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Molecular identification of *Peperomia pellucida* (L.) Kunth Piperaceae

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Abstract

The surge in the use of botanicals for medicinal purposes cannot be over emphasized. Many stakeholders now perpetrate sharp practices for monetary gains at the expense of clients' wellbeing. Therefore, proper plant identification must be adhered to and must be followed by authentication in a recognized herbarium. The aim of the present study was to identify the leaf of *Peperomia pellucida* (L.) Kunth Piperaceae using DNA marker and by employing standard method. A maximum identity of 99% was obtained for *P. pellucida* (L.) Kunth. This is above the correctness level of 95%.

Keywords: Identification; Authentication; DNA; Molecular; Safety; Medicinal Plants

1. Introduction

Medicinal plants are an asset for prevention of ailments as well as healing in rural areas worldwide and yet remain vital to healthcare at primary care level [1]. It still remains very important in drug discovery, with eight-tenth of orthodox drugs derived from them [2]. Correct identification of medicinal plants before they are used in drug preparation is very vital to the safety of the end users. Features for plant identification include leaf colour, shape and texture [3]. Plant identification and selection for medicine and food by man have been predicated on organoleptic evaluation of aptness and quality in the past before the advent of fundamental analytical techniques such as microscopic and chemical techniques. But the knowledge of a taxonomist is required and most often, his/her presence will be needed at the location of the plant because a damaged or immature specimen may be difficult to identify. Chemical profiling also has its own limitation as it can be influenced by improper storage conditions of specimen. So, a new approach that is efficient and more accurate for plant identification is needed. Authentication of plant species by utilizing DNA barcoding can be a technique for surmounting some of the highlighted challenges. This method is a swift and precise means of plant identification through DNA extraction and sequencing [4].

Standardization of botanicals is the procedure of stipulating an array of benchmarks which are mostly intrinsic attributes, invariable specifications; precise qualitative and quantitative standards which give an assurance of the plant quality, efficacy as well as safety and it must be replicable [5]. Standardization of medicinal plants begins with the appropriate identity of the plant [6-7]. Among the angiosperms, the family Piperaceae is vast and comprises approximately 3700 species [8]. This family belongs to one of the oldest plants of the pan-tropical source. The two major species are distributed in the *Piper* and *Peperomia* genera with approximately 2000 and 1600 species, respectively. The genus *Peperomia* comprises mainly perennial herbs [8]. *Peperomia pellucida* (L.) Kunth (Piperaceae) is native to tropical North and South America. It is widely distributed throughout the tropics and it is often naturalized as a weed and occasionally cultivated [9]. It can be epiphyte, terrestrial and occasionally lithophytes [10]. It is commonly known as Pepper elder, Shinny bush, Cowfoot, Silver bush, Rabbit ear in English. In French, it is called Cresson, Salade soda, Salade soldat. In Portuguese, it is known as Coracaozinho, Inguade sapo while it is known as Alumbre in Spanish [11].

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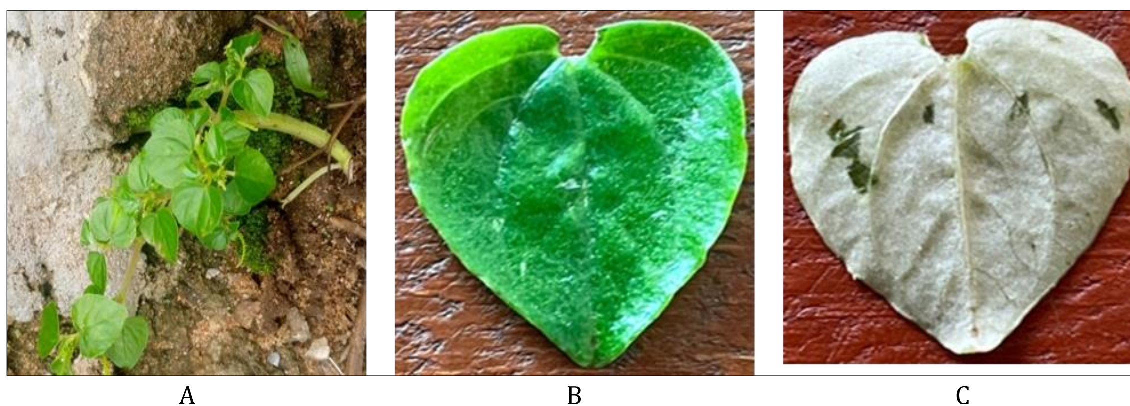
Ethnomedicinally, it is used for rheumatism, cuts, boil and fever in India, diarrhea, insect and snake bites in Bangladesh and dizziness in Indonesia [11]. A lot of these ethnomedicinal claims have been validated pharmacologically. Antipyretic [12], *a*nti-inflammatory **[**13], antisickling [14], antimicrobial [15], anticancer**,** antidiarrheal, antioxidant, hypotensive and antidiabetic [11] activities have been reported. The study is aimed at identifying *P. pellucida* at molecular level.

