED USING THE SCANNING BIOLOGICAL MICROSCOPE, 3000X. AFEW STOMATA WERE OBSERVED ON THE UPPER SURFACE OF THE LEAF TYPICAL OF TERRESTRIAL ANGIOSPERMS (A).

Chemomicroscopical Evaluation of the Powder Stembark of *I. gabonensis*: Chemomicroscopical evaluation of the powdered stembark of *I. gabonensis* revealed the presence of cellulose cell wall, lignified cell wall, tannins, starch, calcium oxalate, suberin and mucilage **Table 3**.

TABLE 3: CHEMOMICROSCOPICAL FEATURES OF *I. GABONENSIS* STEMBARK POWDER

Constituents	Inference
Starch	+
Lignin	+
Tannins	+
Calcium oxalate	+
Calcium carbonate	-
Cellulose	+
Suberin	+
Gums and Mucilage	+

+: present and -: absent.

Evaluation of Physicochemical Parameters of *I. gabonensis* the Stembark: The total ash was 18.58 ± 0.23% w/w, acid-insoluble ash was 8.02 ± 0.17% w/w and water- soluble ash was 13.96 ± 0.66% w/w, to the physicochemical parameters. The ethanol-soluble extractives were 18.20 ± 0.28% w/v and 28.60 ± 0.12 % w/v water-soluble extractives. The moisture content was 5.13 ± 0.11. The plant's swelling index was 4.55 ± 0.32 mL. The plant's foaming index was calculated as 142.9± 0.61% w/v **Table 4.**

TABLE 4: PHYSICOCHEMICAL PARAMETERS OF *I. GABONENSIS* STEMBARK POWDER

Parameters	Values (%w/w or w/v) ± SE
Moisture content	5.13 ± 0.11
Total ash	18.58 ± 0.23
Acid insoluble ash	8.02 ± 0.17
Water soluble ash	13.96 ± 0.66
Ethanol extractive	18.20 ± 0.28
Water extractive	28.60 ± 0.12
Swelling index	4.55 ± 0.32 mL
Foaming index	142.9± 0.61

Data were expressed as means ± SE (n = 10).

DNA Molecular Barcoding Analysis: Results of the gel electrophoresis after visualization show a clear band concerning the ladder as presented in **Fig. 2.**

