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rbcL GENE SEQUENCING AND RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) PROFILING OF *Pavonia senegalensis* (CAV.) LIESTNER (MALVACEAE) A MEDICINAL PLANT COLLECTED FROM GIWA NORTHWEST NIGERIA

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ABSTRACT

Pavonia senegalensis (Cav.) Liestner is a medicinal plant used in African traditional medicine for the treatment of diarrhea, soft and tissue infections, induction of labor and contraception. In this study, ribulose-1, 5-biphosphate carboxylase (rbcL)gene sequence and random amplification of polymorphic DNA (RAPD) profile of the plant were determined. The study showed that the species of the plant is phylogenetically related to *Abutilon sp., Hibiscus micranthus, Hibiscus punalunensis, Hibiscus rosa-sinensis* and *Lagunaria patersonia* all from the same family showing 98% similarity on the basis of rbcL gene sequences. The studied specimen and related taxa inferred from partial rbcL gene sequence demonstrated a distinct lineage; therefore, could distinguish the species as *P. senegalensis* without doubt. RAPD profile of *P. senegalensis* generated amplified products ranging from 320 to 700 bp. A total of 9 bands were observed for the plant species using 3 primers (OP-A07, OP-A10 and OP-C06). The determined rbcL gene sequence and RAPD profile of the *P. senegalensis* will help in quality control for authentication and detection of adulteration in the development of the plant as an herbal medicine.

KEYWORDS: **DNA; Primers; Phylogenetic tree; PCR.**

INTRODUCTION

DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for the identification of chemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remain the same irrespective of the plant part used while the phytochemical content will vary with the plant part used, physiology and environment [1]. The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples [2].

Plastid rbcL (ribulose-1,5-bisphosphatecarboxylase/ oxygenase large sub-unit) is the most commonly sequenced gene for phylogenetic studies of plants [3], in which the success rate of PCR and sequencing is higher compared with the other selected plant characterization genes such as matK (maturase K) [4,5]. RAPD (random amplified polymorphic DNA) provides an easy and rapid method and does not need any DNA probe or sequence information for primer design. RAPD has been used previously for estimation of genetic diversity in plant species [6].

Pavonia senegalensis (Cav.) Liestner synonyms *Pavonia hirsuta* Gull. & Perr., *Pavonia arabica* Hoschst ex Steud and *Pavonia argentina* Gurke. It is called *Tsu* in Hausa. It is found in drier parts of

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tropical Africa on sandy-clayey soils, humid sands and sometimes on rocky screen in savannah areas; often near villages; in woodland with Grewia, Terminalia, etc.; along rivers and in seasonally dry riverbeds; usually in light shade [7]. *Pavonia senegalensis* is usually an annual plant, but occasionally lives longer. A spreading, short-lived perennial with semi-prostrate to ascending branches, up to 1.25 m. Stems somewhat angular with harsh stellate hairs. Leaves suborbicular in outline, angular to shallowly lobed; lower surface densely stellate-hairy. Stipules up to 10 mm, filiform. Flowers solitary in the leaf axils, up to 8 cm in diameter sulphur-yellow with a maroon centre. Epicalyx bracts 12-16, narrowly linear, usually shorter than the calyx lobes [8].

The roots are put into cold water to draw and the infusion is taken as a remedy for diarrhoea in South and East Africa [9]. The powdered seed is taken with milk and used as a contraceptive in Sokoto North-west Nigeria [10]. The infusion of the roots is used in antenatal care for general wellbeign in Katsina North-west Nigeria [11]. A cold water infusion of the dry roots is taken to induce labour, particularly if the onset is being delayed in Botswana [7].

literature on the molecular characterization of *P. senegalensis* is not currently available based on the search carried out. In this study, we attempted to characterize this plant species by rbcL gene sequence and RAPD fingerprinting to ease the identification of the plant.

MATERIALS AND METHOD

Plant Collection and Identification

Leaves of *P. senegalensis* were collected from Rafin Yashi, Giwa Local Government Area of Kaduna State in November, 2018. The plant was identified and authenticated by Taxonomist U.S Gallah at National Research Institute for Chemical Technology (NARICT), Zaria, Kaduna State Nigeria and assigned a voucher number 24011.

