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DNA extraction from therapeutic herbs

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Research Article

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Abstract

The Department of Biochemistry and Molecular Biology at MGM College of Agricultural Biotechnology, Aurangabad, conducted a lab experiment in the summer of 2012–13 to investigate the separation of DNA from medicinal plants without the use of liquid nitrogen. DNA was isolated using five different medicinal herbs. DNA was successfully isolated for a variety of molecular biology applications using an extraction buffer based on Tris (hydroxymethyl) amino methane and varying PVP concentrations. The ratio of absorbance at 260 and 280 nm was used to assess the purity of DNA. The isolated DNA was found to have a ratio between 1.4 and 1.8. Therefore, for a variety of molecular biology applications, the current procedure may be utilized to isolate DNA from medicinal plants such as Neem (Azadirachta indica), Bel (Aegle marmelos), Brahmi (Bacopa monnieri), Tulsi (Ocimum sanctum), and Sandalwood (Santalum album). For restriction digestion and PCR analyses, the purest DNA was used. The isolated DNA's suitability for restriction digestion and PCR was verified.

Keywords: Tacrolimus, olopatadine, immunomodulators, and vernal keratoconjunctivitis

Introduction

Approximately 60% of people worldwide utilize traditional medicines in addition to other forms of treatment. Not only are they employed for basic healthcare in rural parts of underdeveloped nations, but they are also used in wealthy nations where advanced medications are the norm. Herbal medications are made only from medicinal plants, while traditional medicines are made from minerals, organic materials, and medicinal plants. An essential part of India's healthcare system is the hereditary practice of using plants as medication. Since the majority of practitioners in Indian medical systems create and administer their own recipes, appropriate documentation and study are necessary. About 40% of people in the western world report using herbal remedies to address medical conditions in the previous year, indicating a steady increase in the usage of herbal remedies. The rising prevalence of adverse medication responses and the financial load of the contemporary medical system have led to an exponential surge in public, academic, and government interest in traditional remedies. With concentrated hotspots in the Eastern Himalayas, Western Ghats, and Andaman and Nicobar Islands, India is home to over 45,000 different plant species. Although there are about 3000 officially recognized herbs with therapeutic potential, over 6000 are used by traditional healers. India is known as the "botanical garden of the world" and is the world's biggest producer of medicinal plants. Currently, the Ayurvedic system has around 250,000 registered medical practitioners (the total for all traditional systems is roughly 291,000), whereas the contemporary medical system has over 700,000. Seventy percent of people in rural India rely on Ayurveda, a traditional medical practice.The various Indian medical systems are said to use over 80,000 distinct plant species in one way or another. Clearly defined biological metrics such as rasa (taste), vipaka (metabolic characteristic), guna (quality), prabhava (biological impact), and virya (potency) have been used to study plants. Approximately 25,000 plant medication compositions have been developed in the codified traditions as a result of these investigations. Furthermore, it is estimated that the folk and tribal traditions include more than 50,000 formulae. All of them indicate the intense desire for a thorough understanding of the therapeutic herbs that have been present in this country from ancient times due to the negative medication responses and expense of the

contemporary medical system. Medicinal In terms of global health, plants have been crucial. Due to their use in popular medications, medicines, and nutrition, medicinal plant species have significant economic value. Nano grams of the pure DNA are needed for DNA-based methods in order to amplify and produce enough PCR products. Global interest has been

drawn to the use and preservation of medicines (Parrotta, 2001). Northeast India is the origin of neem (Azadirachta indica). It is sometimes referred to as the Persian lilac or Margosa. This plant is known as Mimba in Indonesia. This plant is known as the "village pharmacy" in India because to its capacity to treat a wide range of illnesses, from ulcers and malaria to bed bugs and rotten teeth. The discipline of dentistry is particularly interested in neem since it has a long history of treating gum and tooth issues. Neem may be used in mouthwashes to lessen periodontal disorders and can stop Streptococcus mutans from growing, according to many studies. Tulsi (Ocimum sanctum) is a tiny, multipurpose sub-shrub plant. The significance of its medical use is mentioned in Ayurveda. The leaves work wonders for oral infections and ulcers. Chewing a few leaves will heal certain ailments. The herb helps with dental issues. Its dried leaves

in the sun and powdered, can be used for brushing teeth. It can also be mixed with mustered oil to make a paste and used as toothpaste. This is very good for maintaining dental health counteracting bad breath and for massaging the gums. It is also useful in pyorrhea and other gum disorders. The anti- inflammatory and anti-infectious properties of Tulsi make it a powerful treatment for gum disease