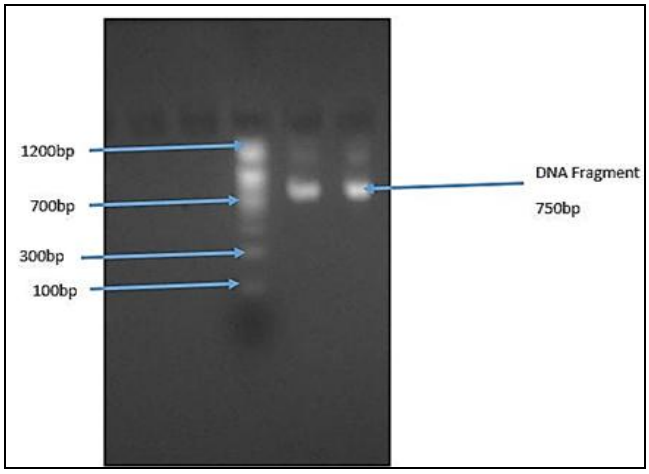
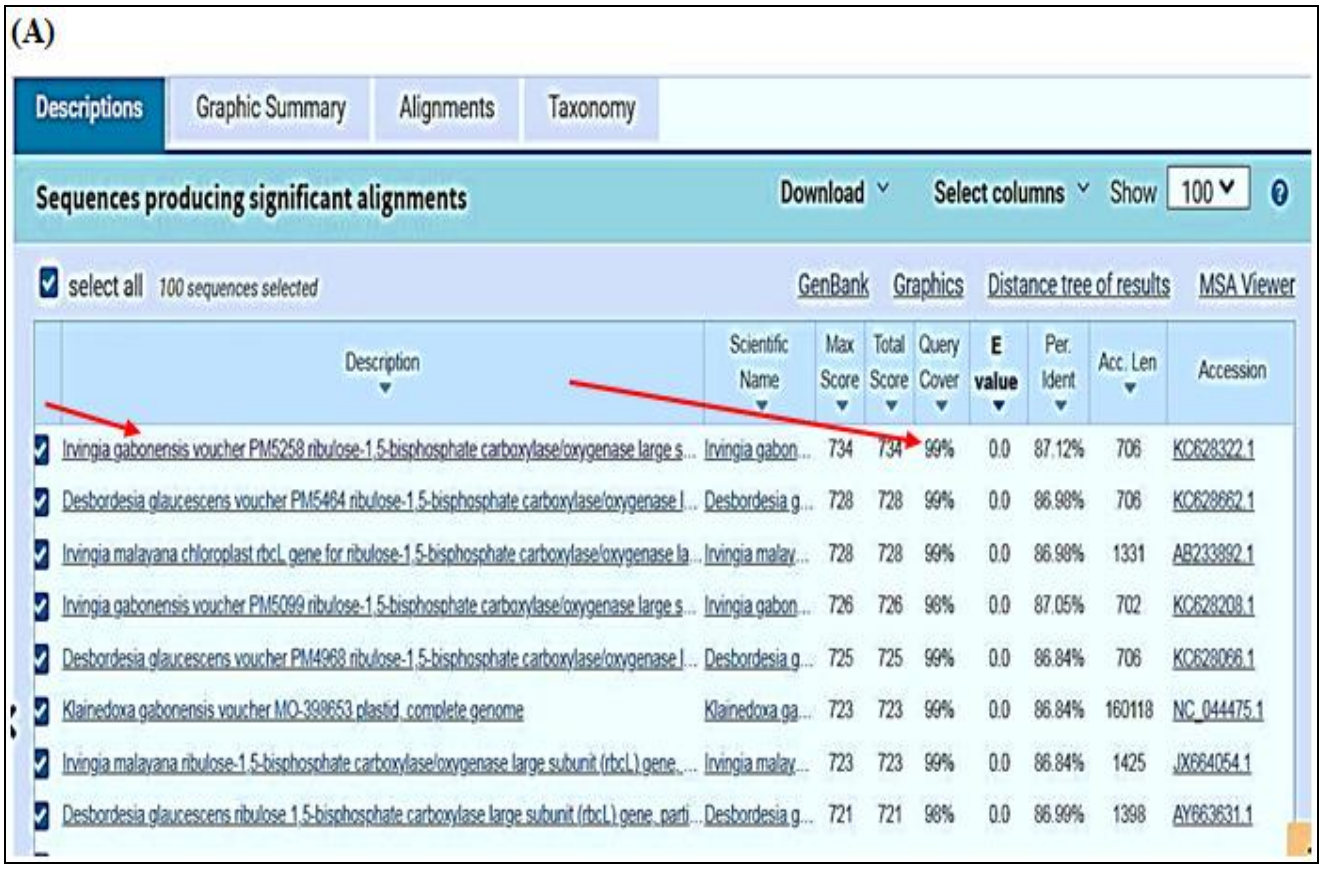


