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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 discovered to have a ratio between 1.4 and 1.8. Therefore, for a variety of molecular biology applications, the current procedure may be utilised 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 utilise traditional medicines in addition to other forms of treatment. Not only are they employed for basic healthcare in rural parts of impoverished nations, but they are also used in affluent 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 recognised 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 (<http://www.nmpb.nic.com>) 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 When powdered and exposed to the sun, it may be used to clean teeth. Additionally, it may be combined with mustered oil to create a paste that can be used as toothpaste. This is excellent for massaging the gums, preventing bad breath, and preserving oral health. Additionally, it helps with gum disease and pyorrhoea. Tulsi is an effective therapy for gum disease because of its anti-inflammatory and anti-infectious qualities. In Ayurveda, Brahmi (*Bacopa monnieri*) is referred to as "Medhya rasayanas" because it improves mental clarity and stimulates the brain (Bhattacharya and Ghosal 1998). The existence of several saponins, including Bacosides A, B, C, and D, known as the "memory chemicals," has been linked to *Bacopa monnieri*'s therapeutic qualities that improve memory-related function (Rastogi, 1994). Additionally, *Bacopa monnieri* includes a number of medicinally useful compounds, including as flavonoids, sapogenins, and stigma sterol. D-mannitol, betulic acid, beta-sisterol, octacosane, nicotine, and amino acids are other substances. Additionally, it has anti-inflammatory, analgesic, antipyretic, insanity, epilepsy, anticancer, and antioxidant properties (Satyavati, 1976). Additionally, it is used to treat blood clotting, water retention, and asthma. In Pakistan, skin conditions, leprosy, epilepsy, eczema, asthma, hoarseness of voice, and nervous system disorders are all treated using the herbal remedy Brahmi-buti (Shakoor, 1994). They had experimented with previously developed DNA isolation techniques, but they produced highly contaminated DNA that was unsuitable for RAPD analysis. As a result, we provide here a process for whole genomic DNA separation that is based on a technique that was first developed for other plants. The separated DNA would be appropriate for use in subsequent processes. High concentrations of polysaccharides, polyphenols, various colours, and other secondary metabolites found in medicinal plants render DNA useless for further molecular biology studies (Wen and Deng, 2002). The yield and purity of extracted DNA may be reduced by polyphenols since they are strong oxidising agents. By blocking Taq polymerase enzyme activity and interfering with proper DNA digestion, polysaccharides cause DNA to become sticky, glue-like, and non-amplifiable in PCR (Porebski et al., 1997) [9]. A good isolation procedure should produce significant volumes of high-quality DNA appropriate for molecular analysis and be quick, easy, and effective. For several plant species, various DNA extraction techniques have been successfully used. Since a lot of RNA in the sample might chelate Mg^{2+} and lower PCR output, DNA has been processed with DNase-free ribonuclease A. Additionally, it entails a series of long-term RNase treatments, all of which are conducted at room temperature. RNase treatment produces RNA-free pure DNA by breaking down RNA into tiny ribonucleosides that do not contaminate the DNA preparation (Jena et al., 2010). There are already a number of commercially available DNA isolation kits; however, the primary issue with these kits is their high cost per sample (Ahmed et al., 2009). For large-scale genomic applications, regular DNA extraction is economically challenging since the majority of existing methods rely on the usage of commercial kits. Thus, the experiment "Isolation of DNA from medicinal plant" has been scheduled for the academic year November 2012 to April 2013 at MGM College of Agricultural Biotechnology, Aurangabad, with the following goals in order to address the issue of DNA isolation from medicinal plants.

1. Standardisation of DNA isolation procedures.
2. To measure the isolated DNA yield.
3. To investigate isolated DNA's potential for PCR analysis.

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 ginseng (*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. *siamensis* 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. *siamensis* 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:

This chapter describes the specific resources utilised and the methodology used to carry out the current inquiry within the relevant headings.