Brahmi (*Bacopa monnieri*) is also known as “Medhya rasayanas” in *ayurveda* as it increases mental clarity and brain stimulating action (Bhattacharya and Ghosal 1998). The medicinal properties of *Bacopa monnieri* responsible for improving memory-related function have been attributed to the presence of different types of saponins such as Bacosides A, B, C, and D called the “memory chemicals” (Rastogi, 1994). *Bacopa monnieri* also contains variety of medically active substances i.e. stigma sterol, sapogenins and flavonoids. Other compounds are D-mannitol, betulic acid, beta-sisterol, octacosane, nicotine and amino acid. It also possesses anti-inflammatory, analgesic, antipyretic, epilepsy, insanity, anticancer and antioxidant activities (Satyavati, 1976). It also used for the treatment of asthma, water retention and blood clearing. In Pakistan, the herbal drugs, Brahmi-buti, is used to treat skin diseases, leprosy, epilepsy, eczema, asthama, hoarseness of the voice, and diseases of the nervous system (Shakoor, 1994). They had tested previously established DNA isolation protocols but these methods resulted in DNA with lot of impurities and not very suitable for RAPD analysis Therefore, we report here a total genomic DNA isolation protocol derived from a method originally developed for other plants. The isolated DNA would be suitable for further downstream applications. Medicinal plants contain high levels of polysaccharides, polyphenols, several pigments, and other secondary metabolites, which makes DNA unusable for downstream work in molecular biology research (Wen and Deng, 2002). Polyphenols as powerful oxidizing agents can decrease the yield and purity of extracted DNA. Polysaccharides make DNA viscous, glue-like and non-amplifiable in PCR by inhibiting Taq polymerase enzyme activity and also interfere with accurate DNA digestion (Porebski *et al*., 1997) [9]. A good isolation protocol should be simple, rapid and efficient, yielding appreciable amounts of high-quality DNA suitable for molecular analysis. Different methods for DNA extraction have effectively been applied for many plant species. DNA has been treated with DNase-free ribonuclease A, since large amounts of RNA in the sample can chelate Mg^{2+} and reduce yield in PCR. It also involves successive long-term RNAse treatment with all steps carried out at room temperature. RNase treatment degrades RNA into small ribonucleosides that do not contaminate the DNA preparation and yields RNA-free pure DNA (Jena *et al*., 2010). At present, there are several DNA isolation kits, but the main problem with these commercially available kits is their high cost per sample (Ahmed *et al*., 2009). Because most of the available procedures are based on the use of commercial kits, routine DNA extraction is economically difficult for large scale genomic applications. Therefore, to solve the problem of DNA isolation from medicinal plant the experiment entitled “Isolation of DNA from medicinal plant” has been planned during academic year Nov 2012 to April 2013 in MGM College of Agricultural Biotechnology, Aurangabad with following objectives.

1. Standardization of protocol for isolation of DNA.
2. To quantify the yield of isolated DNA.
3. To study possibilities of isolated DNA for PCR study.

2. Review of Literature

An attempts has been made in this chapter to review and classify the work done in past on this aspect of present investigation by eminent scientists in India and abroad.