DNA Extraction

The leaf specimen of the plant was washed with distilled water and then crushed using pestle and mortar. AccuPrep® Genomic DNA Extraction Kit (Bioneer Corporation, Germany) was used for the DNA extraction according to the manufacturer's instructions.

DNA Quantification and Quality Assessment

DNA quantification and quality assessment were carried out using UV-VIS spectrophotometry. An aliquot of the DNA was diluted in distilled water (ratio of 1: 100) in a microcuvet. The optical density (OD) was then determined at 260, 280 and 320 nm against a blank. The DNA concentration in the sample was calculated using the formula 1.0 OD_{260} = 50 µg/ml (under standard conditions, i.e., a 1-cm light path). The ratio OD_{260} to OD_{280} provides information about the purity of the DNA sample. [12].

Nucleotide Sequencing

A set of primer, rbcLaF (5'ATGTCACCACAAACAGAGACTAAAGC3'; [13]) and rbcLaR (5'GTAAAATCAAGTCCACCRCG3'; [14]) were used in this study for the amplification of rbcL gene of the chloroplast. A total volume of 20 µl of PCR reaction mixture prepared as follows: 8 µl of DTCS Quick start master Mix, 2 µL of genomic DNA, 1µ of each primer and adjusted with sterile distilled water (to 20 µl). The PCR amplification was performed with a PTC-100™ Programmable Thermal Controller (MJ Research INC.) as follows: 96°C for 20 sec, followed by 30 cycles of 50°C for 20s, 51°C for 30s and followed by an final elongation step at 60°C for 4 min.

A long (20 × 14 cm) 1.5% agarose gel using 1X TAE buffer containing 0.5 µg/mL ethidium bromide was used for electrophoresis of PCR-products. Gel images were obtained using Proxima C16 Phi+ (Isogen Life Science) UV transilluminator and Opticom (version 3.2.5; OptiGo) imaging system. The amplified PCR products were determined on gel for the presence or absence of the band. The size of PCR products resulting from the primer pair were determined by using an Amersham 100-bp ladder (GE Healthcare).

PCR products were purified using a PCR Purification Kit (Thermo scientific) before being sequenced using the dideoxynucleotide chain termination method with a DNA sequencer (ABI 3130XL, Applied Biosystems) and a BigDye Terminator version 3.1 Cycle Sequencing RR-100 Kit (Applied Biosystems). NCBI BLASTN 2.9.0+ online database was applied to the produced sequence for alignment, assignment of taxa and construction of phylogenetic tree. Obtained rbcL gene sequence was submitted to the GenBank database.

Random Amplified Polymorphic DNA (RAPD)-PCR Analysis

Ready-To-Go RAPD analysis beads (GE Healthcare, Buckinghamshire, UK) was used for RAPD-PCR analysis. The PCR mixture of 20 µL contained a single Ready-To-Go RAPD analysis bead, 25 pmol of a single RAPD primer, 50 ng of template DNA and sterile distilled water. The bead contained a thermostable polymerase (AmpliTaq™ DNA polymerase and Stoffel fragment), dNTPs (0.4 mM each), BSA (2.5 µg) and buffer (3 mM $MgCl_2$, 30

mM KCl and 10 mM Tris, pH 8.3). Three commercially available primers were used in this study. Each was a 10-mer of arbitrary sequence: OP-A07 (5-GAAACGGGTG-3), OP-A10 (5-GTGATCGCAG-3) and OP-C06 (5-GAACGGACTA-3).

The PCR amplification was performed with a PTC-100™ Programmable Thermal Controller (MJ Research INC.) as follows: a 1 cycle of 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 29°C for 1 min, 72°C for 1 min and a final extension of 72°C for 5 min. A long (20 × 14 cm) 1.5% agarose gel using 1X TAE buffer containing 0.5 µg/mL ethidium bromide was used for electrophoresis of PCR-products. Gel images were obtained using Proxima C16 Phi+ (Isogen Life Science) UV transilluminator and Opticom (version 3.2.5; OptiGo) imaging system. The amplified PCR products were determined on gel for the presence or absence of the band. The size of PCR products resulting from the primer pair were determined by using an Amersham 100-bp ladder (GE Healthcare).

