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EFFICIENT GENOMIC DNA EXTRACTION AND MOLECULAR ANALYSIS OF MEDICINALLY RICH *VALERIANA JATAMANSI* DRY ROOTS

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ABSTRACT: One of the major problems faced by the pharmaceutical industry is to maintain the quality and efficacy of herbal drugs due to the lack of purity of herbal raw materials. Furthermore, using traditional identification methods to identify the correct plant part needed for the preparation of herbal medicine can be difficult. Creating a novel system to assess the quality of a medicinal herb and to discriminate adulterants from authentic raw material is essential. There is a lot of confusion regarding nomenclature and originality of Valerian available in the market. The objective of the present work is to develop a molecular tool for accurate identification of dry root samples. The isolation of DNA is the first step in developing this technology. The methods employed for extracting DNA from fresh and dried root samples of medicinal plants, however, are time-consuming and yield DNA in lesser quantity and low purity due to the presence of high levels of polysaccharides, phenolics and other secondary metabolites in these samples. In this study, an easy and rapid protocol to extract genomic DNA from the fresh and dry roots of Indian Valerian has been discussed. The method involves a modified CTAB procedure of Doyle and Doyle (1990). The DNA obtained through this method is highly pure and proves to be good for polymerase chain reaction (PCR) with random primers.

INTRODUCTION: Valerian is a plant that is steeped in history. Its original use as a perfume or perhaps even as a source of food was different from its modern use in herbal medicine today, as a sedative and calming agent. Roots are used in the treatment of epilepsy, hysteria, convulsive ailments, and palpitations of the heart, diseases of the eye, itch, boils and diseases of liver, kidney, spleen, and head.

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It is one of the ingredients in the preparation of snake bite cures [1]. *V. jatamansi* is very important for its insect repelling and antihelminthic properties [2]. It consists of different chemical components, but the main bioactive constituents in the plants are coumarins and sesquiterpenes [3].

Valeriana jatamansi, also known as Indian Valerian, is an erect pubescent herb, having horizontal, thick rootstock/rhizomes, with thick descending fibrous roots. The species is found growing on moist slopes in the Himalayas. It is also found in Pakistan to Southwest China, Burma, and South-East Asia. There is a lot of confusion regarding nomenclature and originality of Valerian. Among 250 species of Valerian, very few have commercial importance.

But because of high commerce, traders are subjecting it to adulteration/substitution [4]. With the increasing use of DNA fingerprinting in plants, the preparation of good quality and quantity DNA has become a major concern. The extraction from tissue needs to be simple, rapid, efficient, and inexpensive when many samples are used, such as in population studies, molecular breeding and screening of raw herbal drug materials.

Several methods for extracting plant DNA from different plant parts, including roots, have been developed in the past [5]. The methods employed for extracting DNA from fresh and dried root samples of medicinal plants, however, are time-consuming and yield DNA in lesser quantity and low purity due to the presence of high levels of polysaccharides, phenolics and other secondary metabolites in these samples.

In this communication, we have described an easy and rapid protocol to extract genomic DNA from the dry roots of a medicinal plant. The method involves a modified CTAB procedure. The DNA obtained through this method is highly pure and proved to be good in restriction digestion with endonucleases and also for polymerase chain reaction conducted with random primers. The time taken for DNA extraction using this protocol is also less as compared to the existing protocols.

Very little information exists on the molecular aspects of commercial roots of Valerian, which requires high-quality DNA [6]. Therefore, the present study is planned with the following objectives:

1. Protocol standardization for isolation of DNA from commercially available dry root samples;
2. Validation of commercial dry root samples of Indian *Valerian* with the help of molecular analysis using PCR-RAPD based technique.

MATERIALS AND METHODS: Commercially available dry root samples are collected from various local Ayurvedic shops and markets in Mumbai, Manali, and Nepal. Authenticated Valerian plant is collected from the botanical garden of Department of Botany, University of Jammu and Kashmir, Srinagar Campus, India. Arbitrary decamer primers used during RAPD analysis are synthesized by Eurofins and are based on sequences from Operon Technologies, USA.