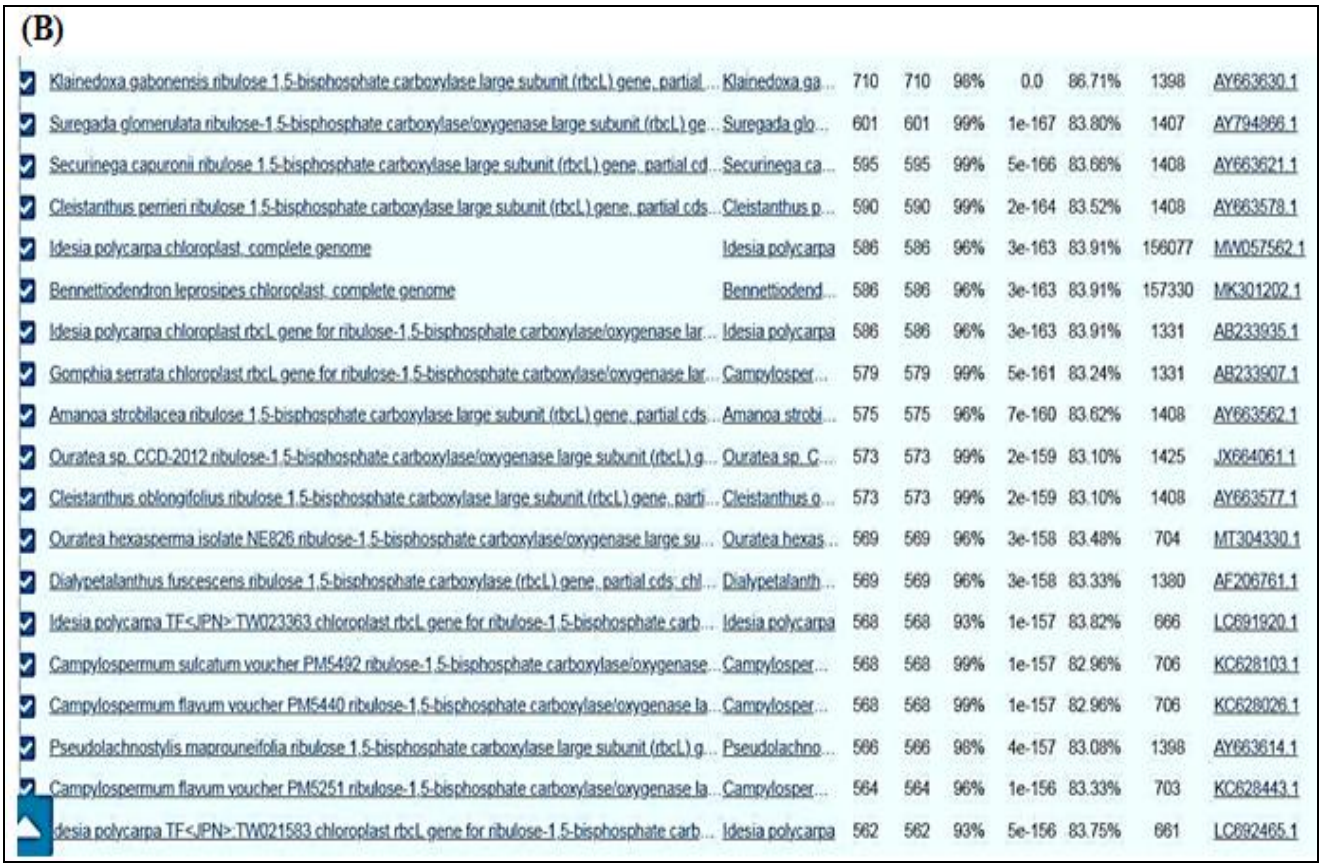
FIG. 2: ELECTROPHORESIS GEL STAINED WITH BROMOPHENOL BLUE UNDER UV TRANSILLUMINATOR COMPARED WITH A 1200BP LADDER