2. Material and methods

2.1. Collection of plant

The leaves of *Peperomia pellucida* (Figure 1) were collected from Amassoma, Southern Ijaw, Bayelsa State Nigeria on the 3rd of January, 2022. They were identified at site by Dr A.T. Oladele of the Department of Forestry and Wild Life Sciences, University of Port Harcourt, Rivers State of Nigeria. Authentication of the plant was carried out at the herbarium of the Department of Pharmacognosy & Herbal medicine, Faculty of Pharmacy, Niger Delta University where an herbarium voucher number NDUP0180 was assigned.



A B C

Figure 1 *Pepperomia pellucida* growing in the College of Health Science, Niger Delta University, Bayelsa State, Nigeria; A- whole plant, B- Leaf Adaxial surface, C –Leaf Abaxial surface

2.2. Molecular Identification

2.2.1 DNA extraction

Extraction was carried out with the use of ZR fungal/bacterial DNA mini prep extraction kit purchased from Inqaba South Africa. Fresh leaves of *P. pellucida* were cut into strips of 1.00 cm x 1.00 cm and suspended in 200 µL of isotonic buffer in ZR BashingBead Lysis tubes, 750 µL of lysis solution was subsequently transferred into the lysis tubes. The cells of the leaves were disrupted using a Disruptor GenieTM for 5 min. The ZR bashing bead lysis tubes with their contents were centrifuged at 10,000 *g* for 1 min. The supernatant (400 µL) was transferred into a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000 *g* for 1 min. after which fungal/bacterial DNA binding buffer (1200 µL) was introduced to the filtrate in the collection tube making it up to 1600 µL. Exactly 800 µL of this was transferred into a new Zymo-Spin IIC column in a collection tube and then subjected to centrifugation at 10,000 *g* for a min, while the content of the collection tube was disposed off. This operation was also carried out for the remaining 800 µL. Fungal/bacterial DNA Pre-Wash Buffer (200 µL) was introduced to Zymo-Spin™ IIC column that was placed in a new collection tube and centrifugation process was performed at 10,000 *g* for a minute. Thereafter, Fungal/Bacteria DNA Wash Buffer (500 µL) was introduced and centrifuged at 10,000 *g* for a minute. The Zymo-Spin™ IIC column was thereafter placed in a sterile microcentrifuge tube (1.5 mL) and DNA Elution Buffer (100 µL) added directly to the matrix of the column so as to elute the DNA. Centrifugation was performed on the latter at 10,000 *g* for half a minute in order to facilitate the DNA elution. The ultra-pure DNA was thereafter kept at 20° C for further study [16].

2.2.2 DNA quantification

The genomic DNA obtained was later quantified using the Nanodrop 1000 spectrophotometer.

2.2.3 Maturase K (MATK-1RKIM-F) Amplification

The Maturase K region of the isolates were amplified using the MATK-1RKIM-F ACCCAGTCCATCTGGAAATCTTGGTTC, MATK-1RKIM-R CGTACAGTACTTTTGTGTTTACGAG primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 µL for 35 cycles. Included in the PCR mix were: the 2X Dream taq Master mix purchased from Inqaba,

South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the DNA of the *P. pellucida* leaf that was extracted served as the template. The PCR conditions were as follows: Initial denaturation, 95ºC for 300seconds; denaturation, 95ºC for half a minute; annealing, 53ºC for half a minute; extension, 72ºC for half a minute for 35 cycles and final extension, 72ºC for 300seconds. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualization was performed in UV transilluminator light.

2.2.4 Sequencing

The arrangement of the nucleotides of the gene (sequencing) of *P. pellucida* was executed by employing the BigDye Terminator kit on a 3510 ABI sequencer provided by Inqaba Biotechnological, Pretoria South Africa. It was done by using 10 µL which included 0.25 µL BigDye® terminator v1.1/v3.1, 2.25 µL of 5 x BigDye sequencing buffer, 10 uM Primer PCR primer, and 2-10ng PCR template per 100bp. The employed conditions of sequencing were: 32 cycles. of 96°C for 10sec, 55°C for 5sec. and 60°C for 4min.

