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Fast and Easy Protocol of DNA Extraction from Fresh and Hard/Dried Plant Samples of Indian Spices

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Authors' contributions

This work was carried out in collaboration between all authors. Author SN designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors RG, PKKS and JTV managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Spices identified using basic techniques like magnifying glasses or microscopic instruments. The DNA based spice detection techniques used in food testing labs for the disclosure of adulterants. The molecular techniques like PCR, Sequencing, and HRM etc. enables the exact disclosure of spices. Plants synthesize more amounts of polysaccharides, flavonoids, polyphenols and other secondary metabolites which interfere with the pure and high-quality genomic DNA extraction. Although plant DNA isolation protocols, extracting DNA from dried plant samples is a challenging task. This present research describes an expeditious, reliable SDS buffer protocol suited for extracting DNA from both fresh and dried plants samples rich in polysaccharides and secondary

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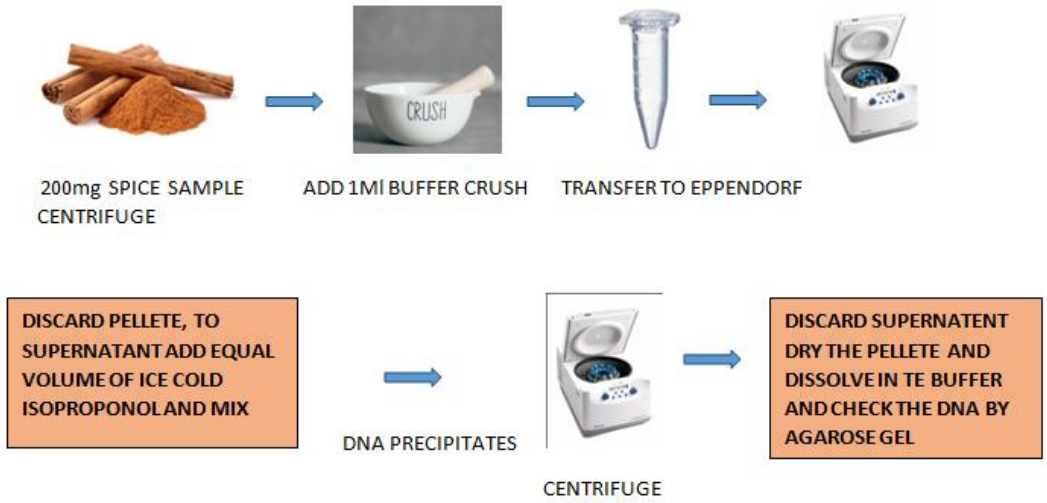
metabolites, and the protocol does not need toxic chemicals like phenols and use of liquid nitrogen. Extracted DNA showed the excellent purity evidenced by UV spectrometer at the ratio of A260/A280 ranging from approximately 1.8-1.85 and the A260/A230 ratio was approximately 2, which explains the preparations are sufficiently free from proteins and polysaccharides. The developed method is satisfactory for both dry and fresh spice samples. The success of the present study is getting high-quality genomic DNA and helps to carry out DNA based experiments with a shorter duration of time.



Keywords: SDS; DNA isolation; corrosive chemicals; dry and fresh plant samples.

ABBREVIATIONS

SDS : Sodium dodecyl sulphate;
DNA : Deoxyribonucleic acid;
CTAB : Cetyltrimethylammonium bromide;

1. INTRODUCTION

Spices are used medicine and food flavorings. Although about 109 species listed as spices in the International Standards Organization (ISO) list, their uses are specific as each of these commodities has different uses in flavoring foods or in medicine. Spices are the most integral part of Indian cooking. But, the rate of adulteration of spices in the country has risen, and it gives rise serious health complications. It is important to find it has adulterated whether the spices which purchased. Adulteration in spices are defined as mixing or substituting the original crude spice with other spurious, inferior, defective, spoiled, useless other parts of the same or different plant or samples which do not conclude the official standards [1]. Food adulterants are slowly becoming epidemic and affecting the human life in a very large scale. Adulteration was dangerous as it degenerates the quality of food and makes it inadequate for human consumption. Adulterated spices can have many adverse effects on our health like nausea, vomiting, and blurred vision [2]. At present, purity of spices are identified morphologically using simple methods like magnifying glasses or microscopic instruments. The development of molecular techniques like PCR, Sequencing, and HRM etc. enables the correct detection of spices. There are many methods like CTAB, Commercial Kits for the isolation of DNA from spices. But these methods are highly time-consuming or more expansive [1,2].