- Anna *et al*. (2001) [1] carried out an experiment and successfully isolated DNA from leaves of milfoil (*Achillea millefolium* L.) and Siberian ginserg (*Eleutherococcus senticosus*) suitable for molecular biology application.
- Nagarajan (2005) [7] developed a modified CTAB based protocol for DNA isolation from leaf tissue of *Phyllanthus emblica* and obtained high quantity genomic DNA. They have obtained yield of DNA ranged from 1-2 µg/µl per gram of the leaf tissue and the purity (ratio) was between 1.6-1.7 indicating minimal levels of contaminating metabolites.
- Padmalatha *et al*. (2005) [8] optimized DNA isolation and PCR based protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India containing high levels of polysaccharides, polyphenols and secondary metabolites. The method involves a modified CTAB extraction employing polyvinyl pyrrolidone while grinding, an overnight RNAse treatment with all steps carried out at room temp. These techniques are ideal for isolation of DNA from different plant species and the DNA isolated was used for randomly amplified polymorphic DNA (RAPD)

analysis. They isolated DNA from eight different medicinal plants for RAPD analysis.

- Khan *et al*. (2007) [6] demonstrated the protocol for isolated genomic DNA from dry and fresh roots of medicinal plant suitable for RAPD and restriction digestion. The research work carried out at Centre for transgenic plant development, Department of Biotechnology, Jamia Hamdard, New Delhi.
- Pandey *et al*. (2008) [10] isolated and characterized microsatellite markers in Indian neem (*Azadirachta indica* var. *indica Azadirachta Juss*) and cross-amplification in Thai neem (*Azadirachta indica* var. *siamenis Valenton*). They are developed eight polymorphic microsatellite loci in Indian neem (*Azadirachta indica* var. *indica Azadirachta Juss*) and cross-amplified in closely related species Thai neem (*Azadirachta indica* var. *siamenis Valenton*).
- Sharma *et al*. (2010) [11] conducted research to study the isolated genomic DNA from medicinal plant without using liquid nitrogen and obtained DNA quantity and quality comparable those isolated with liquid nitrogen. DNA isolated by this method was used for various molecular biology applications.
- Adhikari *et al*. (2011) [3] has been observed to create interference with DNA isolation procedure and downstream reaction, such as DNA amplification, restrictions and cloning and to prepare a simplified high yielding miniprep genomic DNA extraction protocol for medicinal plant. They have developed the protocol was to make this techniques readily available in low facility laboratories and to minimize the duration of plant DNA isolation.
- Alatar *et al*. (2012) [2] discovered Simple and rapid protocol for the isolation of PCR-amplifiable DNA from medicinal plants. They are used SDS-based DNA isolation method to extract DNA from 11 species of different aromatic and medicinal plants collected from Saudi Arabia. They confirmed purity of DNA by agarose gel, restriction endonuclease digestion and microsatellite primed- polymerase chain reaction (MP-PCR). The extracted genomic DNA was found suitable for restriction digestion and PCR amplification. This experimental procedure provides an easy, suitable, non-toxic, cheap, and quick process for the amplification of DNA from medical plant tissue.
- Ghaffariyan *et al*. (2012) [4] improvement the protocol for DNA isolation from the medicinal plant of lemon balm (*Melissa officinalis*). They are used PVP to overcome the problem of polyphenols. DNA yields ranged from 10-20 µg (in 100-µL elution volumes) from all plant material evaluated. The DNA was obtained free of any contaminating proteins, polysaccharides and colored pigments.
- Sahare *et al*. (2012) [12] conducted the experiment for the Isolation of Genomic DNA from Medicinal Plants Producing Large Amount of Secondary Metabolites; the experiment was carried out at Department of Botany, Rashtrasant Tukadoji Maharaj University, Nagpur.

3. Materials and Method

The detail of materials was used and a method was adopted for conducting the present investigation is described in this chapter under appropriate heads.

3.1. Collection of Plant material

1. Five different medicinal plant [Brahmi (*Bacopa monnieri*), Bel (*Aegle marmelos*), Neem (*Azadirachta indica*), Tulsi (*Ocium sanctum*), and Sandalwood (*Santalum album*)] were selected for DNA isolation studies. The suitable plant part for DNA isolation is leaf tissue, because it is available in large quantity and can be easily detached from the plant. The DNA isolation from young leaves is easy comparative to other plant part.
2. Plant material was collected from waluj and MGM CABT Aurangabad. The sample was collected early in the morning before sunrise, the stress level in the leaves of plant increases with rising temperature and the increased stress inside leaves increases level of various secondary metabolites and these secondary metabolites are major hurdles in the DNA extraction protocol.
3. The leaf sample were kept in ice (4^{0C}) and then brought to the Biochemistry and Molecular Biology laboratories for further processing.
4. The leaves were kept at -20^{0C} and processed within 24 h.
5. (Ref. Pallavi Sahare, the International Journal of Engineering and Science (IJES).