2.2.5 Phylogenetic Analysis

The sequences that were obtained were edited using the bioinformatics algorithm Trace edit, closely related sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN and were aligned by the use of MAFFT. The history of the evolution of the plants in the family was deduced with the use of the Neighbor-Joining method in MEGA 6.0 [17]. The bootstrap consensus tree deduced from five hundred replicates [18] was chosen to exemplify history of evolution of *P. pellucida* and the evolutionary distances were computed using the Jukes-Cantor method [19].

3. Results and discussion

The amplification of Mat k gene was successful because it aligned with molecular data at 850bp (Figure 2). The Mat K gene complex is frequently utilized for evolution studies in medicinal plants and has helped to solve many taxonomic challenges [20]. A maximum identity of 99% was obtained for *P. pellucida* (L.) Kunth and 85% for *P. pellicida* and *P. pseudoforcata* C.DC. while less than 85% were recorded for *P. reptilis* C.DC. and *P. metallica* Linden & Rodigas in this study (Figure 3). Maximum identity of plant species is an indication of the measure of similarity of the identified plant species. A maximum identity that is above 95% is an indicator of the correctness of the plant species' identification. However, this may not be so if plant species that are similar to the one to be identified are not incorporated into the reference database. Data gathered from sequenced genes are crucial in molecular systematic studies. Analysis of sequenced DNA of medicinal plant species provides vital taxonomic data. Doubtlessly, The use of sequence data is very instructive in the characterization and phylogenetic relationship of different plant species. [21].

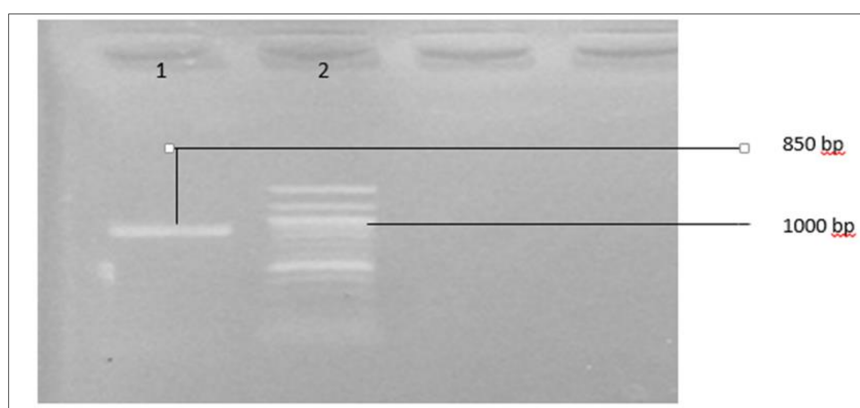


Figure 2 Agarose gel electrophoresis of MAT-K gene of *Pepperomia pellucida*. Lane 1 represents the MAT-K gene band (850bp). Lane 2 represents the 100bp Molecular ladder of 1500bp

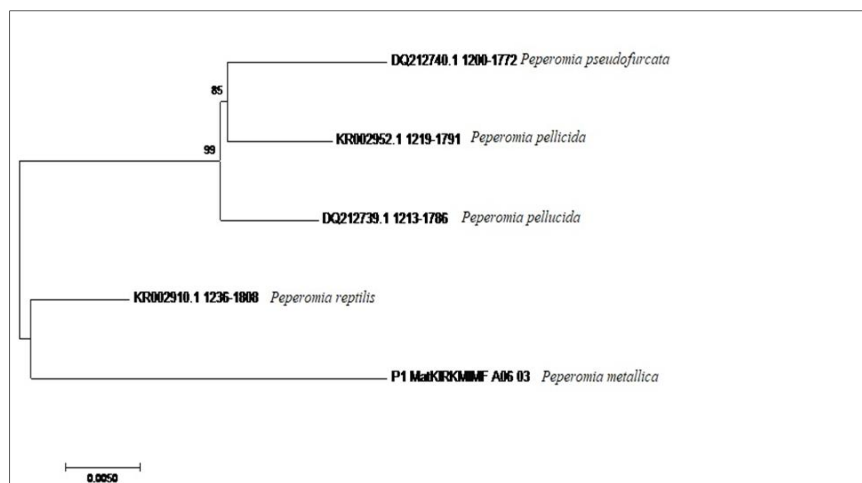


Figure 3 Phyllogenetic tree of *Peperomia pellucida*

4. Conclusion

Some degrees of similarity in sequences were observed in our findings, especially between *P. pellicida* and *P. pseudoforcata*. The findings from this study have shown that medicinal plant and co-generic species can be differentiated with the use of DNA barcoding technique with Mat-K region which is a known DNA marker for plants. Plant characterization with DNA markers is a good method of identification of plant medicines and should be recommended for plants especially those with closely related species. This does not jettison the expertise of taxonomists but will go a long way at validating their work.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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