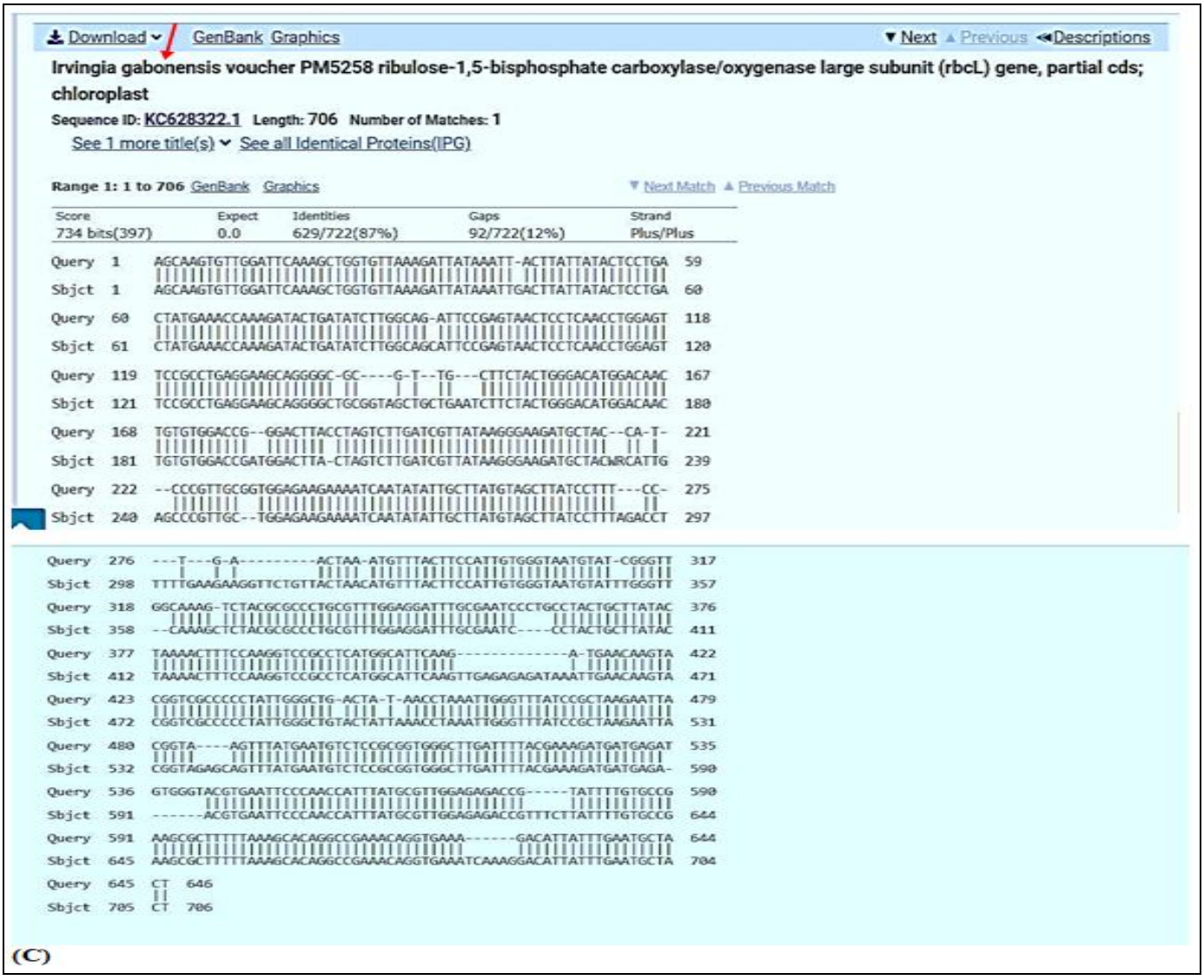
Sequencing and Analysis of DNA Fragments: Fig. 3(A) below shows the consensus DNA after the sequencing process. This sequence was obtained after the purified fragment was trimmed and cleaned to remove any low-quality or ambiguous bases. The consensus DNA obtained was analysed with bioinformatics tools; the DNA was compared with reference sequences available in public DNA barcode databases NCBI and blasted. **Fig. 3(B)** and **(C)** show that the consensus sequence has an alignment like that of *I. gabonensis*. Similarly, **Fig. 3(D)** shows the consensus DNA's phylogenic relationships.

AGCAAGTGTTGGATTCAAAGCTGGTGTTAA
AGATTATAAATTACTTATTATACTCCTGACT
ATGAAACCAAAGATACTGATATCTTGGCAG
ATTCCGAGTAACTCCTCAACCTGGAGTTCC
GCCTGAGGAAGCAGGGGCGCGTTGCTTCTA
CTGGGACATGGACAACTGTGTGGACCGGGA
CTTACCTAGTCTTGATCGTTATAAGGGAAG
ATGCTACCATCCCGTTGCGGTGGAGAAGAA
AATCAATATATTGCTTATGTAGCTTATCCTT
TCCTGAACTAAATGTTTACTTCCATTGTGGG
TAATGTATCGGGTTGGCAAAGTCTACGCGC
CCTGCGTTTGGAGGATTTGCGAATCCCTGC
CTACTGCTTATACTAAAACTTTCCAAGGTCC
GCCTCATGGCATTCAAGATGAACAAGTACG
GTCGCCCCCTATTGGGCTGACTATAACCTA
AATTGGGTTTATCCGCTAAGAATTACGGTA
AGTTTATGAATGTCTCCGCGGTGGGCTTGA
TTTTACGAAAGATGATGAGATGTGGGTACG