3.2. Extraction of DNA from stored plant material

DNA extraction from medicinal plant is very laborious work. The biggest problem faced by researcher is of secondary metabolites, so the protocol has been standardized in a way that the isolated DNA is pure for restriction digestion and RAPD analysis.

1. The stored plant leaves of Leaves Neem (*Azadirachta indica*), Bel (*Aegle marmelos*), Brahmi (*Bacopa monnieri*), Tulsi (*Ocimum sanctum*) and Sandalwood (*Santalum album*) were used for the DNA extraction.

2. Exactly 100mg of plant leaves were grounded with mortar and pastel in the presence of 2 ml of extraction buffer (200m*M* Tris-HCL (PH-8.0), 200m*M* NaCl and 25m*M*
   EDTA, Proteinase K (0.1mg/ml) and PVP (1.5%, 2.0% and 2.5%).
1. The homogenate was transferred to 1.5 ml micro centrifuge tube and vortexed for 30 sec after adding 50 µl of 10% SDS solution.
2. The tube was kept in a water bath at 60°c for 65 minute with gentle shaking after 5 minutes.
3. The tubes were subjected to centrifugation at 17,000 rpm for 15 minutes at 4^0C.
4. The upper aqueous phase was taken into new tube and equal volume of phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v) was mixed and again centrifuged at 15,000 rpm for 10 minute 4^0C.
5. The supernatant was removed, and to which one fifth volume 2 M NaCl and two volumes per chilled ethanol was added and kept in -20◦c for 10 min to precipitate DNA.
6. Again an extra centrifugation was performed at 17,000 rpm for 10 minutes at 4^0C.
7. The DNA was pelleted and transferred to another tube. The DNA was washed with 70% ethanol (3 times) and air dried under laminar flow chamber. The DNA was dissolved in sterile double distilled water and stored at -20 ◦C.
8. RNAse treatment was given as follows

Added 1ul of a 10µg/ml stock solution of RNAse A to DNA. Incubated at 37◦C then added 1/10 volume of 3*M* Sodium acetate and 2 volumes of Isopropanol to the DNA containing solution then incubated on ice for 10 min, and centrifuged at maximum speed for 5 min at room temperature, to pallet the DNA. Then discarded the alcohol, pallet was washed with 70% ethanol and dried the DNA, and dissolved in dH_2O.
(Ref. Adhikari, S. and Ghosh, T. 2011) [3]

3.3. Quantification of yield and purity of DNA using A260/A280 by UV-VIS Spectrophotometer

1. The isolated DNA was quantified by its absorbance at 260_{nm} and $280_{nm,}$ the ideal value for $A_{260}/A_{280\ is}$ 1.8, if the ratio decreases it represented protein contamination and if it increases RNA contamination.
2. The spectrophotometer (UV-1800, schimatzu) has an inbuilt program for quantification of DNA yield and purity.
3. The milli-Q water was used for absorbance blank of both cuvets, then one of the cuvet is replaced by solution contaminant DNA and readings were recorded.

(Ref. Alatar, A. and Mahmoud, M. 2012.) [2]

3.4. Restriction digestion of purified DNA fragment

1. The reaction mixture was prepared by mixing 20µl of DNA sample ,10 µl of reaction mixture, 3µl of restriction enzymes (*Eco*RI) and final volume was maintain 50µl using sterile milli-Q water.
2. The mixture was incubated at 37^0C for 45 min and placed on ice to stop the reaction.