3.1.Collection of Plant material;

1. Five distinct medicinal plants were chosen for DNA isolation research: Brahmi (*Bacopa monnieri*), Bel (*Aegle marmelos*), Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Sandalwood (*Santalum album*). Leaf tissue is an ideal plant part for DNA isolation since it can be readily separated from the plant and is readily accessible in large quantities. Young leaves are easier to isolate DNA from than other parts of the plant.
2. Plant material was gathered from MGM CABT Aurangabad and Waluj. The sample was taken early in the morning, just before sunrise. As the temperature rises, the plant's leaves become more stressed, which raises the levels of several secondary metabolites. These secondary metabolites are significant obstacles to the DNA extraction process.
3. The leaf samples were transported to the biochemistry and molecular biology labs for further processing after being

stored on ice at 40°C.

4. The leaves were treated in less than a day while being stored at -200C.
5. The International Journal of Engineering and Science (IJES) cites Pallavi Sahare.

3.2.Extraction of DNA from stored plant material;

The process of extracting DNA from medicinal plants is very time-consuming. Secondary metabolites are the main issue that researchers deal with, thus the procedure has been standardised such that the extracted DNA is pure for restriction digestion and RAPD analysis.

1. The DNA was extracted from the preserved plant leaves of Neem (*Azadirachta indica*), Bel (*Aegle marmelos*), Brahmi (*Bacopa monnieri*), Tulsi (*Ocimum sanctum*), and Sandalwood (*Santalum album*).
2. In the presence of 2 ml of extraction buffer (200 mM Tris-HCL (PH-8.0), 200 mM NaCl, 25 mM EDTA, Proteinase K (0.1 mg/ml), and PVP (1.5%, 2.0%, and 2.5%), precisely 100 mg of plant leaves were mashed with mortar and pestel.
1. After adding 50 µl of 10% SDS solution, the homogenate was moved to a 1.5 ml micro centrifuge tube and vortexed for 30 seconds.
2. After five minutes, the tube was gently shaken and held in a water bath at 60°C for 65 minutes.
3. The tubes were centrifuged for 15 minutes at 40C at 17,000 rpm.
4. After transferring the upper aqueous phase into a fresh tube, an equal amount of phenol, chloroform, and isoamyl alcohol (25:24:1, v/v/v) was combined, and the mixture was once again centrifuged at 15,000 rpm for 10 minutes at 40C.
5. After removing the supernatant, a fifth volume of 2 M NaCl and two volumes of cold ethanol were added, and the mixture was maintained at -20°C for ten minutes in order to precipitate DNA.
6. An further centrifugation was carried out once more for ten minutes at 40C and 17,000 rpm.
7. After being pelleted, the DNA was moved to a different tube. The DNA was air dried in a laminar flow chamber after being cleaned three times with 70% ethanol. After being dissolved in sterile double-distilled water, the DNA was kept at -20 °C.
8. The following RNase therapy was administered:
added 1 ul of an RNase A stock solution (10µg/ml) to the DNA. After the DNA-containing solution was incubated at 37°C, 1/10 volume of 3M sodium acetate and 2 volumes of isopropanol were added. The mixture was then incubated on ice for 10 minutes and centrifuged at maximum speed for 5 minutes at room temperature in order to extract the DNA. After discarding the alcohol, the pallet was cleaned with 70% ethanol, the DNA was dried, and it was dissolved in dH2O. (Source: 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 absorbance of the extracted DNA at 260 and 280 nm was used to quantify it; an optimum value for A₂₆₀/A₂₈₀ is 1.8; a drop in this ratio indicates protein contamination, while an increase indicates RNA contamination.
 2. A software for measuring DNA yield and purity is included into the spectrophotometer (UV-1800, Schimatzu).
 3. After using milli-Q water as an absorbance blank for both cuvetts, one cuvet was swapped out for a solution containing contaminating DNA, and measurements were noted.
- (Source: Alatar, A. and Mahmoud, M. 2012.) [2]

3.4.Restriction digestion of purified DNA fragment;