DNA Extraction: The optimized protocol is as described below:

1. Extraction buffer is prepared containing 3% CTAB (w/v), 2M NaCl, 25mM EDTA (pH 8), 100 mM Tris HCl (pH 8), 2% PVP and 1% β-mercaptoethanol. Extraction buffer is adjusted to pH 8 and is preheated to 70 ºC;
2. 1 gm dry root sample is mixed with extraction buffer and finely ground in the pre-chilled mortar and pestle. Incubation is carried out for 1 h in a water bath at 70 ºC with intermittent shaking and swirling;
3. After cooling, equilibrated Phenol: Chloroform: Isoamylalcohol mixture (25:24:1) is added and mixed by inversion; centrifuged at 10,000 rpm for 15 mins; Supernatant is precipitated after gently mixing with equal volumes of cold Isopropanol and kept overnight at -20 ºC for maximum DNA precipitation;
4. Next day, the tubes are centrifuged at 10,000 rpm at 4 ºC for 15 min, the pellet is washed with 80% Ethanol; RNAse treatment is given to dried pellet;
5. Step 3 and 4 are repeated to achieve high purity DNA. Lastly, the dried DNA pellet is dissolved in TE buffer.

Analysis of Valerian species through RAPD: In RAPD analysis, both the samples – authenticated plant root and dry root sample from the market - are analyzed and compared simultaneously. For optimization of RAPD reaction, ten arbitrary decamer primers (Universal primers) are used for amplification to standardize the PCR condition. PCR amplifications are performed in 15 µl volumes, using a VERITI 96 well thermal cycler (Applied Biosystems, USA). The analysis conditions are given in the following tables.

TABLE 1: ANALYSIS OF VALERIAN SPECIES THROUGH PCR

Contents	Amount (µl)
Water	6.9
Assay buffer	1.5
dNTPs	1.5
Primers	1.0
Taq polymerase	0.1
Template DNA (sample)	4.0

TABLE 2: THERMOCYCLER PROGRAMME

Steps	Temp (ºC)	Duration
Lid Temp	105	
T1	94	4 min
T1	94	1 min
T2	38	1 min
T3	72	2 min

TABLE 3: PRIMERS USED FOR AMPLIFICATION

S. no.	Primer fragments	Sequence(5'-3')
1	OPP03	CTGATACGCC
2	OPAP8	ACCCCCACAC
3	OPW15	ACACCGGAAC
4	OPW16	CAGCCTACCA
5	OPW17	GTCCTGGGTT
6	OPA04	AATCGGGCTG
7	OPA15	TTCCGAACCC
8	OPK16	GAGCGTCGAA
9	OPP01	GTAGCACTCC
10	OPT18	GATGCCAGAC

Agarose Gel Electrophoresis and Data Analysis: PCR products are electrophoresed on 3% Agarose gels, in 1X TBE buffers at 180V for 1 h and then are stained with Ethidium bromide (0.5µl/ml). Gels with amplification fragments are visualized and photographed under UV light. Bands with similar mobility are treated as identical fragments. The positions of PCR bands are compared with molecular weight standards.

RESULTS AND DISCUSSION: The genus Valeriana comprises of a large number of species. Among the 12 species reported to occur in India, *Valeriana jatamansi* is called as Tagar, Mushkbala or Samao in the local language. The roots or rhizomes with or without stolons are used in Unani and Ayurvedic system of medicine. This species is the native Himalayan region of India, Nepal and Southwest China [7].

For protocol standardization, various ranges of temperature and different concentrations of reagents are tested to get the optimum conditions giving the best results. Following table summarizes these parameters optimized for DNA extraction.