The digestion was confirmed by agarose gel electrophoresis. (Ref. Ghaffariyan, S. and Mohammadi, S. 2012.) [4]

3.5. RAPD PCR Analysis of DNA

1. The DNA was used to perform PCR with RAPD marker.
2. The 25µl of master mix supplied by Genei™ was mixed with 1µl of DNA sample, the PCR was carried out using applied Bio Student PCR machine. The result was analyzed using agarose gel electrophoresis.
3. Master mixture for one reaction was consist of following components

- 10X assay buffer
- dNTP mix
- Taq DNA polymerase
- Genomic DNA
- Primer
- $MgCl_2$

Final volume adjusted using double distilled water.

4. PCR amplification

The DNA was amplified using thermal cycler. It involves following steps-

- Initial Denaturation at 95^0c for 3 min.
- Denaturation at 94^0c for 1 min.
- Primer annealing at 52^0c for 1 min.
- Extension at 72^0c for 2 min.

- Went to step 2 for repeated cycles.
- Final extension at 72^0c for 3 min.
- Hold at 4^0

(Ref. Sharma, P. and Joshi, N. 2010.) [11]

3.6. Agarose Gel Electrophoresis

1. Gel electrophoresis is a widely used technique for the analysis of nucleic acids. Every molecular biology research laboratory routinely uses agarose gel electrophoresis for the preparation and analysis of DNA.
2. Agarose gel electrophoresis consist of following components-

Material

Agarose, TAE Buffer, 6X Sample Loading Buffer, DNA ladder standard, Electrophoresis chamber, Power supply, Gel casting tray and combs, DNA stain, Ethidium bromide, Gloves, Pipette and tips

Protocol

- Measured 1.25 g Agarose powder and added it to a 500 ml flask
- Added 125 ml TAE Buffer to the flask. (The total gel volume well vary depended on the size of the casting tray)
- Melted the agarose in a microwave or hot water bath until the solution becomes clear. (if used a microwave, heat the solution for several short intervals - did not let the solution boil for long periods as it may boil out of the flask).
- The solution cold to about 50-55°C, swirling the flask occasionally to cool evenly.
- Seal the ends of the casting tray with two layers of tape.
- Placed the combs in the gel casting tray.
- Pour the melted agarose solution into the casting tray and let cool until it is solid (it should appear milky white).
- Carefully pull out the combs and removed the tape.
- Place the gel in the electrophoresis chamber.

3. Added enough TAE Buffer so that there is about 2-3 mm of buffer over the gel. Product was separated on agarose gel by electrophoresis. It was visualized in gel documentation system after staining with fluorescent dye and photo was saved for further analysis by using appropriate software.
(Ref. Anna, M. and Laura, Z. 2001.) [9]

4. Results and Discussion

The data on yield and purity of DNA as influenced by different extraction method is given in Table 1 (1.5 % PVP), Table 2 (2%
PVP) and Table 3 (2.5% PVP).

4.1. Effect of Different extraction methods on Yield and purity of DNA

a) **Table 1:** In presence of 1.5% PVP

Plant	od260	od280	A260/A280	DNA yield (µg/g)
Azadirachta indica	0.152	0.086	1.76	1086
Aegle marmelos	0.085	0.049	1.73	1025
Bacopa monnieri	0.045	0.033	1.36	0856
Ocimum sanctum	0.187	0.109	1.71	1016
Santalum album	0.196	0.117	1.67	0993

b) **Table 2:** In presence of 2.0 % PVP

Plant	od260	od280	A260/A280	DNA yield(µg/g)
Azadirachta indica	0.140	0.096	1.45	1050
Aegle marmelos	0.109	0.080	1.36	1002
Bacopa monnieri	0.102	0.065	1.56	0965
Ocimum sanctum	0.125	0.086	1.47	1056
Santalum album	0.163	0.102	1.59	0975

c) **Table 3:** In presence of 2.5% PVP

Plant	od260	od280	A260/A280	DNA yield(µg/g)
Azadirachta indica	0.106	0.073	1.45	0986
Aegle marmelos	0.143	0.096	1.48	0845
Bacopa monnieri	0.156	0.091	1.71	1025
Ocimum sanctum	0.087	0.065	1.33	1006
Santalum album	0.146	0.096	1.52	0975