1. 20µl of DNA sample, 10µl of reaction mixture, 3µl of restriction enzymes (EcoRI), and 50µl of sterile milli-Q water were combined to create the reaction mixture.
 2. After 45 minutes of incubation at 370C, the mixture was put on ice to halt the process.
- Agarose gel electrophoresis was used to verify the digestion. (Source: Ghaffariyan, S. and Mohammadi, S. 2012.) [4]

3.5.RAPD PCR Analysis of DNA;

1. PCR using the RAPD marker was carried out using the DNA.
2. A Bio Student PCR equipment was used to do the PCR after 25µl of Genei™'s master mix and 1µl of DNA sample were combined. Agarose gel electrophoresis was used to analyse the outcome.
3. The following ingredients made up the master combination for one reaction:
10X assay buffer
The dNTP mix

Taq DNA polymerase

Primers and genomic DNA

Double-distilled water was used to modify the final amount of MgCl₂.

1. PCR amplification;

A thermal cycler was used to amplify the DNA. It entails the following actions:

First denaturation for three minutes at 950°C.

Denaturation for one minute at 940 degrees Celsius.

Annealing the primer for one minute at 520 degrees Celsius.

Extension for two minutes at 720 degrees Celsius.

For repeated cycles, I went to step 2.

Final extension for three minutes at 720 degrees Celsius.

(Ref. Sharma, P. and Joshi, N. 2010.) [11] Hold at 40

3.6. Agarose Gel Electrophoresis;

1. One popular method for analysing nucleic acids is gel electrophoresis. Agarose gel electrophoresis is a standard procedure used in all molecular biology research labs to prepare and analyse DNA.

2. The components of agarose gel electrophoresis are as follows:

Material;

Agarose, TAE Buffer, 6X Sample Loading Buffer, DNA ladder standard, Electrophoresis chamber, power supply, combs and gel casting tray, DNA stain, Ethidium bromide, gloves, pipette, and tips

Protocol;

A 500 ml flask was filled with 1.25 g of measured agarose powder.

125 millilitres of TAE buffer were added to the flask. (The size of the casting tray will affect the overall gel volume.)

The agarose was melted in a hot water bath or microwave until it was transparent. (If using a microwave, heat the mixture for a few brief intervals; avoid letting it boil for extended periods of time as it can spill out of the flask).

The solution cools to between 50 and 55 degrees Celsius, and the flask is periodically swirled to ensure uniform cooling.

Use two layers of tape to seal the ends of the casting tray.

The combs were placed in the gel casting tray.

Fill the casting tray with the melted agarose solution, then let it to cool until it solidifies (it should look milky white).

Remove the tape and carefully remove the combs.

Put the gel into the electrophoresis chamber.

Enough TAE buffer was added to cover the gel by two to three millimetres.

Electrophoresis was used to separate the product on an agarose gel. After being stained with fluorescent dye, it was visualised in a gel documentation system, and the image was stored for further examination using the relevant software.

(Source: Laura, Z., and Anna, M. 2001.) [9]

1. Results and Discussion;

Tables 1 (1.5% PVP), 2 (2% PVP), and 3 (2.5% PVP) provide information on DNA yield and purity as affected by various extraction techniques.

1.1. Effect of Different extraction methods on Yield and purity of DNA;

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

Plant	od ₂₆₀	od ₂₈₀	A ₂₆₀ /A ₂₈₀	DNA yield (µg/g)
<i>Azadirachta indica</i>	0.152	0.086	1.76	1086
<i>Aegle marmelos</i>	0.085	0.049	1.73	1025
<i>Bacopa monnieri</i>	0.045	0.033	1.36	0856
<i>Ocimum sanctum</i>	0.187	0.109	1.71	1016
<i>Santalum album</i>	0.196	0.117	1.67	0993

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

Plant	od ₂₆₀	od ₂₈₀	A ₂₆₀ /A ₂₈₀	DNA yield(μg/g)
<i>Azadirachta indica</i>	0.140	0.096	1.45	1050
<i>Aegle marmelos</i>	0.109	0.080	1.36	1002
<i>Bacopa monnieri</i>	0.102	0.065	1.56	0965
<i>Ocimum sanctum</i>	0.125	0.086	1.47	1056
<i>Santalum album</i>	0.163	0.102	1.59	0975