The separation of DNA from proteins membranes and other cellular materials contained by cells is called DNA extraction. This extraction process is one of the most important parts of DNA analysis. Some of the extraction methods may require incubation for overnight some can be done in a day, some can be done in two days and some protocols can be done in minutes also [3]. The DNA extraction procedures require the careful handling of biological samples to prevent the contamination of other biological DNA and cross over. DNA extraction tubes should be carefully labeled, especially when transfers are required [4]. DNA is highly negatively charged because it contains more phosphate groups and it is

stabilized by magnesium in the cell when unwound. DNA is organized into chromosomes by coiling around positively charged proteins called histones these coiled forms are known as nucleosomes. Magnesium very important to the function of proteases, enzyme proteins that cut up DNA [5].The lipid structures present in the cell membrane are responsible for the presence of proteases, magnesium, coiling of DNA around histones [6].

Many of the DNA extraction procedures have common mechanisms that are as follows [7].

Lysis of cell, Separation of cells from other cellular components, Purification of DNA, Isolate the DNA The disruption of the cell membrane is carried out by many procedures that include using heat to increase fluidity, disulfide bonds are reduced by using dithiothreitol (DTT) or a detergent, such as sodium dodecyl sulfate (SDS, CTAB) etc.[7]. Extraction of pure and high-quality DNA is very important in molecular biology studies, plant DNA is mostly contaminated by excessive secondary metabolites. The DNA isolation protocols require to be modified to each species and sometimes for each plant tissue because of the presence of high level of plant metabolites [8], the search for a more efficient DNA extraction method for both higher quality and yield has led to the development of several protocols [9]. Many factors which affect the isolation of good quality DNA that includes the degradation of DNA due to endonucleases which interfere with the enzymatic reactions [10]. Polysaccharides are the most problematic when it's present in DNA samples. Their presence may also inhibit the activity of many enzymes like *Taq* polymerase and restriction enzymes [11]. The presence of polysaccharides in the DNA sample results in the formation of a highly viscous solution. Polyphenols bind covalently to the DNA and change color to brown and reduce the maintenance time, makes the DNA useless for molecular studies [12]. Many of the researchers have carried out experiments to eliminate the use of corrosive chemicals like phenol, high economic kits and labor-intensive steps for high throughput DNA extraction [13]. The extraction protocols recommend for fresh spice samples seems impractical when the samples are collected from remote and rare locations.

The objective of this study was to develop a simple, fast, low cost and easy protocol to isolate DNA in an open laboratory environment avoiding corrosive chemicals like liquid nitrogen and toxic phenol. The resulting optimized buffer protocol that enables us to isolate high-quality genomic DNA amenable to PCR and other DNA experiments like restriction digestion, and amplification of plant barcode genes like *matK* and *rbcl* etc. with reduced cost and health concerns.

2. MATERIALS AND METHODS

2.1 Plant Materials and DNA Isolation

For this study, commonly used Indian spice samples were collected from different geographical regions of India and Genomic DNA were isolated from 200–250 mg dried and fresh plant material using Extraction buffer: 1.5% SDS +250mM Tris HCl+300 mM NaCl+25mM EDTA, the concentration of SDS was of 0.5 %, 1%,1.5%.

2.1.1 Protocol

200mg of plant sample was taken and ground the sample using mortar and pestle in 500uL of extraction buffer and added some buffer (approximately 200 uL, final volume is 700 uL) to get enough homogenate, homogenate was transferred to the 1500 uL microfuge (centrifuge tube) tube and incubate the tubes for 15 minutes at 65°C. centrifuged samples for 10min at 13000 rpm, discard the pellet, supernatant was transferred to another microfuge tube (centrifuge tube) and equal volume of ice-cold isopropanol was added and inverted tubes slowly. DNA starts precipitating, after precipitation again centrifuge the tubes for 10 min at 13000 rpm discard the supernatant. Wash the pellet four times with 70% ethanol by centrifuging at 5000 rpm for 5 min, dry the pellet and dissolve in 100 uL of TE buffer.

