DEVELOPMENT OF RAPD MARKERS FOR AUTHENTICATION OF *MOKSHAKA* (*SCHREBERA SWIETENIOIDES* ROXB.)

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Abstract: *Schrebera swietenioides* Roxb. commonly known as Mokshaka in Ayurvedic classical texts like *Charaka Smhita,Sushruta samhi*ta etc., is well-known for its *Kshara karma*. It is also used to treat in many diseases like *Kantha roga, Grahani, Prameha, Gulma* etc. The fresh young leaves of *Schrebera swietenioides* Roxb. were collected from its natural habitat and used for molecular characterization and DNA fingerprints, by standard and most convenient Random Amplified Polymorphic DNA (RAPD) markers at Food testing laboratory, *Junagadh* Agriculture University, Gujarat, India. In RAPD study primer 3,4,5,6,7 and 9 showed bright bands and most of them having more number of light and bright bands matching characters with plant. Observed RAPD marker can be used to differentiate genuine as well as adulterated samples. The results may be used for the further research purposes and also required DNA Barcoding studies for further authentication.

Key words: *Mokshaka, Schrebera swietenioides* Roxb. RAPD, DNA fingerprints.

INTRODUCTION

Worldwide, medicinal plants have been used to treat the disease and maintain the health of an individual from centuries. However, adulteration and use of specious materials as substitutes have become a major concern for users and industry for the safety and efficacy of the drug. Therefore, authentication of medicinal plants is of very importance. Morphological[i], anatomical[ii], chemical[iii] and DNA markers[iv] solve the problem by differentiating the genuine material from the adulterants, substitutes and spurious drugs[v]. Recently, Random Amplified Polymorphic DNA (RAPD) method has been used for the estimation of genetic diversity of medicinal plants and certain plants have been reported and recounted for their molecular characterization through RAPD markers[vi]. Hence their identification through molecular characters is needed. Review of literature reveals that the plant *Schrebera swietenioides* Roxb. has not been intended for its molecular characterisation. The present study was carried out to establish certain botanical standards for identification and authentication of *Schrebera swietenioides* Roxb. through RAPD analysis and also introduce *Schrebera swietenioides* Roxb. as a mother source plant of *patala (stereospermum souveloens) by its* phyto pharmacognostical profile. *Mokshaka* is mentioned in *Ayurvedic* classical texts like Charaka Smhita,Sushruta samhita for many pathological condition or as a part of formulations. It is well-known for its *Kshara karma*. The study also done for DNA finger printing to compare it with *Patala for* substitution. Here results for DNA fingerprinting of *Schrebera swietenioides* Roxb.are published for authenticating plant by its DNA profile for standardization.

MATERIALS AND METHODS

Collection and preservation of the sample:

Fresh young leaves of *Schrebera swietenioides* Roxb. were collected from its natural habitat Datar hills, Junagadh in April 2018, identified and authenticated by local taxonomist with the help of botanical flora[vii]. A sample specimen was authenticated by expert taxonomist of Pharmacognosy Department of G.A.U., Jamnagar and deposited to institutes Pharmacognosy museum ((Phm 6245-46/16-17)) for future references. The collected leaf samples were washed under running fresh tap water to remove adherent soil and dirt.

Molecular characterization (DNA fingerprints):

Fresh leaves were used for molecular characterization and DNA fingerprints, by standard and most convenient RAPD method[viii]. The RAPD reaction was performed following standard procedures at Food testing laboratory, Junagadh Agriculture University, Gujarat, India.

DNA isolation: Young leaves were selected, cut into small pieces without cutting the veins washed with distilled water and ethanol, frozen with dry ice and crushed. To that, 2 ml of plant DNA extraction buffer was added. The samples were ground thoroughly, transferred into centrifuge tube and added 10 ml plant DNA extraction buffer. 50 µl of BME added to each tube mixed well. Incubated at 65ºC for 1 hour with intermittent mixing and centrifuged for 15 minutes at 10 K (10000 rpm). Supernatant was transferred carefully into fresh tube and added equal volume of chloroform and mixed well. Centrifuged for 15 minutes at 10 K (10000 rpm). Aqueous layer was carefully pipetted into fresh tube and precipitated with isopropanol. DNA pellet suspended in 300 µl of TE and subjected to column purification.

Column purification:

Silica spin columns and buffers were from Qiagen. The column was placed in collection tube, 400µl of equilibration buffer was added to the column and centrifuged at 10000 rpm for 1min. Collected buffer was discarded. 400 µl of equilibration buffer was added to the DNA samples, mixed and loaded into the column (This step was repeated till the DNA sample was completed). Flow through was collected. 500 µl of wash buffer 1 was added, centrifuged at 10000 rpm for 1minute and buffer was collected. 500 µl of wash buffer 2 was added, centrifuged at 10000 rpm for 1 minute and buffer was collected. The empty column was centrifuged with collection tube to completely remove the wash buffer for 2 minute. 50 µl of elution buffer was added to the column placed in new collection tube. Incubated at room temperature for 2 minutes and centrifuge at 10000 rpm for 1 minute and eluted sample was saved (elution 1). Previous step was repeated (elution 2). Quantization of eluted DNA samples was done by loading into the agars gel.

