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*Original Research Article*

# Molecular Discrimination and Phylogenetic Relationship of some Medicinal Plants Using the Plastidial *rbcL* Gene: A Case Study of *Lannea* species

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ABSTRACT

The conventional taxonomic system is not without inherent limitations, and this often leads to misidentification of medicinal plants and, consequently, adulteration. To overcome this challenge, sequencing of genomic Deoxyribonucleic acid (DNA) has been used as a standardized method of plant identification. Thus, this study was carried out to discriminate some members of the *Lannea* species (*L. welwitschia*, *L. coromandelica*, *L. microcarpa* and *L. barteri*) towards providing genetic data for proper identification and quality control of these medicinal plants. Genomic DNA was isolated using the cetyltrimethylammonium bromide (CTAB) method, and its purity and concentration were measured with a spectrophotometer. Polymerase chain reaction (PCR) amplification, purification of PCR products, and quality assessment were conducted following standard procedures. PCR products sequencing was conducted using a sequencing kit. Sequence alignment and phylogenetic analysis were performed utilizing molecular evolutionary genetics analysis (MEGA v6) and BioEdit software. PCR amplification of the *rbcL* gene marker was significantly successful. The *rbcL* amplicons generated high-quality sequences, and basic local alignment search tool (BLAST) analysis correctly matched *L. welwitschia* and *L. coromandelica* with hits >95%. Phylogenetic relationship analysis revealed clear differentiation in *Lannea* species. This study generated genetic data that can be used to authenticate these *Lannea* species members, thereby enhancing the safety and effectiveness of these medicinal plants within Nigeria's traditional medical system.

***Keywords:*** Molecular marker, *Lannea*, Taxonomy, Phylogenetic tree, Adulteration

## Introduction

Authentication of medicinal plant species based on molecular gene markers (DNA barcoding), owing to their standardization, efficiency, and adaptability has become a widely used method.[1] Among potential gene markers, the *rbcL* gene stands out for its ability to discriminate plants, particularly at the family and genus levels.[2] One of the remarkable advantages of this gene is its high discrimination power.[1] Traditional taxonomic methods, which rely heavily on phenotypic traits, encounter significant challenges due to their susceptibility to environmental variations, which leads to inconsistencies and ambiguities in plant species authentication. The limitations encountered make it essential to adopt more advanced methods, like the molecular approach of DNA barcoding —a molecular technique —to address these challenges. By utilizing molecular techniques, the precision and reliability of plant classification are significantly improved, overcoming the major drawbacks of depending solely on visible phenotypic traits.[3]

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Moreover, the emerging practice of combining morphological, chemical, and molecular gene markers for species identification emphasizes the increasing relevance of DNA barcoding as one of the most effective tools for identifying medicinal plant species.[4] Recent studies used this barcode to authenticate and explore phylogenetic relationships of medicinal plants in different genera including *Withania*,[5] *Drynaria*,[6] and *Gossypium*.[7]

Substitution and adulteration in the trade of economically important medicinal plants have been a challenge over time. This is largely due to a break in the demand and supply chain of raw materials (medicinal plants) and, in many cases, results in adulteration and substitution of high-quality raw materials.[8] This practice results in poor quality of medicinal plants in herbal stores, in addition to huge economic losses for pharma industries that rely on these raw materials. It is therefore necessary that proper identification of medicinal plants using advanced techniques such as genome-based methods (DNA barcoding) towards enhancing quality control and achieving therapeutic value be incorporated into the system.

The genus *Lannea* belongs to the family Anacardiaceae, comprising 81 genera and 800 species distributed across Africa, the Middle East and Southeast Asia. Distinct species of this family have been used in traditional medical systems since time immemorial, benefiting communities where these species are predominant.[9] The genus *Lannea* comprises only 36 accepted species, including *L. coromandelica*, *L. barteri*, *L. microcarpa* and *L. welwitschia* being discussed in this study. Traditionally, the most used plant parts are the stem, bark, and leaf. However, the root and fruit are sometimes used also for the management of various diseases.[9] In Nigeria, like many other African countries, *L. coromandelica*, *L. barteri*, *L. microcarpa* and *L. welwitschia* are used specifically for the treatment of various ailments, including rheumatism, diarrhea, gastroenteritis, malaria, bacterial infection, anemia, convulsion, diabetes, hypertension, pulmonary, and heart disease.[10-14] A major drawback in the application of these plant species in the traditional medical system is the overlap in their uses

which often results in misidentification subsequently hampering their safety and efficacy in the management of various diseases. Additionally, the anatomical and morphological characteristics of members of the genus- *Lannea* are quite similar, which can often lead to misidentification. Therefore, this study was carried out to discriminate *L. coromandelica*, *L. barteri*, *L. microcarpa* and *L. welwitschia* using molecular techniques towards their proper identification and quality control. This study marks the first attempt at sequencing the DNA of members of the *Lannea* species in Nigeria, and has provided genetic data for accurate identification of *Lannea* species members in the Nigerian traditional medical system.