2.2 DNA Quality and Quantity Measurement

The DNA yield was measured by using a UV-Visible spectrophotometer (Nanodrop) at 260 nm. DNA purity was determined by calculating the absorbance ratio A260/280. Contamination of polysaccharides was assessed by calculating absorbance at A260/A230. Quality of DNA was checked by using 1% agarose gel stained with ethidium bromide and bands were observed in the gel documentation system [14,15] 2 uL of isolated DNA was loaded into the gel.

3. RESULTS

DNA was isolated from both fresh and dried samples collected (56 samples), with varying concentrations of SDS viz., 0.5%, 1%,1.5%,2%.

0.5% of SDS buffer working fine with fresh samples giving good quality of DNA but it was failed to work for dried samples and commercially available powders (Few samples showed in Fig. 1).

1% of SDS buffer worked fine with fresh samples and few of dried samples gave good quality of DNA but it is failed to work for very hard dried samples and commercially available powders (Few samples showed in Fig. 2).

1.5% of SDS buffer worked fine with fresh samples and all dried samples and also commercially available powders gave good quality of DNA (Few samples showed in Fig. 3).

The results of DNA extraction from various samples of spices are shown in Table 1

The results of DNA UV-Visible spectrophoto meter quantification of samples (Nanodrop) shows the extract free from protein and polyphenolic compounds, shown in Table 1 (1.5% SDS buffer extracted samples quantification).

4. DISCUSSION

The plant DNA extraction protocols already available in present research field are very lengthy or high economic or it does not suitable for isolation of DNA from dried samples probably due to the presence of secondary metabolites. Although the CTAB method and commercially available kits give high-quality DNA they are all either very lengthy or expensive and don't give positive results to very hard/dried samples [15, 17,18,19].

CTAB method worked fine with fresh samples and some of dried leave and fruits but it didn't work for very hard samples like plant dried barks, roots etc [18]. Commercially available Kits works for almost all samples but those are very expansive [19].

In CTAB method hazardous chemicals like liquid nitrogen, phenol-chloroform etc. is used. CTAB method takes a minimum of 6 hours to complete DNA extraction [20] but our method takes 25-30 minutes. In comparison to the CTAB method, our protocol is safe enough to be performed laboratory bench top with limited resources.