TABLE 4: OPTIMIZATION OF CTAB DNA EXTRACTION BUFFER COMPONENTS FOR *V. JATAMANSI* ROOTS

S. no.	Parameters	Tested Range	Optimum conditions	Inferences
1	CTAB (wt)	1%, 2%, 3%, 4%, 5%	3%	Disrupts cell membranes
2	PVP (wt)	1%, 2%, 3%, 4%, 5%	2%	Affects DNA quality
3	Water Bath temperature	50ºC, 60ºC, 65ºC, 70ºC, 75ºC, 80ºC	70 ºC	Lesser temperature increases chances of contamination
4	Duration on a water bath	15 min, 30 min, 1h	1 h	Less duration affects DNA purity
5	Duration for precipitation with isopropanol at -20 ºC	30 min, 1 h, 4 h, overnight	overnight	Extended duration increases precipitation and yield

(CTAB: Cetyl Trimethyl Ammonium Bromide; PVP: Polyvinyl pyrrolidine; wt: weight)

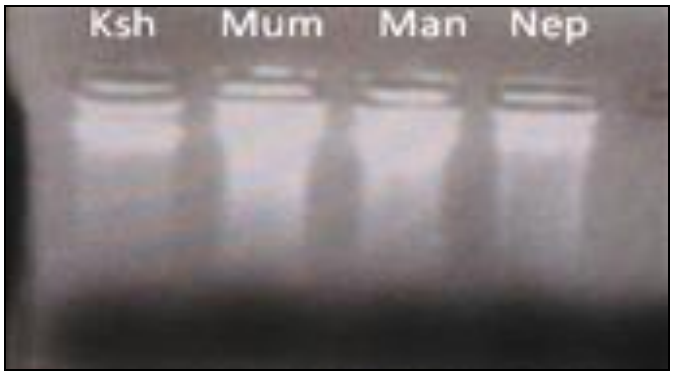

FIG. 1: GENOMIC DNA ISOLATED FROM *VALERIANA JATAMANSI* DRY ROOTS. Legends: Ksh: Kashmir; Mum: Mumbai; Man: Manali; Nep: Nepal

Extraction, Visualization, and Purity of Genomic DNA: After calculating the ratio of 260/280 nm absorbance, the extracted DNA shows reading between 1.7-1.9. The DNA yield obtained ranges from 300-500 ng/µl. The DNA bands obtained do not show any smearing or RNA contamination. DNA isolated by this method yields strong and reliable amplification products showing it's compatibility for RAPD-PCR using universal primers.

As the commercially available dry root samples are rich in polyphenols, it needs a lot of troubleshooting by changing various parameters for extraction and purification of DNA. Use of PVP along with CTAB helps in removal of impurities like polyphenols and long term Phenol: Chloroform: Isoamylalcohol treatment helps in removal of chlorophyll and other coloring substances such as pigments or dyes. Treatment with RNAse helps in degradation of RNA and yields RNA free pure DNA sample. Overnight DNA precipitation with Isopropanol at -20 ºC helps

to increase the yield of DNA [8]. The modifications in procedure make the extraction more effective and give a good yield of DNA from dry roots. The DNA obtained from dry root samples is compared and matched with DNA from standard, authenticated fresh plant material.

TABLE 5: SOLUTIONS FOR SOME OF THE PROBLEMS RELATED TO DNA ISOLATION DURING PRESENT STUDY

Problem	Cause	Solution
DNA pellet colored	Contamination with secondary metabolites or polyphenolic compounds	Use of PVP and β-mercaptoethanol in extraction buffer. Washing with Phenol: Chloroform: isoamylalcohol (25: 24: 1)
Low yield	Dry tissue or cell lysis not done properly	Take more amount of tissue and homogenize properly. Increase in temperature and period of incubation of tissue in extraction buffer. Keep in -20 mini cooler for a longer time for DNA precipitation
Smear in gel	DNA fragmentation	Do not mix vigorously and do not vortex. Mix gently

PCR amplification of DNA extracted from root samples are carried out with selected RAPD primers. The results are shown in the following figures.