## Materials and Methods

*Sample Collection and DNA Extraction*

The stem bark, fruit, flower and the leaf of *L. coromandelica, L. barteri, L.welwitschii* and *L. microcarpa* were collected from Karau-Karau area in Zaria local area government, Kaduna state, Nigeria, located at latitude11° 06` 00`` and longitude 7° 30` 00``, with the help of a plant taxonomist. The plant specimens were identified at the herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University in Zaria. Identified samples with voucher numbers; *L. coromandelica* (ABU0900127), *L. barteri* (ABU155), *L.welwitschii* (ABU1832) and *L. macrocarpa* (ABU88) were deposited at the herbarium unit. Genomic DNA was extracted from the leaf of each plant species using the CTAB method.[5] Purity and concentration of each extracted genomic DNA were determined using a spectrophotometer (Thermo Scientific, USA) and 0.8% agarose gel electrophoresis stained with ethidium bromide. Purified genomic DNA was stored at -80℃.[15] All experiments were run in triplicate.

*PCR Amplification and Purification*

A qualitative DNA template was employed for the PCR amplification using selected primers (Table 1). The reaction mixture comprised 10 µL of 5× GoTaq colorless reaction buffer, 3 µL of 25 mM $MgCl_2$ (Sigma-Aldrich, molecular biology grade), 1 µL of a 10 mM dNTP mix, 1 µL of each 10 pmol primer, and 0.3 units of Taq DNA polymerase (Promega, USA). Sterile distilled water was added to adjust the total volume to 42 µL, along with 8 µL of DNA template.

**Table 1:** PCR primers for *rbcL* marker

| Loci | Primer | Sequence |
|---|---|---|
| *rbcL* | F | 5`-ATGTCACCACAAACAGAGACTAAAGC-3` |
| *rbcL* | R | 5`-GTAAAATCAAGTCCACCRCG-3` |

F= forward, R= reverse

PCR was performed on a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystems Inc., USA) with an initial denaturation at 94°C for 5 minutes, followed by 30 cycles—each consisting of 94°C for 30 seconds, an annealing phase at 54°C, and a final extension at 72°C for 10 minutes. The amplified fragments were then purified using ethanol (Sigma-Aldrich, ACS reagent, ≥99.5% purity) to remove any residual PCR reagents.

The purified PCR fragments were separated on a 1.5% agarose gel stained with ethidium bromide for 60 minutes to verify the presence of the purified PCR products. Their concentrations were quantified using a spectrophotometer (Thermo Scientific, USA). PCR products sizes of samples were determined based on comparison in their mobility with that of a 100 bp ladder (Fermentas, USA).[16]

*Sequence Analysis*

The PCR products were sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Thermo Fisher Scientific, USA) in accordance with the manufacturer's guidelines. Sequence alignment and phylogenetic analysis of gene fragments were performed using (MEGA 6)[17] and BioEdit 7.2 (Informer Technologies, USA) software, with 1000 bootstrap replicates. After sequencing, the sequences were manually curated and subsequently aligned using multiple sequence alignment.[18] Evolutionary distances were computed with the maximum composite likelihood (MCL) method, and any ambiguous positions in each sequence pair were removed to ensure precise SNP detection. Only sequences displaying variation at a single base pair across all experiments were selected for SNP detection.