Observation and Result:

Table 1: List of RAPD primers used for the analysis of Medicinal Plant (*Schrebera swietenioides*) DNA sample

Sr. no.	Primer	Sequence 5' – 3'
1.	M-9	GTCTTGCGGA
2.	N-3	GGTAACTCCC
3.	N-6	GAGACGCACA
4.	N-9	TGCCGGCTTG
5.	N-10	ACAACTGGGG
6.	O-1	GGCACGTAAG
7.	O-2	ACGTAGCGTC
8.	O-3	CTGTTGCTAC
9.	O-4	AAGTCCGCTC
10.	O-5	CCCAGTCACT

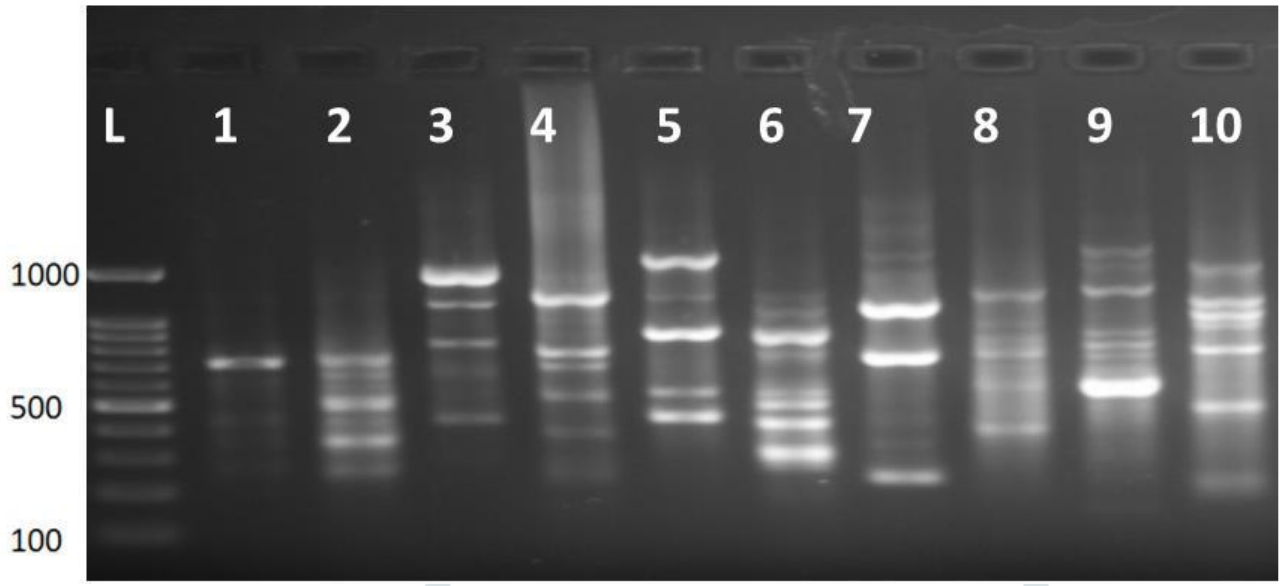

Figure 1: RAPD banding pattern on agarose gel for Sample

TABLE: 2. BAND OBSERVATION FROM FIGURE 1

PRIMER NO:	BAND OBERVATION RANGE (bp)
1	250 to 700
2	250 to 700
3	450 to 1000
4	350 to 900
5	400 to 900
6	250 to 900
7	200 to 800
8	250 to 800
9	500 to 1000
10	150 to 1000

Result and Dicussion:

Medicinal Plant sample was analyzed for DNA fingerprinting through RAPD markers. DNA was extracted by using Doyle and Doyle (1990) method with minor modifications. DNA quantification was done using a Pico drop spectrophotometer and DNA sample was diluted using TE buffer up to 50 ng/ µl. Quality of sample DNA was checked by 0.8% Agarose gel electrophoresis. RAPD-PCR was carried out in Veriti ABI thermal cycler. The resolved amplification products were visualized by illumination under UV light in Gel document system.

The fingerprinting patterns of *Schrebera swietenioides* Roxb., seen as vertical columns with horizontal light bands on a dark background have been depicted in the Figure 1. For the analysis of DNA sample of *Schrebera swietenioides* Roxb. 10 primers were used (1 to 10 RAPD Primers mentioned in Table 1). Primers have been loaded from left to right. Figure 1 for *Schrebera swietenioides* Roxb. contains

primer 1 to 10 with DNA samples for analysis. In DNA sample of *Schrebera swietenioides* Roxb. all primer give good enough result. Primer 3,4,5,6,7 and 10 showed the bright band at 1000bp and 5,6 and 9 showed most bright band at 500bp. And primer 2,4,6,9 and 10 shows more no. of bands in the sequence.

Conclusion:

Establishing the standards for any of the substance or drug is an essential part of finding the correct identity and quality of that drug. The unique bands obtained in Polymerase Chain Reaction (PCR) amplification are clearly differentiated having many bright and light bands indicating the genuinety of the plant *Schrebera swietenioides* Roxb. Observed RAPD marker can be used to differentiate genuine as well as adulterated samples. The results may be used for the further research purposes and also required DNA Barcoding studies for further authentication.

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