Tables 1, 2, and 3 showed that variations in PVP concentration affected DNA yield and purity. Neem (Azadirachta indica), Tulsi (Ocimum sanctum), Bel (Aegle marmelos), and sandalwood (Santalum album) had the best output and purity at 1.5%. For Brahmi (Bacopa monnieri), a PVP concentration of 2.5% was appropriate. The A260/A280 ratio showed the degree of DNA purity in each of the three PVP concentrations in extraction buffer. Neem (Azadirachta indica), Tulsi (Ocimum sanctum), Bel (Aegle marmelos), and sandalwood (Santalum album) leaves had the greatest DNA yields at 1.5% PVP, or 1086 µg/g, 1016 µg/g, 1025 µg/g, and 993 µg/g, respectively. The yieldmaximum at a PVP content of 2.5%, or 1025 µg/g.Four plants had the best DNA production and quality at a low PVP concentration of 1.5%. The low PVP concentration was the cause of this. that the DNA precipitation in the pellet is increased by the greater PVP content (2.5%). Compared to

the other four plants, Brahmi (Bacopa monnieri) requires a higher PVP content (2.5%) to achieve greater purity. Brahmi (Bacopa monnieri) requires a higher PVP content (2.5%) in the extraction buffer due to its greater concentration of secondary metabolites. Adhikari et al. (2011) [3] and Sahare et al. (2012) found similar kinds of findings.

However, the amount and type of tissue, the genomic size, the size of cells at various phonological stages of the plant, the ratio of large amounts of tissue to the volume of the extraction buffers used in three different concentrations of PVP, and the impact of certain substances in the extraction buffer that preserve the stability of pure DNA have all affected the amount of isolated DNA.

As it has previously been reported that adding SDS after one hour of incubation in extraction buffer, rather than introducing it straight to buffer, might enhance efficiency, this experiment demonstrated that the concentration of PVP in the solution and the sequence in which the chemicals are added could be crucial. In order to solubilize the cell membrane and release the contents, the current research changed the usual approach by employing SDS as a detergent with NaCl. PVP was added to extraction buffer at several concentrations (1.5%, 2.0%, and 2.5%) to eliminate polyphenols, which, when oxidized, may attach to DNA and proteins, turning them brown and rendering them unsuitable for use in any kind of study. To get rid of coloring materials like pigment and dyes, phenol, chloroform, and isoamyl alcohol (25:24:1) was used. Any remaining phenol residues were eliminated from the nucleic acid preparation by a further extraction using chloroform. DNA was precipitated and polysaccharides were eliminated using ethanol and a high concentration of NaCl. When ethanol is present, DNA precipitation often produces a sizable gelatinous substance with an unknown composition. Although it is difficult to separate, DNA is readily evident amid the bulk. The easiest way to solve the issue has been to either increase the NaCl extraction before alcohol precipitation or dilute the aqueous phase produced after chloroform extraction. The genomic DNA of these five medicinal plants was successfully isolated using a high-salt buffer (1.5–2.0 M NaCl). The amount of polysaccharides in the precipitated DNA decreases at this stage because the polysaccharides stay in solution and are eliminated with the ethanol supernatant. High concentrations of NaCl may also neutralize acidic polysaccharides that would otherwise interfere with the acidity of Taq DNA polymerase or the Hind111 enzyme in subsequent applications.

4.2. Restriction digestion of purified DNA fragment

5. Restriction digestion analysis was performed on the extracted DNA with the maximum purity and yield. The EcoR1 restriction enzyme was used to perform the restriction digestion. The purity and clean nature of DNA samples could be confirmed through complete digestion EcoRI (3U/µg DNA). This suggested that the separated DNA may be processed further for use in DNA fingerprinting and other molecular biology applications. The clear bands were not visible but DNA was restricted by enzymes and it formed a smear on. Agarose gel (8%).

5.1. RAPD PCR Analysis of DNA

The DNA was used to perform PCR with RAPD marker. The 25µl of master mix supplied by Genei™ was mixed with 1µl of DNA sample, the PCR was carried out using applied Bio Student PCR machine. The result was analyzed using agarose gel electrophoresis. The good utility of the DNA in PCR amplification for RAPD primer (5'GCACGCCGGA3') and with preparation of all the medicinal plant species tested. Only one single RAPD primer was used and it was tested that whether the DNA quality is suitable for RAPD PCR or not. It is found that the DNA got random amplification using PCR and the DNA quality is suitable for the further PCR studies.