RESULT

DNA Quality Asssessment

The genomic DNA isolated from *P. senegalensis* was found to have a ratio of OD_{260} to OD_{280} of 1.6.

Nucleotide Sequence of *P. senegalensis*

The rbcL nucleotide PCR-product is shown is Figure 1. The rbcL nucleotide sequence was submitted to the Genbank and assigned an accession number MN1225. The top Blast results of the rbcL nucleotide sequence of the plant are shown in Table 1.

The Neighbor Joing Tree of *P. senegalensis* and Related Taxa

The neighbor joining tree of *P. senegelensis* and related taxa using partial rbcL sequence is shown in Figure 2.

RADP Profile of *P. senegalensis*

The RAPD banding patterns of *P. senegalensis* are illustrated in Figure 3. The RAPD profile using the sample generated amplified products ranged from 320 to 700 bp. A total of 9 bands were observed for the plant species using 3 primers (OP-A07, OP-A10 and OP-C06).

DISCUSSION

The genomic DNA isolated from *P. senegalensis* was found to have a ratio of OD_{260} to OD_{280} of 1.6, according to Sambrook *et al*., [12] the ratio OD_{260} to OD_{280} provides information about the purity of the DNA sample, 1.8 value will show the highest purity, if more than 1.8 shows the presence of RNA contamination and less than that indicates protein contamination.

BLAST (Basic Local Alignment Search Tool) search, genetic distance and tree-based methods have been commonly used for identification of species. In this process, sequence is assigned on the basis of its similarity to a set of reference (identified) sequences [15]. We conducted NCBI BLASTN 2.9.0+ online database-search to determine the approximate identification and related taxa of the studied specimen which showed 98% sequence similarity with five plant species (Table 1). The tree that was inferred from partial rbcL gene sequence of the studied specimen and related taxa demonstrated a distinct lineage of the studied specimen; thus, could distinguish the species of *P. senegalensis* without doubt. Absence of *P. senegalensis* sequence and related genera in the database may be responsible for such tree-topology. Assignment of an unknown specimen under valid taxa primarily depends on the availability of the sequence in the database. Molecular genetic techniques for species identification based on single-gene sequence similarity or phylogenies are rapidly gaining wide use except few criticisms [16].

Sequence-based analyses sometimes fail to distinguish between species because of the significant similarity between their DNA sequences in the amplified region. In such instances, RAPD primers are able to distinguish taxa [17], because RAPD analysis includes both of the coding and non-coding regions of the genome [18]. The RAPD-primers (OP-A07, OP-A10 and OP-C06) used in this study were able to amplify the DNA obtained from the plant-specimen (Figure 1). Some of the reported problems with RAPD are related to reproducibility, designing appropriate primers and amplification of RAPD-PCR products [19]. PCR conditions constitute another crucial factor for obtaining amplified products, especially for plants [20]. However, if the overall temperature profiles (annealing temperature) inside the PCR tubes are identical, RAPD fragments are then likely to be reproducible [21,22].

CONCLUSION

The rbcL gene of *P. senegalensis* was sequenced and submitted to the Genbank and also the RADP profile of the plant using three primers (OP-A07, OP-A10 and OP-C06) was determined. The rbcL gene