TGAATTCCCAACCATTTATGCGTTGGAGAG ACCGTATTTTGTGCCGAAGCGCTTTTTAAA GCACAGGCCGAAACAGGTGAAAGACA TTATTTGAAT GCTACTCGTC







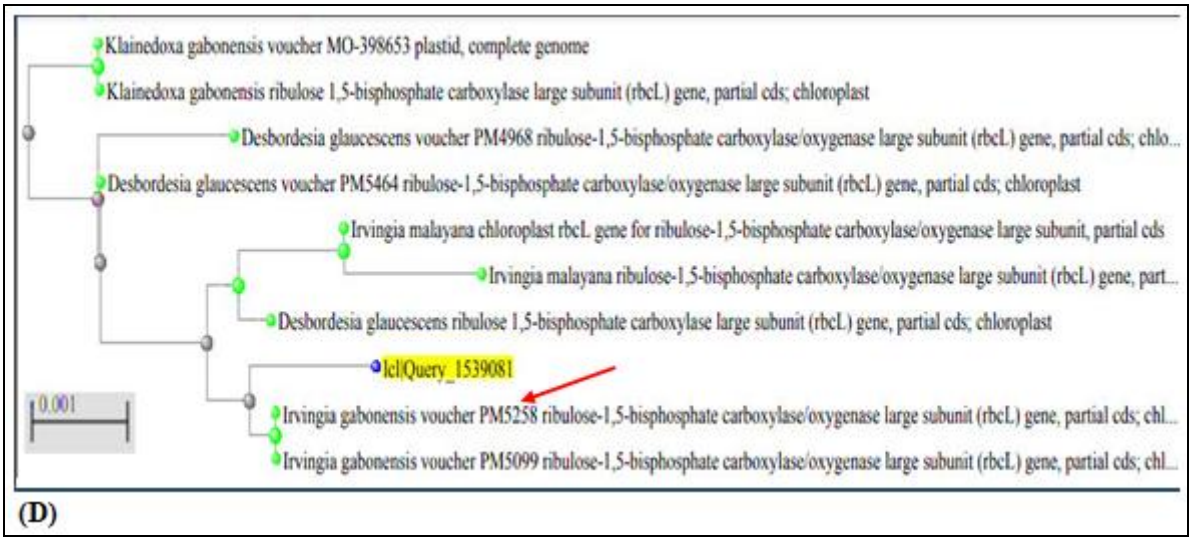


FIG. 3: CONSENSUS DNA OBTAINED FROM SEQUENCING OF THE DNA FRAGMENTS BY THE PCR MACHINE(A), CONSENSUS DNA MATCHING WITH *I. GABONENSIS* SPECIES IN THE GENBANK (B), CONSENSUS DNA ALIGNMENT WITH SPECIES OF *I. GABONENSIS* PRESENT IN THE GENBANK GRAPHICS (C), AND PHYLOGENY TREE SHOWING THE TAXONOMIC RELATIONSHIP BETWEEN *IRVINGIA SPECIES* AND THE CONSENSUS DNA FRAGMENTS(D). THE RED ARROW INDICATES THE TAXON OF *I. GABONENSIS* FROM THE GENBANK.

DISCUSSION: Macroscopical evaluation of the stembark of *I. gabonensis* reveals a greyish-brown colouration. The stembark is known for its distinctive greyish-brown colouration. This colouration is a result of tannins and flavonoids that are present in the plant, which was observed and reported by Ojo [22]. Tannins are a type of plant compound that is often responsible for the astringency or bitterness of certain foods [23]. The organoleptic evaluation of the stembark of *I. gabonensis* showed that the bark is greyish-brown in colour. The colour of the stembark is usually associated with the age of the plant, as it matures plant naturally takes up a brownish coloration [24-25]. Environmental conditions are also implicated in the colour of the stembark as described by Savage and Vellend [26]. Other factors such as the composition of minerals in the soil have also proven to be significant as a determinant of the colour of the stembark [27].

This research has also discovered that the odour of the stembark of *I. gabonensis* is acrid, this was also described in a research conducted in 2020 by Egwunatum, and co-workers [28], to study the effect of variegated forest soil amendments on the germination and early growth of *I. gabonensis* where the odour of the stembark was one of the parameters evaluated and was concluded to be acrid. Otitolaiye*et al.* [29], in research conducted to evaluate the phytochemical activity and in-vitro antioxidant potential of aqueous and ethanol extracts of *I. gabonensis* stembark, reported the odour as bitter. The odour of *I. gabonensis* stem bark can be associated with the presence of resins in the cells [30].