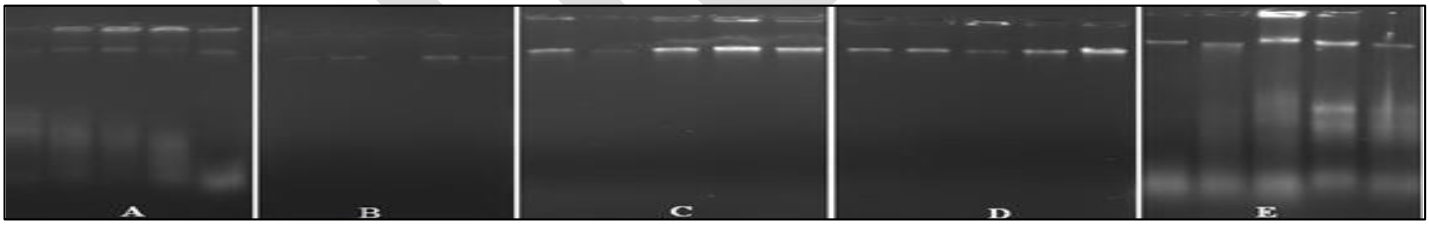

Fig 1: Genomic DNA isolation from five different medicinal plants. (a) Brahmi (*Bacopa monnieri*), (B) Bel (*Aegle marmelos*), (C) Neem (*Azadirachta indica*), (D) Tulsi (*Ocium sanctum*), and (E) Sandalwood (*Santalum album*).

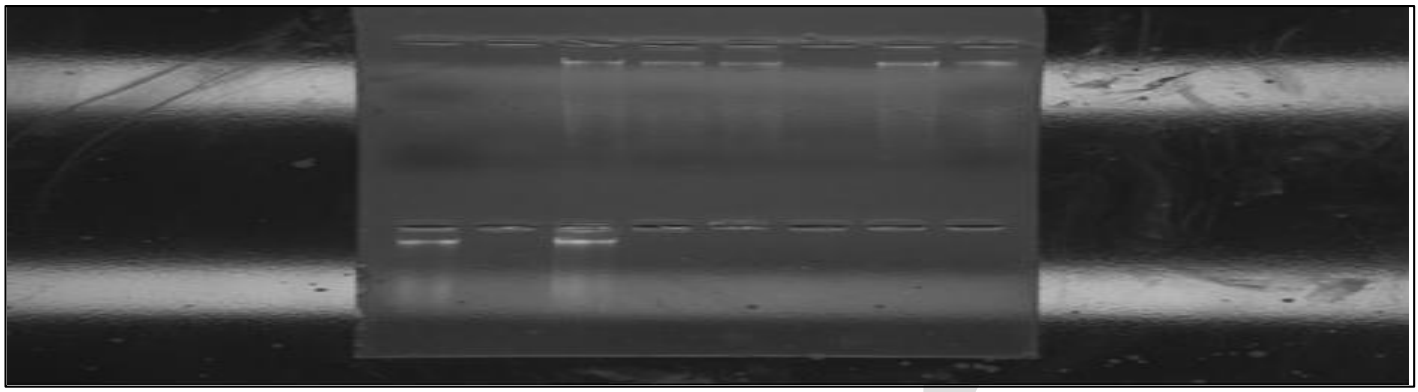

Fig 2: The restricted DNA produced smear on (0.8%) agarose gel, indicating complete digestion of DNA samples. (After restriction digestion, DNA sample was compared to remaining DNA solution (remaining DNA solution did not subjected for restrictions digestion treatment)