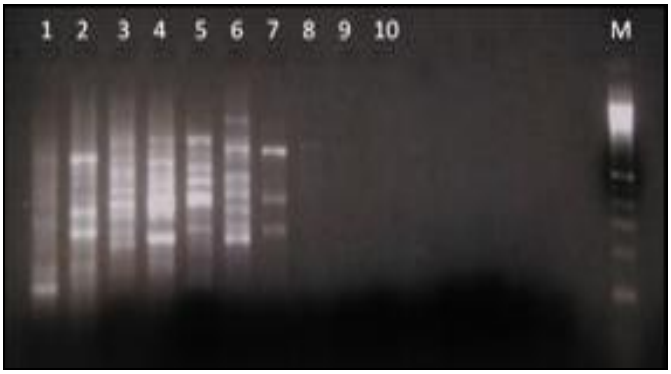

FIG. 2: AMPLIFICATION PATTERN OF *V. JATAMANSI* KASHMIR ROOT DNA WITH ALL TEN RAPD PRIMERS

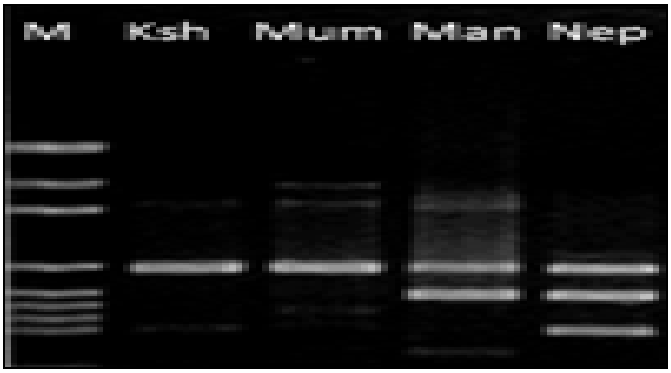

FIG. 3: AMPLIFICATION BY PRIMER OPW15

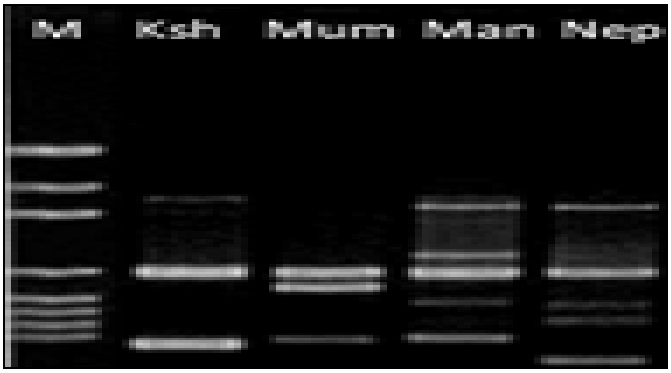

FIG. 4: AMPLIFICATION BY PRIMER OPW16

FIG. 5: AMPLIFICATION BY PRIMER OPW17

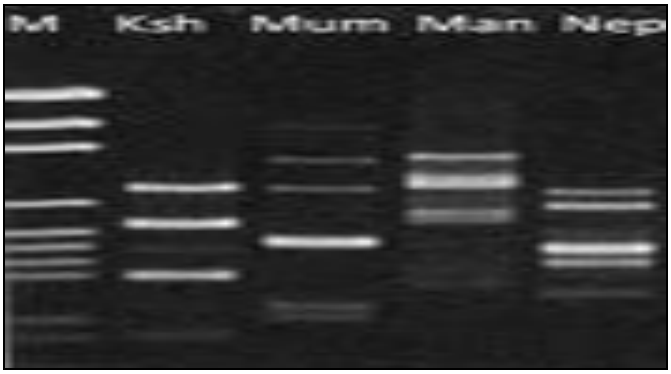

FIG. 6: AMPLIFICATION BY PRIMER OPA4

CONCLUSION: The paper presents the protocol to efficiently extract DNA from dry root material. This method will help determine the purity and quality of herbal drug sources. The dry root samples contain a high level of secondary metabolites, which make DNA extraction more

difficult. However, optimized protocol isolates genomic DNA of good quality that holds promise for further high-throughput molecular techniques, including PCR-RAPD. Comparison of market samples with standard Valerian plant using RAPD primers suggests the validity of collected market samples. Further analysis can be carried out for more samples from different regions.

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CONFLICT OF INTEREST: Nil

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