A study conducted by Godayol *et al.* [31], which aims to study odour-causing organic compounds in wastewater treatment plants, reported that the presence of certain phenols and aldehydes in the stem bark which they postulated is responsible for its odour. Kola-Mustapha *et al.* [32], reported that environmental conditions play a significant contribution to the odour that is associated with stembarks. The texture of *I. gabonensis* as determined by this research is coarse, this was also the conclusion drawn by Nuhu and co-workers in 2020 [33], in research that aimed to establish the macroscopic profile of *I. gabonensis* in Wistar rats. Cell division that is influenced by the environment can affect the texture of the stembark Funada *et al.* [34]. A tough fibrous sclerenchyma can also be implicated in the texture of the stembark, this was described in research conducted by Zhang, and co-workers [35]. The chemomicroscopic analysis of the stem bark of *I. gabonensis* revealed the presence of cellulose, suberin and lignin, this is consistent with research conducted by Nuhu *et al.* [33] while investigating the safety profile of *I. gabonensis* rootbark extract. Mgbemena, and co-workers in 2019 [36], studying the chemical composition, proximate and phytochemical analysis of the peels, seed coat, leaves and seeds of *I. gabonensis*, reported the presence of cellulose, suberin and lignin in their chemomicroscopic analysis.

Cellulose typically adds to the structural support of the plant hence its presence is integral for strength and rigidity Pauly *et al.* [37], this discovery was made while studying the molecular domains of the cellulose/xyloglucan network in the cell walls of higher plants. Cellulose also serves as a barrier against pathogens that could damage tissues involved in the transportation of water and minerals Souza *et al.* [38]. Therefore cellulose plays a significant role in regulating water and nutrient exchange in the plant, this is made possible through small pores present in them that assist in the movement Franca *etal.* [39]. Suberin which was present in the stembark is a waxy hydrophobic substance that helps to prevent water loss in the plant especially when environmental conditions are not favourable de Silva *et al.* [21]. Suberin also protects from pathogens and extends the lifespan of the plant Shukla and Barberon [40]. Like cellulose, suberin found in the stem bark plays an important role in regulating the movement of water and nutrients in and out of plant tissues. Baxter *et al.* [41].

A moisture content of 5.13%for the stembark of *I. gabonensis* indicates that 5.13% of the weight of the bark is water. Moisture content is an important parameter in the processing and storage of plant materials. A high moisture content can lead to microbial growth and spoilage, while a low moisture content can result in brittleness and loss of quality. The ideal moisture content for the stembark of *I. gabonensis* would depend on the intended use of the material. For medicinal use, a moisture content of 5.13% is relatively low and indicates that the bark has been properly dried and stored.

This low moisture content helps to preserve the active compounds in the bark and ensures its effectiveness. All these and other pharmacognostic fingerprints evaluated in this study perform various functions in plants and are used in the pharmaceutical industry for quality assurance.

The obtained DNA sequence of 650 base pairs after sequencing was subjected to a BLAST (Basic Local Alignment Search Tool) analysis. BLAST is a widely used bioinformatics tool that compares the query sequence against a vast database of known sequences to find similarities. The BLAST analysis has shown that the 650-base pair sequence from *I. gabonensis* aligns significantly with an entry in the GenBank database. This suggests that the sequence obtained from the organism shares similarities with a previously documented sequence in GenBank. The fact that the sequenced DNA aligns with a known sequence in the GenBank database supports the accuracy and validity of the sequencing process. It indicates that the DNA sample extracted from *I. gabonensis* is consistent with genetic information previously documented for this species. While the alignment with an existing sequence is a positive indication, further analysis may be required to fully characterize the genetic makeup of *I. gabonensis*. This will involve sequencing comparison with additional sequences, and functional analysis of specific genes or regions.

CONCLUSIONS: Our study comprehensively evaluated various pharmacognostic parameters and DNA barcoding of *I. gabonensis* stembark, shedding light on its botanical and genetic characteristics to provide accurate taxonomic identification. Through detailed analysis, essential parameters such as moisture content, acid-insoluble ash, and total ash among others were determined, providing crucial insights into the plant's quality and purity. Moreover, the successful extraction and sequencing of a DNA fragment from the chloroplast gene further enriched our understanding of the genetic makeup of *I. gabonensis*. The deposition of this DNA sequence in GenBank enhances accessibility for future studies and facilitates accurate species identification and authentication. By integrating traditional pharmacognostic methods with modern molecular techniques, this research contributes valuable data to the field of Pharmacognosy and genetic resource conservation. Moving forward, the findings from this study serve as a foundation for further research into the medicinal and genetic potential of *I. gabonensis*.

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