## Results and Discussion

*PCR amplification and purification*

The specific genomic fragments showed a significant success rate in PCR amplification with an average read length of 600 bp, and having bands with no shearing or contamination for all selected *Lannea* species (Fig. 1). This result was consistent with previous findings on PCR amplification of *rbcL* gene for the authentication of a Chinese medicinal herb, "Gusuibu"[6] and endangered medicinal plants sold in Indian markets.[8] This indicated the potential universality of the *rbcL* gene marker, which is thought to be linked to its highly conserved nature and low evolutionary rate.[19]

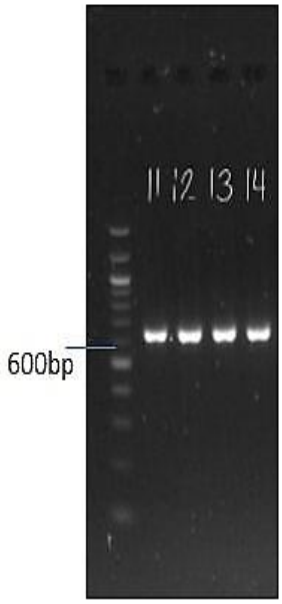


**Figure 1:** PCR amplified products for *rbcL* marker using specific primers. First lane to the left represents DNA ladder, laneI1-I4 represents PCR amplified products of *L. welwitschia*, *L. coromandelica*, *L. macrocarpa*, *L. barteri* respectively

*Sequence Analysis*

In post-genomic era, where sequencing technologies are advancing rapidly, molecular gene markers provide a dependable and consistent approach to accurately distinguish closely related medicinal plant species through their DNA sequences.[18] To address the challenges in morphology-based taxonomy, sequencing of genomic DNA has served as a standardized method of plant identification due to the presence of homologous DNA sequences among related species.[20] In this study, plastidial *rbcL* marker gene of selected *Lannea* species was used to discriminate the species towards authentication of the medicinal plants. In this study, the *r bcL* amplicons generated high -quality sequences that ranged between 517-549 bp with an average of 525 ± 15 bp, consistent with a previous report.[6] BLAST in NCBI nucleotide database employed in sequence data analysis to retrieve top hits for each sample species showed maximum similarity between 98.15-100 % for the top hits (Table 2). Samples I1 and I2 matched correctly with *L. welwitschia* and *L. coromandelica* with a pairwise identity (PI) of 99.81 and 100 % respectively (Table 2). Sample I3 matched *Lannea* sp. with a PI of 99.61 % (Table 2), thus indicating that sample I3 could not be resolved at the species level but showed it shares a close relationship with species of the genus *Lannea.*

**Table 2:** BLAST results for *rbcL* sequences of *Lannea* species

| Sample ID | Species similarity (BLAST) | Pairwise identity (%) | Accession no. |
|---|---|---|---|
| I1 | *Lannea welwitschii* | 99.81 | MN366879.1 |
| I2 | *Lannea coromandelica* | 100.00 | AB925480.1 |
| I3 | *Lannea* sp. | 99.61 | MN216440.1 |
| I4 | No sequence similarity | - | - |

This implies that *rbcL* marker was genus specific for sample I3, and this was consistent with previous findings on the amplification and sequencing of *rbcL* marker gene in traded medicinal plants.[8] The *rbcL* marker gene has been shown in some studies on species identification to possess modest discriminatory power.[20-22] Similarly, no sequence homology was observed for sample I4 (Table 2). Thus, the combination of multiple gene markers could be applied in resolving samples I3 and I4 to species level is suggested. This strategy has been previously used to successfully identify and authenticate medicinal plants.[5]

Utilizing the *rbcL* gene marker, a total of 15 distinct SNP-based variant sites were detected within the 1–450 bp region across all selected *Lannea* species (Fig. 2). The number of unique sites identified for each species is as follows: *L. welwitschia* (1) *L. coromandelica* (3), *L. macrocarpa* (2), and *L. barteri* (9). Through these unique SNPs, *rbcL* marker identified *Lannea* species differently in this study, supporting the use of this marker in species identification. The plastid *rbcL* gene marker demonstrates strong potential as a reliable barcode locus for identifying *Lannea* species due to its recoverability, high-quality sequencing, universality, and robust species discrimination capabilities.[21]