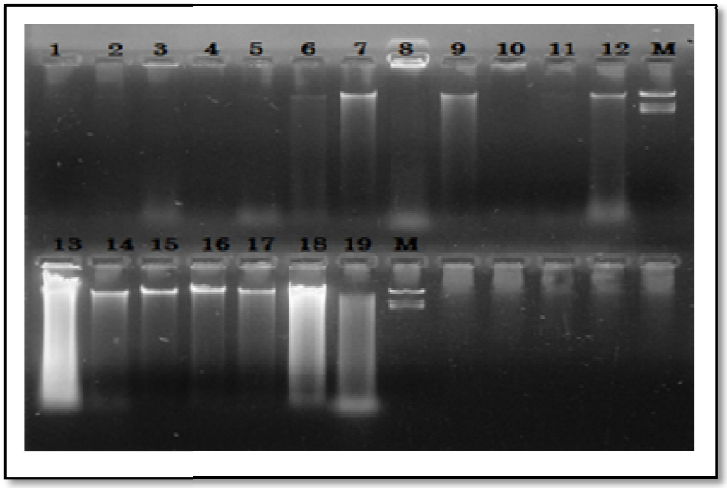


Fig. 1. Shows the genomic DNA loaded in 1% agarose gel

Genomic DNA of plant samples: Lane M-λ ladder, Lane 1- Ginger (Kochi), Lane 2- Turmeric (Salem), Lane 3- Cinnamon bark (Kerala), Lane 4- Cinnamon bark(Kerala), Lane 5- Ginger (Bangalore), Lane 6- Ashwagandha (Yelahanka), Lane 7 – Mango Ginger (Yelahanka), Lane 8-Mustard seed (Gujarat) , Lane 9 – Ginger (Gujarat), Lane 10 Turmeric (Mizoram), Lane 11 Turmeric (Meghalaya), Lane 12- Amla fruit (Kolar), Lane 13 – Mint Leaf (Kolar), Lane 14- Mint leaf (Saudi), Lane 15-Amla leaf (Puttur), Lane 16- Amla fruit (Kuppam), Lane 17- Star Amla leaf (Kolar), Lane 18- Amla leaf (Mangalore), Lane 19- Menthe leaf (Kolar)

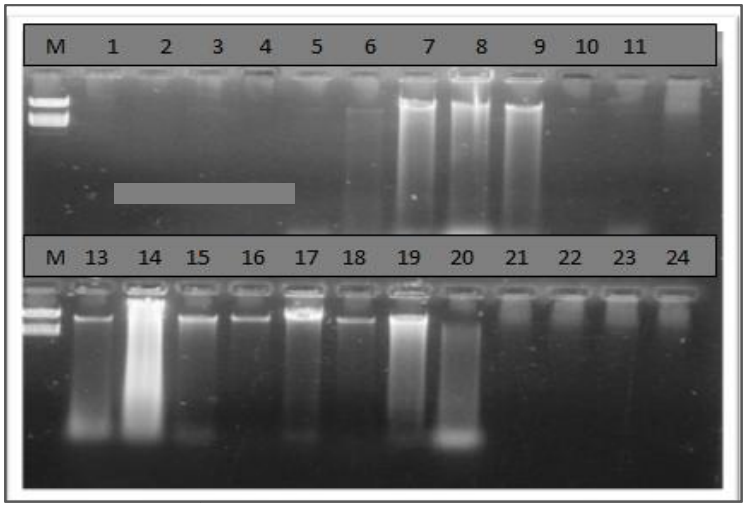


Fig. 2. Shows the genomic DNA loaded in 1% agarose gel

Genomic DNA of plant samples: Lane M-λ ladder, Lane 1- Ginger (kochi), Lane 2- Turmeric (Salem), Lane 3- Cinnamon bark (Kerala), Lane 4- Cinnamon bark(Kerala), Lane 5- Ginger (Bangalore), Lane 6- Ashwgandha (kolar), Lane 7 – Mango Ginger (Yelahanka), Lane 8-Mustard seed (Gujarat) , Lane 9 – Ginger (Gujarat), Lane 10 Turmeric (Mizoram), Lane 11 Turmeric (Meghalaya), Lane 12- Ashwaganda (Bangalore), Lane 13 – Black Pepper (Kerala), Lane 14- Black Pepper (kochi), Lane 15-Musturd (Davanagere), Lane 16- Papaya (Bangalore), Lane 17- Star Amla leaf (Kolar), Lane 18- Amla leaf (Mangalore), Lane 19- White musturd (Kolar)