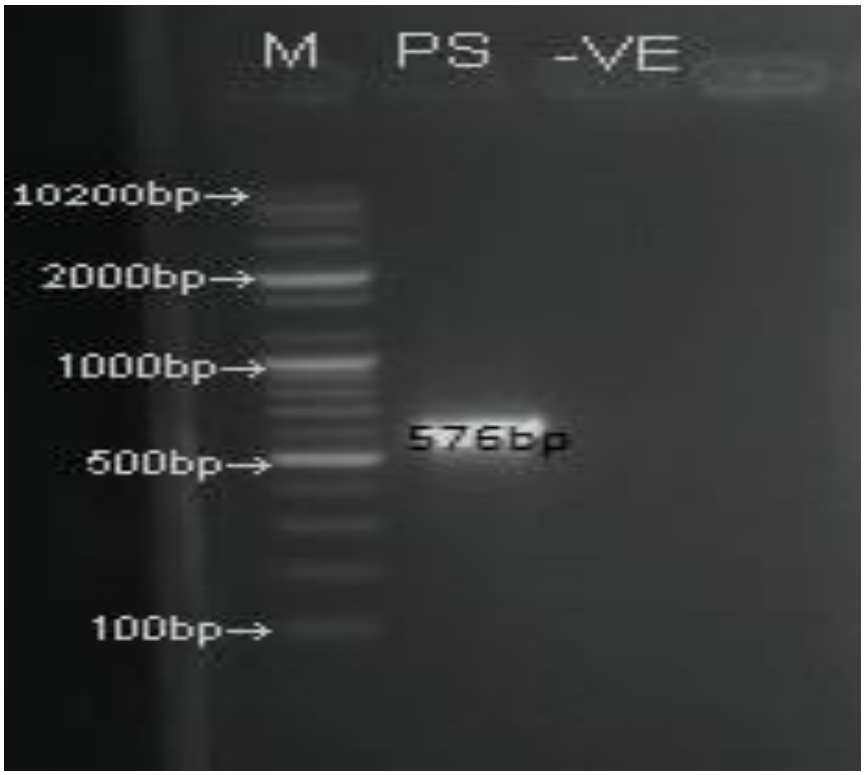

Figure 1: The amplified rbcL-PCR product of *P. senegalensis*.
Key: M= 100 bp ladder molecular weight maker, PS= rbcL primers, -VE= negative control.