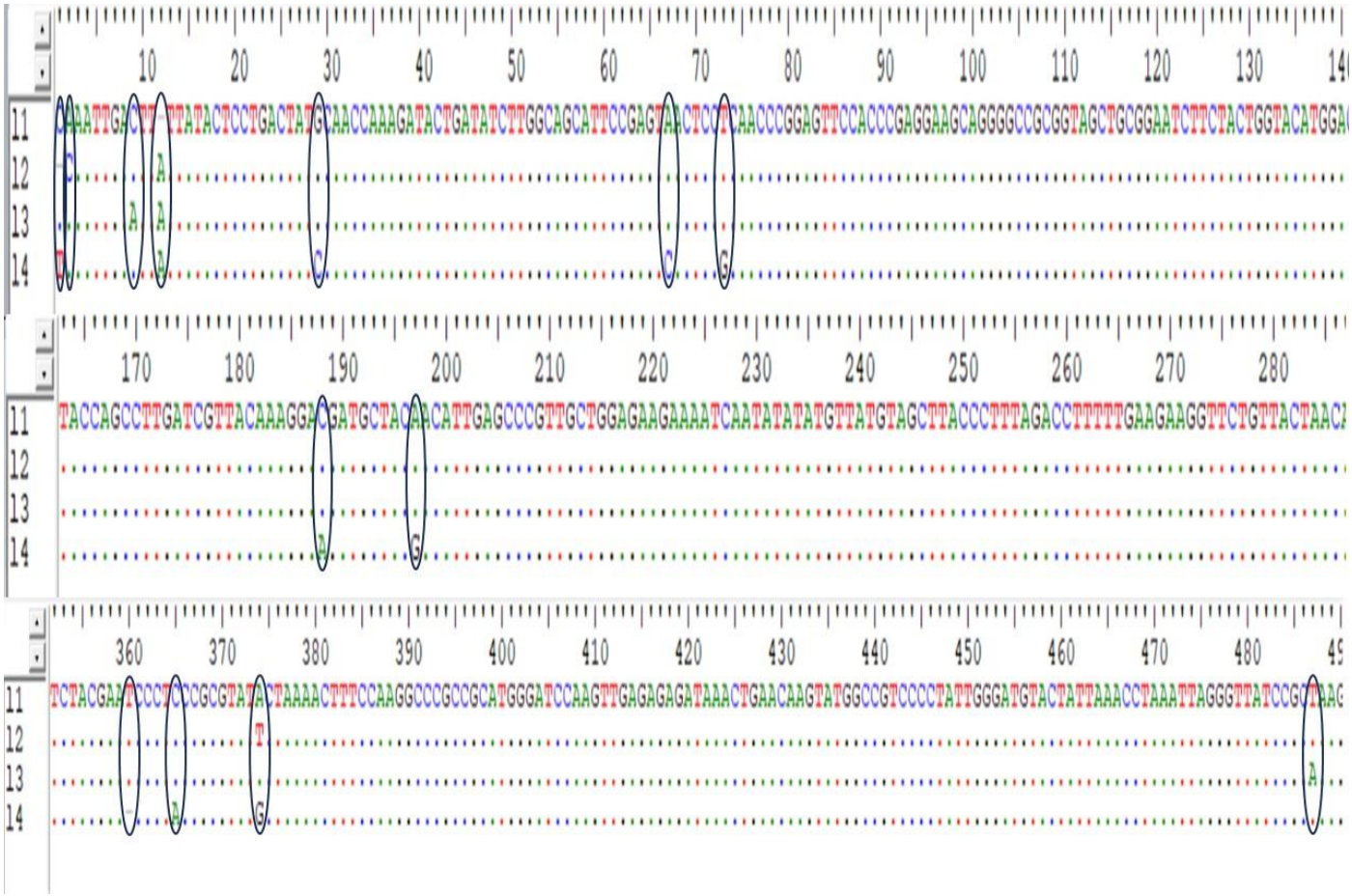

**Figure 2:** Barcode of selected *Lannea* species showing unique SNP variations

*Phylogenetic Relationship*

The ability of a barcode to distinguish and identify species relies on detecting interspecific variations in DNA sequences. A species is deemed resolved when its individuals form a distinct monophyletic branch.[22] In this study, MCL was effective in the classification of *Lannea* species. This method has been successfully used in the authentication of several plant species, including members of the jewel orchid.[22] Additionally, the sequences derived from the *rbcL* gene marker were aligned to assess the phylogenetic relationships between the species. The result indicated that samples I1, I2 and I3 were clustered in the same clades as *L. welwitschia*, *L. coromandelica* and *Lannea* sp. indicating they are closely related (Fig. 3). Furthermore, visual observation showed that samples I1 and I2 corresponding to *L. welwitschia*, *L. coromandelica* are closely related comparative to sample I3 that corresponded to *Lannea* sp. (Fig. 3). Sample 14 had genetic distance far from the other species with clear differentiation as shown in the phylogenetic tree (Fig. 3) suggesting the sample was a distant relative of the other three species, possibly the oldest and most primitive. However, more markers are required to facilitate its discrimination. These findings were consistent with the BLAST analysis results, confirming that the *rbcL* marker gene was highly effective in distinguishing *Lannea* species. This is in agreement with previous findings on *Acacia* species,[18] selected medicinal plants of Caryophyllales class,[23] and the Chinese medicinal herb "Gusuibu".[6]