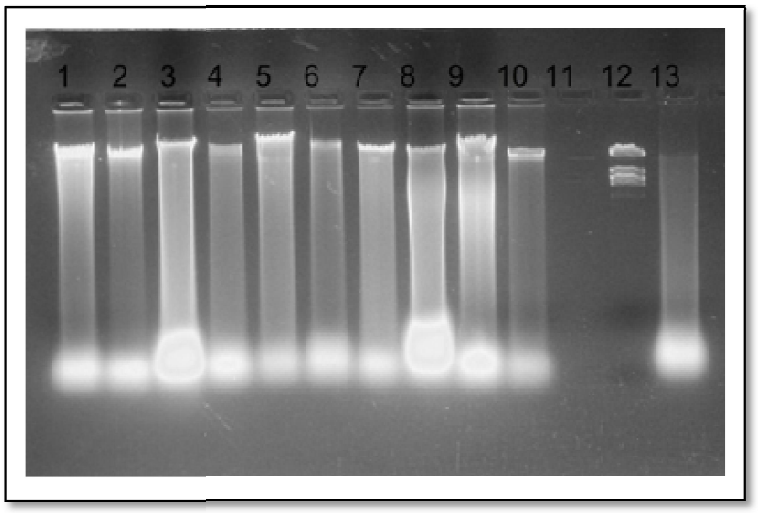


Fig. 3. Shows the genomic DNA loaded in 1% agarose gel

Genomic DNA of plant samples: Lane 1- Ginger (Kochi), Lane 2- Turmeric (Salem), Lane 3- Cinnamon bark (Kerala), Lane 4- Cinnamon bark (Kerala), Lane 5- Ginger (Bangalore), Lane 6- Ashwagandha (Kolar), Lane 7 – Elachi (cooge), Lane 8-Mustard seed (Gujarat), Lane 9 – Ginger (Gujarat), Lane 10 Turmeric (Mizoram), Lane 11 no sample loaded), Lane 12- λ ladder, Lane 13 – Black Pepper (Kerala),

The present method advantages over the CTAB method and other commercially available kits are. It requires less time with good quality DNA isolation, no use of hazardous chemicals, low cost method including chemicals cost and DNA extraction possible from very hard/dried plant samples Extracted DNA showed the excellent purity evidenced by UV spectrometer at the ratio of A260/A280 ranging from approximately 1.8-1.85 and A260/A230 ratio was approximately 2, which shows the preparations are sufficiently free from proteins and polysaccharides.

Table 1. Details of the sample analyzed and results of DNA extraction, absorbance, and quantification

Sl. no	Sample name	Sample type	Sample condition	Place of collection	DNA extraction at percent level of SDS buffer			DNA absorbance ratio (1.5% SDS buffer)		Nucleic acid (dsDNA), ng/Ul (1.5% SDS buffer)
					0.5%	1%	1.5%	260/ 280	260/ 230	
1.	Turmeric	Rhizome	Dried	Mizoram			✓	1.83	1.99	915.0
2.	Turmeric	Rhizome	Dried	Bangalore			✓	1.79	2.22	895.0
3.	Turmeric	Rhizome	Dried	Meghalaya			✓	1.75	1.93	875.8
4.	Ginger	Rhizome	Dried	Bangalore			✓	1.62	2.21	810.4
5.	Turmeric	Rhizome	Dried	Shivamogga			✓	1.97	2.30	985.3
6.	Turmeric	Rhizome	Dried	Puttur			✓	1.55	1.95	775.5
7.	Turmeric	Rhizome	Dried	Salem			✓	1.82	1.97	910.2
8.	Wild turmeric	Rhizome	Dried	Chintamani			✓	1.78	1.82	890.4
9.	Ginger	Rhizome	Dried	Kochi			✓	1.68	1.95	840.0
10.	Ashwagandha	Root	Dried	Gujarat			✓	1.81	1.89	905.4
11.	Ashwagandha	Root	Dried	Bangalore			✓	1.77	1.94	885.0
12.	Ashwagandha	Root	Dried	Kolar			✓	1.75	1.91	878.8
13.	Black Pepper	Seed	Dried	Kerala			✓	1.90	1.95	950.8
14.	Black Pepper	Seed	Dried	Kerala			✓	1.85	1.9	925.5
15.	Black Pepper	Seed	Dried	HB Halli			✓	1.94	2.08	970.3
16.	Elachi	Seed	Dried	Kochi			✓	1.84	2.10	937.3
17.	Papaya	Seed	Dried	Kolar		✓	✓	1.80	2.07	864.1
18.	Fenugreek	Seed	Dried	Kolar			✓	1.77	2.04	885.0
19.	Papaya	Seed	Dried	Bangalore		✓	✓	2.09	1.79	1045.2
20.	Mustard	Seed	Dried	Davanagere		✓	✓	1.99	1.92	752.7
21.	Elachi	Seed	Dried	Coorg			✓	2.03	1.88	1076.7
22.	Black Pepper	Seed	Dried	Kochi		✓	✓	1.75	1.98	878.8
23.	White Pepper	Seed	Dried	Puttur		✓	✓	1.99	1.88	995.0
24.	Elachi Spice Tray	Seed	Dried	Kerala			✓	1.79	1.79	895.0
25.	Elachi	Seed	Dried	HB halli			✓	1.73	1.54	872.8
26.	Mustard	Seed	Dried	Gujarat		✓	✓	1.71	1.92	862.4
27.	White mustard	Seed	Dried	Kolar		✓	✓	1.90	2.21	950.8
28.	Papaya	Seed	Dried	Chintamani			✓	1.88	2.85	940.8
29.	Amla	Fruit	Dried	Puttur			✓	1.86	1.95	930.0
30.	Garcinia 1	Fruit	Dried	Coimbatore			✓	1.85	2.72	928.2
31.	Garcinia 2	Fruit	Dried	Coimbatore			✓	1.89	2.09	945.5
32.	Garcinia 3	Fruit	Dried	Coimbatore			✓	1.74	2.10	870.4
33.	Garcinia 4	Fruit	Dried	Coimbatore			✓	2.00	2.62	1000.0
34.	Amla	Fruit	Dried	Andra Pradesh			✓	1.56	2.04	780.8