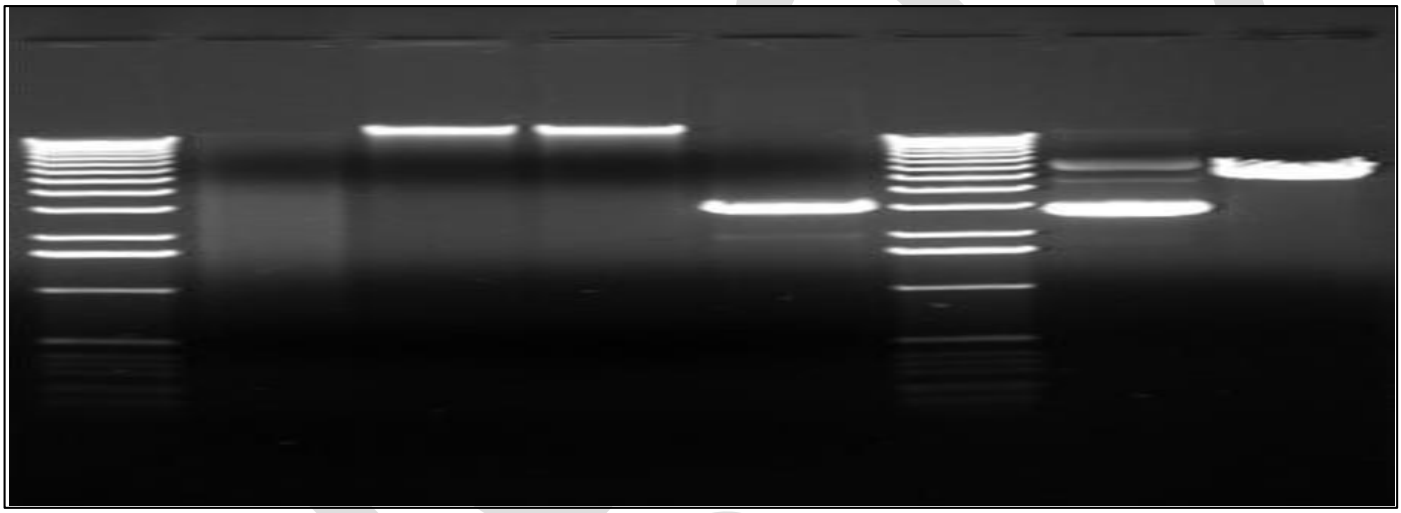

Fig 3: Optimization of the RAPD-PCR reaction parameters for five different medicinal plants. Brahmi (*Bacopa monnieri*), Bel (*Aegle marmelos*), Neem (*Azadirachta indica*), Tulsi (*Ocium sanctum*), and Sandalwood (*Santalum album*).

Conclusion

The data above make it evident that variations in PVP concentrations (1.5%, 2.0%, and 2.5%) have an impact on DNA yield and purity. The five distinct medicinal plants used in the research produced higher amounts of high molecular weight, high-quality, undamaged DNA. However, the species, genomic size, cell size at various physiological stages of the plant, amount and type of tissue, the ratio of large amounts of tissue to the volume of the extraction buffers used, and the impact of certain substances in the extraction buffer that preserve the stability of pure DNA have all influenced the amount of isolated DNA. Azadirachta indica produced the highest yield of 1086 µg/g in this investigation when compared to tissue samples from other medicinal plants. The DNA extraction procedure for plants rich in secondary metabolites or derivatives of polysaccharides has solved the majority of the issues in the current investigation. Large numbers of samples could be handled in parallel using the current approach, which only needed a modest quantity of tissues for effective extraction. DNA was purified using chloroform/phenol instead of the more costly cesium chloride or CTAB utilized in previous methods. Liquid nitrogen was not required for crushing the plant material, and it took around one hour to produce DNA for any molecular biology application. A costly and very dangerous reagent was not needed for this process. Even in low-tech labs, it could function.Thousands of PCR-based reactions may be carried out using the high quality and amount of DNA extracted using this approach, which could also be used to other DNA manipulation methods. With very few modifications, the approach might be expanded to include related species. The goal of developing this process was to reduce the cost of DNA

extraction by using commercially available kits and to make this approach easily accessible in facility labs. Most importantly, a significant amount of high-quality genomic DNA that is appropriate for PCR-based markers and other genomic investigations has been effectively extracted.

6. References

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