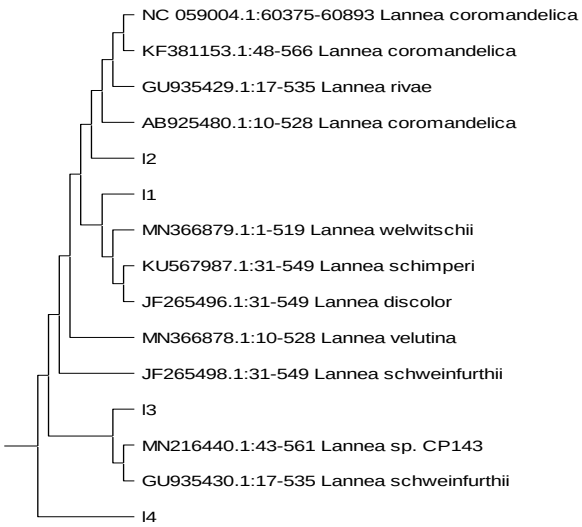


**Figure 3:** Phylogenetic relationship between selected *Lannea* species based on *rbcL* marker.

## Conclusion

The *rbcL* gene marker was successfully isolated and amplified, generating a high-quality sequence. *L. welwitschia*, and *L. coromandelica* were correctly identified using the *rbcL* marker. Sample I4 was inferred to be the oldest and most primitive of the three species and was distantly related to samples I1, I2 and I3. The short sequence of the *rbcL* gene in this study proved to be a valuable and potentially effective molecular marker for identifying *Lannea* species, with the exception of sample I4. It is therefore recommended that more genetic markers such as *matK* be used in combination with other marker genes to further discriminate and identify samples I3 and I4 to species level. Furthermore, the findings in this study provide genetic resources towards developing conservation and sustainable management strategies for these medicinal plants.

