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Molecular Identification of *Phyllanthus amarus* Schumach. & Thonn. Phyllanthaceae

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Abstract

The upswing in the exploitation of plants for prevention and treatment of diseases in human and animal cannot be overstressed. This upsurge has drawn the attention of the users, health care professionals and policy holders in health care delivery to the safety and therapeutic efficacy of these products. Every issue that has to do with these is initiated by identifying the plant(s) used as the raw material correctly. The aim of the present study is to identify the leaf of *Phyllanthus amarus* Schumach. & Thonn. Phyllanthaceae using DNA marker by employing standard method. *Phyllanthus amarus* is a very useful plant made into various preparations such as decoction, infusion and maceration, among others and used folklorically to treat miscellaneous ailments such as jaundice, hepatitis, dysentery, dropsy, diarrhoea, fevers, inflammation, urinary issues, diabetes, gonorrhoea, kidney ailments, measles, influenza and tuberculosis among many others.

Keywords: DNA; Safety; Identity; Efficacy; Barcoding

Introduction

Errors have been detected in using only morphological characters for plant identification [1-5]. The use of DNA-based technique, therefore, would provide proper authentication for discrimination of plant species [6]. It is a new trend for quick technique of identifying and evaluating organisms, including plants, animals and microorganisms based on the sequence of the DNA obtained from a small fragment of the individual organism [7]. This method also helps in detecting any species that are possibly novel [8-9], it is also a potential tool for detecting any error often faced in identifying species since this method often combines similarity-based method of DNA barcoding and morphology to solve taxonomical challenges and any stage plant's growth can be employed [10-12]. The method is based on comparism of DNA sequence with established reference databases [13]. *Phyllanthus amarus* has its origin in the tropical parts of America and widely distributed throughout the tropical and subtropical regions globally, occurring as a weed and used for gonorrhea, jaundice, diabetes, stomachache, malaria, skin rashes, itches and dysentery [14] in ethnomedicine. Numerous biological activities have been carried out on the plant, some of which are anti-inflammatory [15], antioxidant [16], antinociceptive [17], immunostimulant [18], antiviral [19], antibacterial [20], antihyperglycemic [21], anti-diarrheal [22], gastro protective and antiulcer activity [23], contraceptive [24] among many others. Phyllanthin, hypohyllanthin, nirtetralin, nitanthin and phytetralin are some isolated chemical constituents of the plant [25]. The study is aimed at identifying *P. amarus* at molecularlevel.

Methodology

Collection of plant

The leaves of the *P. amarus* (Figure 1) were harvested from the premise of Madonna University campus, Elele, Rivers State, Nigeria on the 10th of May 2022 after it has been properly identified at site by Dr. A. T. Oladele of the Department of Forestry and Wildlife Sciences, University of Port Harcourt, Rivers State, Nigeria. Mr, Frelix I. Nwafor of Department of Pharmacognosy, Faculty of Pharmacy, University of Nigeria, Nsukka authenticated the collected plant samples where a voucher specimen with voucher number PCG/UNN/0436 was thereafter deposited.

Figure 1: *Phyllanthus amarus* growing in Madonna University campus, Elele, Rivers State, Nigeria.

Molecular Identification

The extraction of the deoxyribonucleic acid (DNA) of *P. amarus* leaf sample was effected by employing Zymo Research (ZR) fungal/bacterial deoxyribonucleic acid (DNA) Mini-Prep™ isolation/recovery kit obtained from 'Inqaba', South Africa and carried out using the method described by Alade et al. [26]. It was thereafter quantified and subjected to Maturase K region amplication with the use of PCR [26]. Chromatography of the amplified-product was run by using Gel electrophoresis technique with agarose-gel (1%) as the stationary phase at 120V for 15 minutes. It was finally visualized and removed in UV transilluminator light. ZR fungal/bacterial DNA mini prep recovery kit was then used to recover the DNA for sequencing, which was carried out at Inqaba, South Africa. The resulting amino acid sequences of the DNA were engineered (edited) by employing the 'bioinformatics algorithm Trace edit' in which sequences that were related or showed similarities were downloaded from the 'National Center for Biotechnology Information' (NCBI) data-base by blasting the DNA sequence and aligning [27]. This was used to synthesize evolution trend of the *P. amarus* in the Phyllanthaceae family.

Result

The phylogenetic tree of *P. amarus* is as shown in Figure 2.

Discussion

The success of amplication of mat k gene was deduced from the 900 bp obtained for the extracted DNA of the plant (Figure 3). Issues of concern or challenges arising from Plant taxonomy are mostly tacked with the use of *mat-K* gene complex [8]. A size of 800 – 900 base pair indicates successful amplication which in turns shows uncontaminated DNA. Maturase K genes are employed for the study of 'sequence variations' and 'evolutionary trends' at the level of the genus [2]. The upsurge in the use of the gene is as a result of its fast evolution at the nucleotide and analogous amino acid levels [10]. The measure of similarity of a plant species to an already identified one in the data base is known as its maximum identity. Accuracy of identification is specified by a maximum similarity that is not less than 95%. One hundred percent (100%) maximum identity was observed for *P. amarus* (Figure 2). The use of DNA sequence data has been seen to be informative in characterizing and synthesizing phylogenetic relationship of different medicinal plant species. This trend contributed immensely to adequate plant classification [28]. Information obtained from sequenced genes is crucial in molecular systematic studies [29] Analysis of obtained DNA sequence can provide taxonomic information of medicinal plants [13]. Similarity of DNA sequence indicates that the various sequences have the same homologs [30]. DNA sequences with percentage identity above 70 means that they have ≥ 90% probability to share the same biological processes and functions. Data gathered from sequenced genes is crucial in molecular systematic studies. Analysis of sequenced DNA of medicinal plant species provides vital taxonomic data. Doubtlessly, genomic regions differ significantly in their prospective phylogenetic instructiveness and their beneficence in solving a taxonomy challenges [31].