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

Plant	od ₂₆₀	od ₂₈₀	A ₂₆₀ /A ₂₈₀	DNA yield(μg/g)
<i>Azadirachta indica</i>	0.106	0.073	1.45	0986
<i>Aegle marmelos</i>	0.143	0.096	1.48	0845
<i>Bacopa monnieri</i>	0.156	0.091	1.71	1025
<i>Ocimum sanctum</i>	0.087	0.065	1.33	1006
<i>Santalum album</i>	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. It was discovered that Brahmi (*Bacopa monnieri*) DNA production peaked at 2.5% PVP concentration, 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) [12] revealed similar kinds of findings. However, the amount and type of tissue, the genomic size, the size of cells at various phenological 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 solubilise 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 oxidised, may attach to DNA and proteins, turning them brown and rendering them unsuitable for use in any kind of study. To get rid of colouring 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 neutralise acidic polysaccharides that would otherwise interfere with the acidity of Taq DNA polymerase or the HindIII enzyme in subsequent applications.

1.2. Restriction digestion of purified DNA fragment;

Restriction digestion analysis was performed on the extracted DNA with the maximum purity and yield. The EcoRI restriction enzyme was used to perform the restriction digestion. Complete digestion EcoRI (3U/μg DNA) might be

used to verify the cleanliness and purity of DNA samples. This suggested that the separated DNA may be processed further for use in DNA fingerprinting and other molecular biology applications. DNA was constrained by enzymes and created a smear, but the distinct bands were not visible. Agarose gel (8%).

1.3. RAPD PCR Analysis of DNA:

PCR using the RAPD marker was carried out using the DNA. A Bio Student PCR machine was used to do the PCR after 25 μ l of GeneiTM's master mix and 1 μ l of DNA sample were combined. Agarose gel electrophoresis was used to analyse the outcome. The DNA's usefulness in PCR amplification for the RAPD primer (5'GCACGCCGGA3') and in the manufacture of every examined medicinal plant species. The suitability of the DNA quality for RAPD PCR was verified using a single RAPD primer. It is discovered that the DNA underwent random amplification using PCR, and the quality of the DNA is appropriate for more PCR investigations.

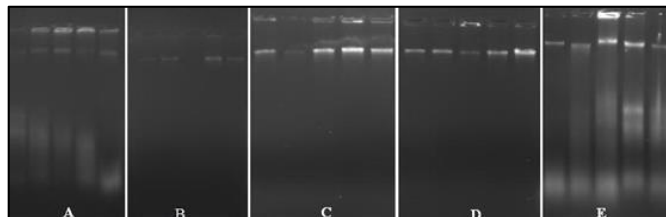


Fig 1: Genomic DNA isolation from five different medicinal plants. (a) Brahmi (*Bacopa monnieri*), (B) Bel (*Aegle marmelos*), (C) Neem (*Azadirachta indica*), (D) Tulsi (*Ocimum sanctum*), and (E) Sandalwood (*Santalum album*).

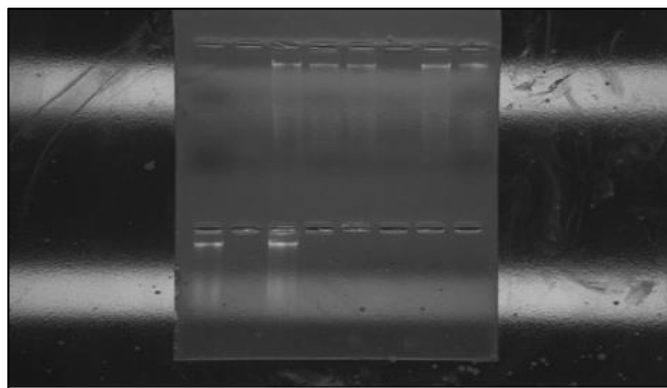


Fig 2: The restricted DNA produced smear on (0.8%) agarose gel, indicating complete digestion of DNA samples. (After restriction digestion, DNA sample was compared to remaining DNA solution (remaining DNA solution did not subjected for restrictions digestion treatment))