## Conflict of Interest

Authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

1. Zhu S, Liu Q, Qiu S, Dai J, Gao X. DNA barcoding: an efficient technology to authenticate plant species of traditional Chinese medicine and recent advances. Chin Med. 2022; 17: 112. DOI: 10.1186/s13020-022-00655-y.
2. Tnah LH, Lee SL, Tan AL, Lee CT, Ng KKS, Ng CH, Nurul Farhanah Z. DNA barcode database of common herbal plants in the tropics: a resource for herbal product authentication. Food Control. 2019; 95: 318-326. DOI: https://doi.org/10.1016/j.foodcont.2018.08.022.
3. Sawarkar AD, Shrimankar DD, Kumar M, Kumar P, Kumar S, Singh L. Traditional System Versus DNA Barcoding in Identification of Bamboo Species: A Systematic Review. Mol Biotechnol. 2021; 63: 651-675. DOI: 10.1007/s12033-021-00337-4.
4. Safhi FA, Alshamrani SM, Bogmaza AFM, El-Moneim DA. DNA Barcoding of Wild Plants with Potential Medicinal Properties from Faifa Mountains in Saudi Arabia. Genes (Basel). 2023; 14. DOI: 10.3390/genes14020469.
5. Bare NB, Jadhav PS, Ponnuchamy M. DNA barcoding for species identification and phylogenetic investigation employing five genetic markers of Withania coagulans. J Appl Biol Biotechnol. 2023; 12: 69-76. DOI: 10.7324/jabb.2023.145330.
6. Shen Z, Lu T, Zhang Z, Cai C, Yang J, Tian B. Authentication of traditional Chinese medicinal herb "Gusuibu" by DNA-based molecular methods. Ind Crops Prod. 2019; 141. DOI: 10.1016/j.indcrop.2019.111756.
7. Iqbal A, Ur Rehman MZ. Characterization, Amplification, and Phylogenetic Analysis of Gossypium herbaceum Using rbcL Molecular Marker. Pak Biomed J. 2023; 6: 09-16. DOI: 10.54393/pbmj.v6i05.891.
8. Malik S, Priya A, Babbar SB. Employing barcoding markers to authenticate selected endangered medicinal plants traded in Indian markets. Physiol Mol Biol Plants. 2019; 25: 327-337. DOI: 10.1007/s12298-018-0610-8.
9. Malu Q, Caldeira GI, Catarino L, Indjai B, da Silva IM, Lima B, Silva O. Ethnomedicinal, Chemical, and Biological Aspects of Lannea Species-A Review. Plants (Basel). 2024; 13: 1-24. DOI: 10.3390/plants13050690.
10. Maroyi A. Lannea microcarpa: A Review of its Medicinal Uses, Phytochemistry and Pharmacological Properties. J Pharm Nutr Sci. 2018; 8: 168-177.
11. Mbaoji FN, Behnisch-Cornwell S, Ezike AC, Nworu CS, Bednarski PJ. Pharmacological Evaluation of the Anticancer Activity of Extracts and Fractions of Lannea barteri Oliv. (Anacardiaceae) on Adherent Human Cancer Cell Lines. Molecules. 2020; 25: 1-19. DOI: 10.3390/molecules25040849.
12. Garba K, Yaro AH, Ya'u J. Anticonvulsant effects of ethanol stem bark extract of Lannea barteri (Anacardiaceae) in mice and chicks. J Ethnopharmacol. 2015; 172: 227-231. DOI: 10.1016/j.jep.2015.06.039.
13. Osafo N, Boakye YD. A Review: Ethnomedicinal, Phytochemical and Pharmacological Investigations of Lannea welwitschii (Hiern) Engl. J Adv Med Pharm. 2016; 11: 1-10.
14. Nitiema M, Soleti R, Koffi C, Belemnaba L, Mallegol P, Ouedraogo N, Kini FB, Ouedraogo S, Guissou IP, Andriantsitohaina R. Ethyl Acetate Fraction of Lannea microcarpa Engl. and K. Krause (Anacardiaceae) Trunk Barks Corrects Angiotensin II-Induced Hypertension and Endothelial Dysfunction in Mice. Oxid Med Cell Longev. 2019; 2019: 1-13. DOI: 10.1155/2019/9464608.
15. Alaklabi A, Ahamed A, Al Qthanin RN, Arif IA, Panneerselvam A, Al-Khulaidi AW. Molecular characterization of endangered endemic plant Aloe pseudorubroviolacea using chloroplast matK and plastid rbcL gene. Saudi J Biol Sci. 2021; 28: 1123-1127. DOI: 10.1016/j.sjbs.2020.11.042.
16. Thakur VV, Tiwari S, Tripathi N, Tiwari G. Molecular identification of medicinal plants with amplicon length polymorphism using universal DNA barcodes of the atpF-atpH, trnL and trnH-psbA regions. 3 Biotech. 2019; 9: 188. DOI: 10.1007/s13205-019-1724-6.
17. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013; 30: 2725-2729. DOI: 10.1093/molbev/mst197.
18. Ismail M, Ahmad A, Nadeem M, Javed MA, Khan SH, Khawaish I, Sthanadar AA, Qari SH, Alghanem SM, Khan KA, Khan MF, Qamer S. Development of DNA barcodes for selected Acacia species by using rbcL and matK DNA markers.

Saudi J Biol Sci. 2020; 27: 3735-3742. DOI: 10.1016/j.sjbs.2020.08.020.
19. Kang Y, Deng Z, Zang R, Long W. DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. Sci Rep. 2017; 7: 12564. DOI: 10.1038/s41598-017-13057-0.
20. Letsiou S, Madesis P, Vasdekis E, Montemurro C, Grigoriou ME, Skavdis G, Moussis V, Koutelidakis AE, Tzakos AG. DNA Barcoding as a Plant Identification Method. Appl Sci. 2024; 14. DOI: 10.3390/app14041415.
21. Maloukh L, Kumarappan A, Jarrar M, Salehi J, El-Wakil H, Rajya Lakshmi TV. Discriminatory power of rbcL barcode locus for authentication of some of United Arab Emirates (UAE) native plants. 3 Biotech. 2017; 7: 144. DOI: 10.1007/s13205-017-0746-1.
22. Ho VT, Tran TKP, Vu TTT, Widiarsih S. Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. J Genet Eng Biotechnol. 2021; 19: 93. DOI: 10.1186/s43141-021-00188-1.
23. Jamdade R, Mosa KA, El-Keblawy A, Al Shaer K, Al Harthi E, Al Sallani M, Al Jasmi M, Gairola S, Shabana H, Mahmoud T. DNA Barcodes for Accurate Identification of Selected Medicinal Plants (Caryophyllales): Toward Barcoding Flowering Plants of the United Arab Emirates. Diversity. 2022; 14. DOI: 10.3390/d14040262.