

Genomic DNA Isolation and Purification of Two
Endemic Medicinal Plants (*Pterocarpus Santalinus*
Linn.F & *Pimpinella Tirupatiensis* Bal&Subr) of
Seshachalam Hills, Tirumala.

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Abstract

Pterocarpus santalinus is an important endemic medicinal plant with high medicinal values that interfere with DNA extraction procedures and qualitative, quantitative agarose gel electrophoresis. An effective and low-cost protocol for isolating genomic DNA from the roots of *Pterocarpus santalinus* and *Pimpinella tirupatiensis* was described in this paper. It involved a modified CTAB method with distilled water pretreating samples. The A_{260}/A_{280} absorbance ratio of extracted DNA was found to be free from polysaccharide, poly-phenols and tannins contaminants ranged from 2.2 to 2.8 within the high level of purity.

Keywords: *Pterocarpus santalinus*, DNA extraction, Secondary metabolites

Abbreviations: CTAB, hexadecyltrimethylammonium bromide

1. Introduction

The genus *Pterocarpus santalinus* Linn.F. belongs to the family papilionaceae/ fabaceae and popularly known as 'Red sander's (Naidu et al. 2001). It was traditionally used in treatment for headache, skin diseases, fever, boils, scorpion-sting and to improve sight (Chopra et al. 1956; Latheef et al., 2008). Previous chemical constituents revealed the presence of triterpenes, isoflavone, glucosids, savinin and calocedrin (Krishnaveni et al. 2000). The methanolic extract of *Pterocarpus santalinus* was capable of scavenging the free radicals and inhibited the tested bacteria, suggested the potential antibacterial and antioxidant effect (Krishnamurthy et al. 2011). *Pterocarpus santalinus* Linn.F. is an endemic and endangered species of Andhra Pradesh, Seshachalam hills in India.

Pterocarpus santalinus is endemic to Eastern ghats of Andhra Pradesh and in the adjoining regions of Karnataka and Tamil Nadu. The Heartwood is also used for treating skin diseases, bone fracture, leprosy, ulcers and mental aberration. Wood paste applied on skin eruptions, inflammations and forehead to relieve headache, wood powder is used to control hemorrhage, bleeding piles and inflammation (Ravi Kumar et al. 2000).

Pterocarpus santalinus is one of the plants used for treatment of diabetes. Molecular markers provide the best estimates of genetic diversity that are independent of effect of various environmental factors. RAPD techniques were successfully used in determining genetic diversity in various plants from western ghats such as *Dendrocalamus strictus* and *Bambusa bambos* (Biradar et al., 2005), *Gnelia arborea* (Shankar et al., 2009), *Sweet sorghum* (Kacahapur et al., 2009) and *Allium stracheyi* (Ranjan et al., 2010). *Pterocarpus santalinus* being a tree species is a difficult plant for DNA isolation due to its high polyphenolic content that may interfere with the quantity and quality of DNA. We report here the modified CTAB protocol derived from the method that was originally developed for other plants (Doyle and Doyle, 1987). Modification was made to yield sufficient and pure DNA (Staden et al., 2008). The protocol optimizes the various conditions for RAPD such as annealing temperature, DNA concentration and the amount of Tag Polymerase (Padmalatha and Prasad 2006).

2. Material and Methods

2.1 Isolation of Genomic DNA from Plant Tissue

The isolation of Genomic DNA was extracted from 8 months-old young leaves of *Perocarpus santalinus* and *Pimpinella tirupatiensis* maintained at the BIOTRIM, Forest Research Institute, Tirupati. Initially, the available protocol was used several times but poor quality and quantity of DNA was extracted. Hence, modifications were made in the protocol viz., the use of higher volume of extraction buffer, increased incubation temperature, use of polyvinyl pyrrolidone repetition of purification step with chloroform: isoamyl alcohol and washing of DNA pellet with wash buffer and ethanol (95%). The detailed modified protocol is given below.

2.2 Solutions and Reagents

2 X CTAB Buffer (EB): 2% CTAB (w/v), 100 mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 1% (w/v) PVP (Polyvinylpyrrolidone), Autoclaved CHCl₃ – IAA: Chloroform: isoamylalcohol (24:1), 10% CTAB solution: 10% CTAB Buffer (CPB): 1% CTAB, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0) Ethanol: absolute or 95% ethanol, Salt solution: 3 M sodium acetate.

Procedure:

All the steps were carried out at room temperature. Rinse harvested leaves with autoclaved water and blot dry. Grind 2 g leaves to a fine powder. Extract in 3 volumes of EB and incubate at 65°C for 30 min. Centrifuge at 10,000 rpm for 15 min and collect supernatant. Extract with equal volumes of CHCl₃: IAA (24:1), collect upper aqueous Phase after centrifugation at 8,000 rpm for 10 min. Add one-tenth volume of warm (55°C) 10% CTAB solution and re-extract with CHCl₃: IAA as above. Add at least 1.5 volume of CPB buffer, mix gently and collect the precipitate by centrifugation at 10,000 rpm for 10 min. Precipitate the DNA by adding 2 volumes of ethanol and one-tenth volume of 3M sodium acetate. Incubate the sample at -20°C for 15 min. Centrifuge the sample at 10,000 rpm for 15 min. Collected the pellet, air dried and added 30-50ul of nuclease free water to dissolve the pellet.