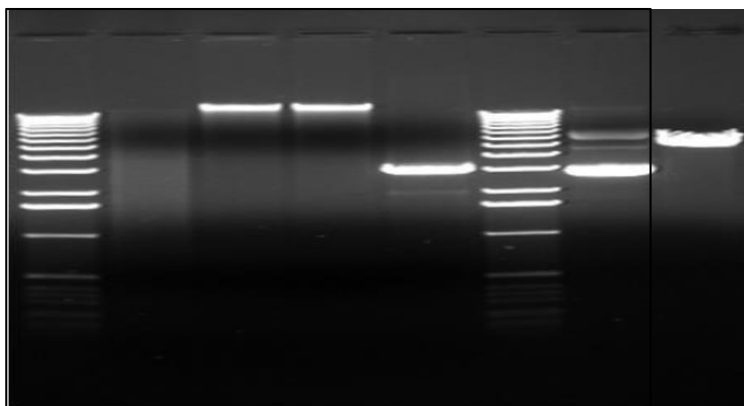


Fig 3: Optimization of the RAPD-PCR reaction parameters for five different medicinal plants. Brahmi (*Bacopa monnieri*), Bel (*Aegle marmelos*), Neem (*Azadirachta indica*), Tulsi (*Ocimum sanctum*), and Sandalwood (*Santalum album*).

2. Summary and 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 caesium chloride or CTAB utilised 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.

3. References:

1. DNA Isolation Techniques for Medicinal and Aromatic Plants, Anna M., Laura Z. Reporter for Plant Molecular Biology. 2001; 19(5):273.
2. Alatar A, Mahmoud M. A quick and easy method for separating PCR-amplifiable DNA from therapeutic herbs. 2012; 11(1):348-354; Genetics Molecular Research.
3. A streamlined, high-yielding miniprep genomic DNA extraction method for medicinal plants, Adhikari S, Ghosh T. Indian Journal of Biotechnology. 2011; 11(8):337-340.
4. Ghaffariyan S, Mohammadi S. Lemon balm (*Melissa officinalis*) DNA isolation procedure. Molecular research in genetics. 2012; 11 (2): 1049-1057.
5. Javornik B. Sturdy CTAB-activated charcoal method for extracting plant DNA. Acta Slovenica Agriculture. 87(1):427-436, 1987.
6. Khan S, Alam T. Method for isolating genomic DNA from fresh and dried medicinal plant roots that is appropriate for restriction digestion and RAPD. African Journal of Biotechnology. 2007; 6(3):175-178. An enhanced method for isolating genomic DNA from *Phyllanthus emblica* leaf tissues was developed by Nagarajan S and Kavitha M. Iranian Biotechnology Journal. 2005; 9(4):56-98.
7. Padmalatha K, Prasad M. DNA isolation and PCR process optimisation for RAPD analysis of certain aromatic and medicinal plants of conservation significance in Peninsular India. African Journal of Biotechnology. 5(3):230-234, 2005.
8. Porebski. The yield and purity of extracted DNA may be reduced by polyphenols, which are potent oxidising agents. Biotechnology Journal. 1997; 6(3):175-178
9. Pandey M, Changtragoon S. Microsatellite marker isolation and characterisation in Indian neem (*Azadirachta indica* var. A. Juss) and cross-amplification in Thai neem (*A. indica* var. *Siamensis valenton*). 48(1):117-123, Springer Science Business Media, 2008.
10. Sharma P, Joshi N. Genomic DNA isolation from medicinal plants without the use of liquid nitrogen. Indian Journal of Experimental Biology. 48 (2): 610-614.
11. Srinivasu, T., and Sahare, P. (2012). A technique for isolating genomic DNA from medicinal plants that produce a lot of secondary metabolites. The International Journal of Science and Engineering. 2010; 1(2):37-39.
12. Tiwari K, Jadhav S. DNA isolation from several medicinal plants using a modified CTAB technique. Journal of Medicinal Plant Research. 2012; 6(5):65-73.