35.	Cinnamon	Bark	Dried	kerala			✓	1.91	1.94	955.9
36.	Cinnamon spice tray	Bark	Dried	Kerala			✓	1.96	1.71	980.2
37.	Cinnamon ss spice	Bark	Dried	Kerala			✓	1.41	1.92	705.2
38.	Cinnamon	Bark	Dried	HB halli			✓	1.87	1.01	935.3
39.	Cinnamon	Bark	Dried	kochi			✓	1.98	0.52	990.0
40.	Saffron		Dried	Bangalore			✓	1.73	2.01	865.23
41.	Mango Ginger	Rhizome	fresh	Yelahanka	✓	✓	✓	2.50	2.77	1255.3
42.	Ginger	Rhizome	fresh	Gujarat	✓	✓	✓	1.83	1.83	915.4
43.	Ginger	Rhizome	fresh	Hasan	✓	✓	✓	1.79	1.93	895.0
44.	Zingiber zerumbet	Rhizome	fresh	Yelahanka	✓	✓	✓	1.55	1.73	775.0
45.	Ashwagandha	Leaf	fresh	Yelahanka	✓	✓	✓	1.78	1.96	894.0
46.	Mint	Leaf	fresh	Saudi	✓	✓	✓	1.97	1.93	985.3
47.	Tulsi	Leaf	fresh	Yelahanka	✓	✓	✓	1.55	2.00	775.0
48.	Mint	Leaf	fresh	Kolar	✓	✓	✓	1.82	1.83	910.0
49.	Tulsi	Leaf	fresh	Kolar	✓	✓	✓	1.79	1.43	895.0
50.	Ocimum G	Leaf	fresh	Yelahanka	✓	✓	✓	1.68	1.98	840.5
51.	Ocimum T	Leaf	fresh	Yelahanka	✓	✓	✓	1.75	1.59	875.6
52.	Amomum Subulatum	Leaf		Yelahanka	✓	✓	✓	1.86	2.24	930.0
53.	Amla Star	Fruit	fresh	Kolar	✓	✓	✓	1.75	191	878.8
54.	Amla	Fruit	fresh	Antaragange	✓	✓	✓	1.85	1.95	925.5
55.	Turmeric MTR	powder	powder	Bangalore			✓	1.75	1.90	875.6
56.	Turmeric Everest	powder	Powder	Bangalore			✓	1.80	1.94	905.0

5. CONCLUSION

Here we have described a simple, safe, reliable, and cost-efficient SDS DNA extraction method that provides high-quality DNA from both fresh and dried hard plant samples which contain elevated concentrations of polysaccharide and polyphenolic compounds this method does not require the use of expensive liquid nitrogen and environmentally hazardous phenol to obtain high-quality genomic DNA. The present method enables the extraction of DNA not only from fresh samples but even from hard/dried samples of the plant too. Therefore this method is recommended even in low-technology laboratories for high-throughput sample preparation in various molecular analytical techniques.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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