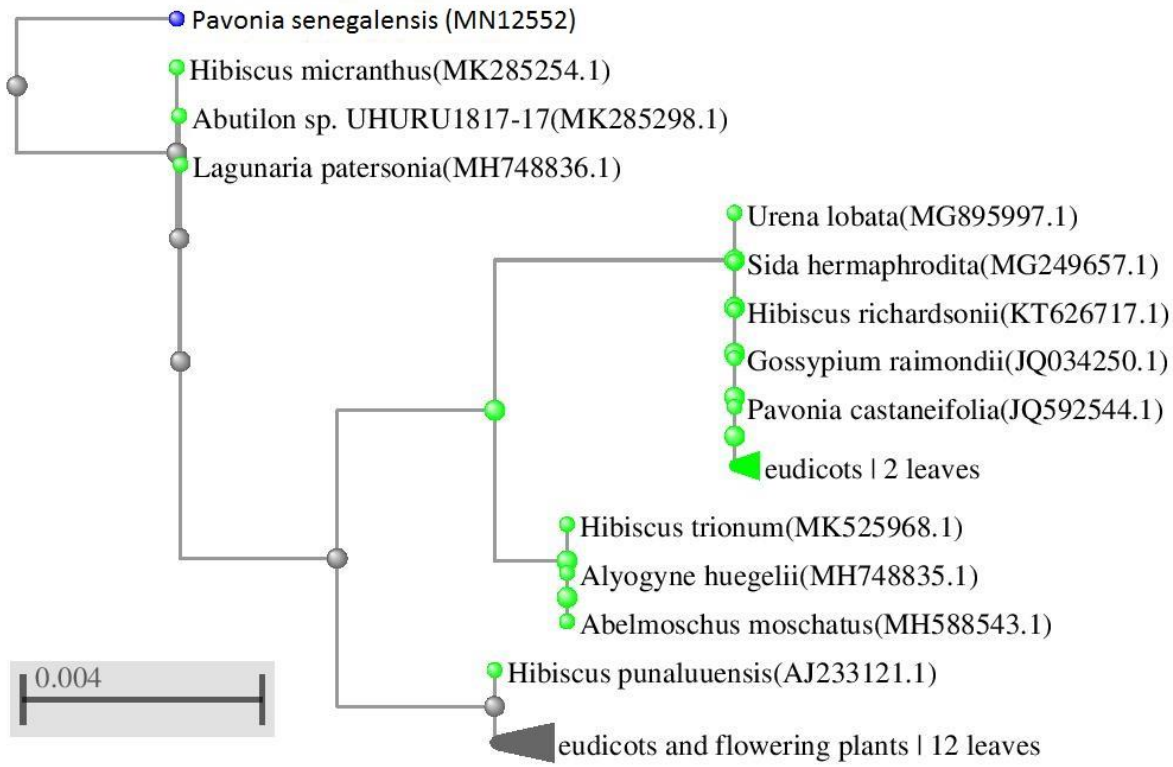

Figure 2: The Neighbor Joing tree showing the relationship of *P. senegalensis* with the related taxa.
GenBank accession numbers of the corresponding taxa are written in parentheses. The scale bar represents the branch length measurement in the number of substitutions per site.

Table 1: The top Blast result of rbcL sequence of *P. senegalensis* from the NCBI BLASTN 2.9.0+ online database.

S/N	Plant Name	Family	Percentage Similarity (%)	Accession Number
1	*Abutilon sp.*	Malvaceae	98.01	MK285298.1
2	*Hibiscus micranthus*	Malvaceae	98.01	MK285254.1
3	*Hibiscus punalunensis*	Malvaceae	97.61	AJ233121.1
4	*Hibiscus rosa-sinensis*	Malvaceae	97.61	MH588538.1
5	*Lagunaria patersonia*	Malvaceae	97.60	MH748836.1

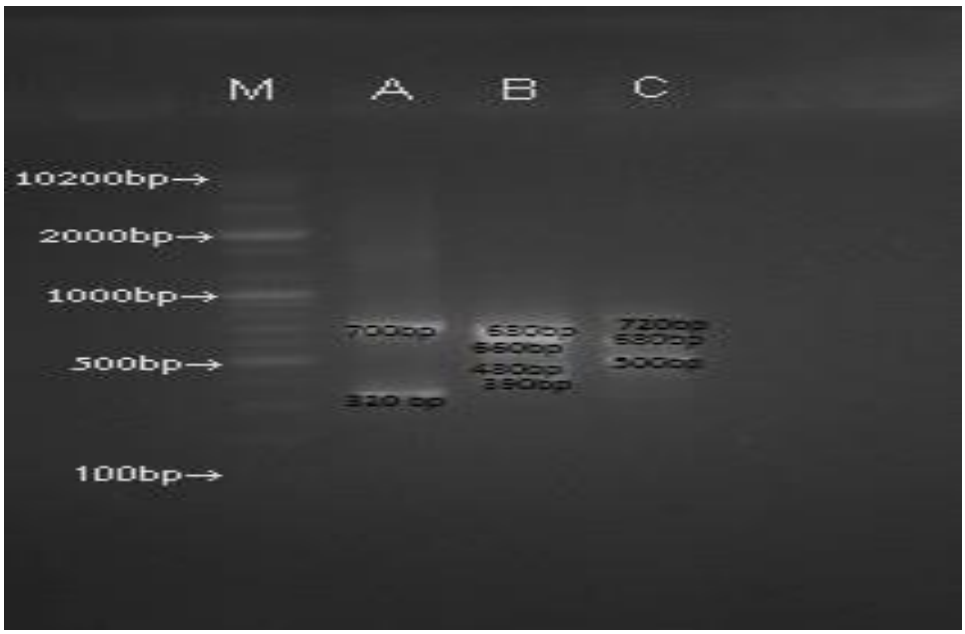

Figure 3: RADP-PCR products of *P. senegalensis* for different primers.
Key: M= 100 bp ladder molecular weight maker, A= OP-A07, B= OP-A10 and C= OP-C06 primers.

was found to be able to distinguish the species of the plant from closely related species unequivocally. The combination of rbcL gene sequence and RAPD profile of *P. senegalensis* may amend the identification process and help in quality control for authentication and detection of adulteration in the development of the plant as an herbal medicine.

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