2.3 Purification of Isolated DNA

3M Sodium Acetate (pH-5.2), Absolute Alcohol, Nuclease free water

Procedure:

Add 1µl of RNase to the DNA mixture and incubate the mixture at 37°C for one hour. After incubation, add 1/10th volume of 3M Sodium acetate (pH-5.2) and 2.5 volumes of absolute alcohol (90%). Keep it on ice for 10 min. Spin at 12,000 rpm for 15- 20 minutes. Collect the pellet and wash it with 150µl of 70% ethyl alcohol. Keep at room temp for 15-20 minutes. Then the pellet was air dried and dissolves in appropriate volume of Nuclease free water.

AGAROSE GEL ELECTROPHORESIS

2.4 Solutions and Reagents

50X TAE BUFFER, 2M-Tris, 1M-Acetate, 50mM- EDTA, Dilute this to 1X TAE with distilled water. Agarose, Ethidium Bromide (10mg/ml), Bromophenol Blue: 0.025%Bromophenol Blue, 10% Glycerol

Procedure

Make a 1% agarose solution by dissolving 0.2gms agarose in 20 ml of 1X TAE. Bring the solution to the boil to dissolve the agarose. Let the solution cool down to room temperature and add 2 µl ethidium bromide stocks for 20 ml gel solution. Stir the solution to disperse the ethidium bromide, and then pour it into the gel rack. Insert the comb at one side of the gel, about 5-10 mm from the end of the gel. When the gel has cooled down and become solid, carefully remove the comb. The holes that remain in the gel are the wells or slots. Put the gel, together with the rack, into a tank with 1XTAE buffer. Add 5µl of Bromophenol blue to the 10µl of Sample and load it into the wells. Allow them to electrophoresis for a while at 100volts. Observe the gel under gel documentation system for the presence of DNA.

2.5 Qualitative and Quantitative Analysis of DNA

Procedure

Take 2 ml Distilled water in a cuvette and calibrate the spectrophotometer at 260 nm as well as 280 nm. Add 10 µl of each DNA sample to 1990µl Distilled water and mix well. Use Distilled water as a blank in the other cuvette of the spectrophotometer. Note the OD₂₆₀ and OD₂₈₀ values on spectrophotometer.

Calculate the OD₂₆₀/OD₂₈₀ ratio. The concentration is calculated using the following formula

Concentration = OD₂₆₀ X 50ug/ml X Dilution factor

A ratio between 1.8-2.0 denotes that the absorption in the UV range is due to nucleic acids. A ratio lower than 1.8 indicates the presence of proteins and/or other UV absorbers. A ratio higher than 2.0 indicates that the samples may be contaminated with chloroform or phenol. In either case (<1.8 or >2.0) it is advisable to re-precipitate the DNA.

3. Results and Discussion

The endemic medicinal plants DNA preparations is the presence of polysaccharides and certain secondary metabolites, which have been observed to interfere with DNA isolation procedure and inhibit the activity of DNA modifying enzymes (Angeles et al. 2005; Padmalatha et al. 2006; Mishra et al. 2008; Yangting Hu et al. 2009). DNA extraction was improved by modifying some steps in the original Doyle's (Doyle and Doyle, 1987) CTAB method to remove these secondary metabolites. Samples were pretreated at 100°C to remove water-soluble contaminations. Buffer I was used to get purity and qualitative, qualitative DNA by removing cell wall debris, proteins, liquid and many secondary compounds at low speed centrifugation. DNA was further purified by removing polysaccharides with CTAB/NaCl buffer in which DNA was soluble but polysaccharides were insoluble (Murry and Thompson, 1980). The RNase treatment, the extracted DNA

could be used for restriction and ISSR-PLR analysis, so it is unnecessary to purify the DNA with RNase (Murry and Thompson, 1980). The A_{260}/A_{280} absorbance ratio of extracted DNA was found to be free from polysaccharide, poly-phenols and tannins contaminants ranged from 2.2 to 2.8 within the high level of purity

Table 1. The purity of DNA extract with modified CTAB method

Sample	Name of the plant	A_{260}	A_{280}	Concentration ($\mu\text{g/ml}$)	Purity (A_{260}/A_{280})
Sample 1	<i>Pterocarpus santalinus</i>	0.032	0.019	320	1.68
Sample2	<i>Pimpinella tirupatiensis</i>	0.021	0.014	210	1.5

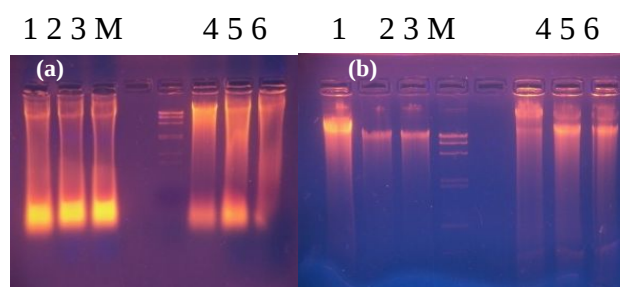


Figure a. Extraction of DNA from plant sample with 100bp DNA marker on 1% Agarose gel

Lane: 1, 2, 3:- *Pimpinella tirupatiensis*
Marker

Lane: M: 100bp DNA

Lane: 4, 5, 6:- *Pterocarpus santalinus*

Figure b. Purified DNA with 100bp DNA marker on 1% Agarose gel

Lane: 1, 2, 3:- *Pimpinella tirupatiensis*
Marker

Lane: M: 100bp DNA

Lane: 4, 5, 6:- *Pterocarpus santalinus*

4. Conclusion

The modified CTAB method was proved to be an efficient method of DNA extraction from *Pterocarpus santalinus* and *Pimpinella tirupatiensis* samples and which is reliable compared to the standard extraction methods from other tissues like leaves.

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