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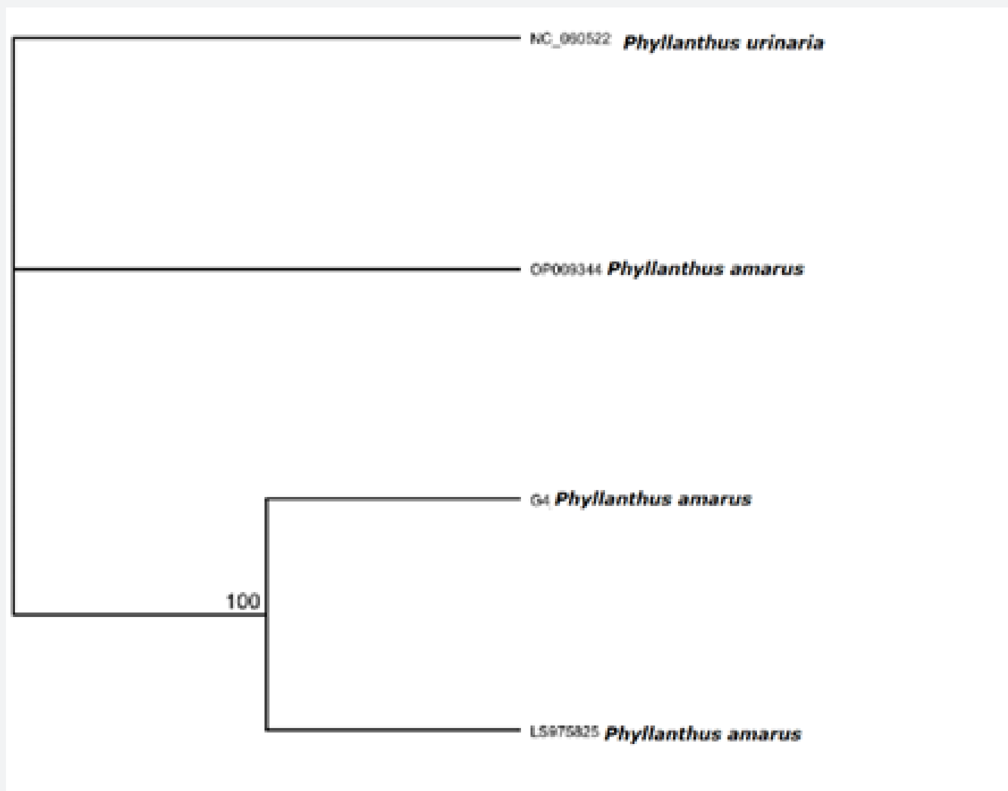

Figure 2: Phylogenetic tree of *Phyllanthus amarus*.

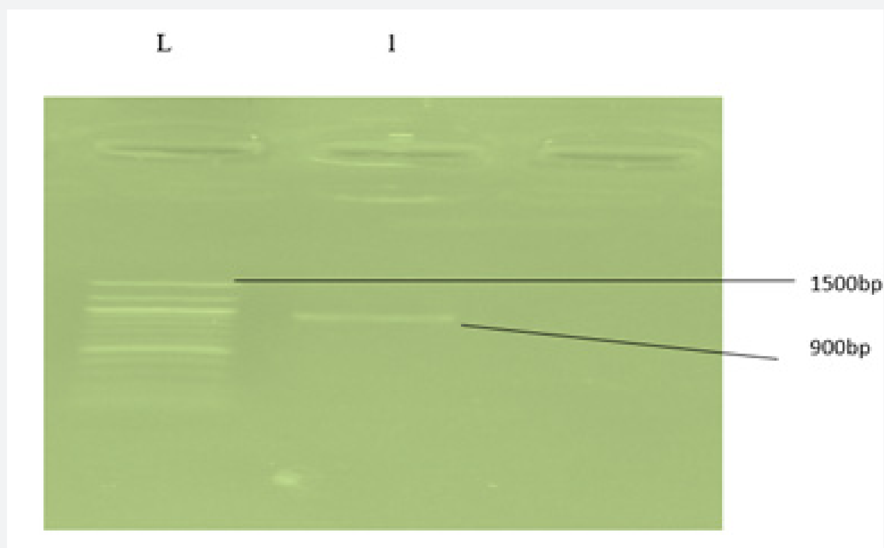

Figure 3: Agarose gel electrophoresis of MAT-K gene of *P. amarus*

Lane 1 represents the MAT-K gene band (900bp). Lane L represents the100bp Molecular ladder of 1500bp.

Conclusion

The findings from this study have shown that medicinal plants and cogenerics 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 for 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.

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