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(RESEARCH ARTICLE)

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Optimization and extraction of DNA from medicinally important plant species *Phyllanthus emblica*

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Abstract

Indian gooseberry has two scientific names *Phyllanthus emblica* and *Emblica officinalis.* Gooseberry has cookery and herbal medicine uses. It grows abundantly in the forests of India and many South Asian countries. Indian gooseberry supplements are used in anti-aging, cancer, heart health, blood sugar levels, liver damage and immune health. Fruit is reputed to probably have the highest content of vitamin C compared with any other naturally occurring substances in nature. Active extracts of *P. emblica* have been shown to possess several pharmacological properties, e.g., analgesic, anti-inflammatory, antioxidant and chemo protective activities *P. emblica* being a perennial crop, molecular tools can be of great utility to support the conventional breeding. Therefore, isolation of intact high-quality DNA is essential for carrying out molecular studies. DNA extraction is the isolation of DNA from a sample. The reason includes its role in understanding the plant's genetic makeup, identifying specific genes and developing improved varieties with desirable traits. They contribute to molecular biology research and to the broader understanding of plant genetics and evolution. Different methods include Lysis, Precipitation, Purification and Concentration. DNA is extracted from Indian gooseberry for various purposes such as genetic studies, plant breeding and research. The reagents used in this process are Tris EDTA buffer, cetyltrimethylammonium bromide buffer and some chemicals.

Keywords*: Phyllanthus emblica;* Genetic makeup; Molecular biology; Tris EDTA buffer*;* Cetyltrimethylammonium bromide buffer.

1. Introduction

The use of medicinal and aromatic plants (MAPs) has a long history in the world. According to the World Health Organization, 20,000 species are used for medicinal and aromatic purposes. Today, 4000 drugs are widely used and 10% of them are commercially exploited or produced. Around 500 plant species are used for medicinal and aromatic purposes in Turkey. However, all these species are neglected or underutilized and only a few are cultivated or subjected to research [1]. *Phyllanthus emblica Linn* or *Emblica officinalis Gaertn* commonly known as Indian gooseberry or Amla is one of the most important medicinal plants in Indian traditional systems of medicine (Ayurveda, Unani and Siddha). It is a well-known fact that all parts of Amla are useful in the treatment of various diseases. Among all, the most important part is fruit. Amla fruit is widely used in the Indian system of medicine as diuretic, laxative, liver tonic, refrigerant, stomach, restorative, anti-pyretic, hair tonic, ulcer preventive and Quercetin and ascorbic acid are found to be biologically effective [2] . *Emblica officinalis Garten* or *Phyllanthus emblica,* generally called the Indian gooseberry or amla, is an extensively used nutraceutical in treating various diseases because it is known to have immunity-boosting ability. It has immense importance in indigenous traditional medicinal systems, for its medicinal and nutritional benefits.

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Aim

The primary aim of this project is to successfully extract high-quality DNA from *Phyllanthus emblica*, commonly known as Indian gooseberry.

- We aim to obtain DNA that is suitable for various genetic analyses and molecular biology research.
- This project aims to contribute to the broader understanding of the genetic makeup of *Phyllanthus emblica*, which has significant implications for medicinal and botanical research.

Objective

- The primary objective of this project is to successfully extract high- quality DNA from *Phyllanthus emblica*.
- To ensure that the extracted DNA is of high purity and integrity, free from contaminants that might affect downstream applications [7].

Figure 1 *Phyllanthus emblica*

2. Materials and methods

2.1. Raw materials

A sample of *Phyllanthus emblica* was collected from an alma tree. Fresh samples are taken for DNA isolation protocol [3]. The following chemicals were used for the isolation of DNA from the sample, ethanol, CTAB buffer, chloroform isoamyl mixture, sodium acetate, TE buffer was used as solubilizing DNA and protect nucleic acids from enzymatic lysis.

2.2. Sample preparation

A healthy *Phyllanthus emblica* plant growing under normal environmental conditions was selected. Fruit samples were collected, frozen immediately in liquid nitrogen and stored at –80 degree Celsius till further use [4]. Fruits were chopped off into small pieces, so that the tissue is grinded easily with ethanol in a mortar and pestle. Store them in a centrifuge tube [9].

2.3. Buffer preparations

2.3.1. CTAB buffer solution

- CTAB 2 g
- 1M Tris base (pH 8.00) 10 ml
- 5M EDTA (pH 8.00) 4 ml
- 5M NaCl 28 ml
- H_2O 40 ml
- PVP 1 g

Adjust all to pH 5.0 with HCL and make up to 100ml with H2O [5].

2.3.2. TE Buffer solution

TE Buffer (pH 7.4)

- 10mM Tris-HCl, (pH 7.4), 1mM EDTA, (pH 8.0)

  10mM Tris-HCl, pH 7.4
  Mass (g) =Molarity (M) ×Volume (L) ×Molar Mass (g/mol)
  Mass (g) =0.01M x 0.1L x 121.4 g/mol
  Mass (g) =0.1214g.

Weigh 0.1214 grams of Tris and dissolve it H_2O and make up to 100ml.

1mM EDTA, pH 8.0

Mass (g) =Molarity (M) ×Volume (L) ×Molar Mass (g/mol)

Mass (g) =0.001M x0.1Lx292.2438g/mol

Mass (g) =0.0292g.

Weigh 0.0292 grams of EDTA and dissolve it H_2O and make up to 100ml [6].

2.3.3. TAE Buffer solution

5Ox TAE (Tris-Acetate Buffer)

- Dissolve 242 g of Tris base, 57.1 mL of glacial acetic acid, and 100 mL of 0.5 M.
- EDTA (pH 8.0) in H20 up to 1 liter.
- The 5Ox TAE is the concentrated stock solution. Use 1x TAE as working solution.
- (0.04 M Tris-acetate, 0.001 M EDTA) [6].

2.3.4. Other reagents

Chloroform Isoamyl alcohol (24:1), 70% of ice cooled ethanol/iso propanol, 5M sodium acetate [8].

3. Methodology

Take 20g of Indian gooseberry and grind it using mortar and pestle with 10ml of CTAB buffer [8].

- Grind it well to a fine paste.
- Put the fine paste into a micro-centrifuge tube.
- Centrifuge the sample at 10000 rpm for 2mins [10].
- Discard the supernatant.
- Add 100µl of sterile distilled water to the pellet.
- Vortex mix/pipette mixes the sample.
- Prick the hole on the top of micro-centrifuge tube and keep the sample tube in the water bath at 45°C for 20mins.
- Centrifuge the tube at 10000 rpm for 2mins.
- Take the clear supernatant.
- Add an equal amount of isoamyl-chloroform mixture (1:24) into the tube [11].
- Centrifuge the tube for 10000 rpm for 2mins.
- After centrifugation two phases are obtained (upper aqueous phase and lower organic phase with an interface layer).
- Transfer the upper phase carefully using pipette into another tube.
- Add sodium acetate 100µl into the tube.
- Add 70% of ice cooled ethanol/iso propanol (500µl) to each tube [12].
- Centrifuge the samples at 10000rpm for 2 minutes.
- Remove all the supernatant from the tube and air dry the pellet (Do not over-dry the pellet it will be difficult to re-dissolve.)

- Then add 100µl of TE buffer to re-suspend the DNA in the tube [14].

Figure 2 Mortars and Pestle

Figure 3 Micro-centrifuge

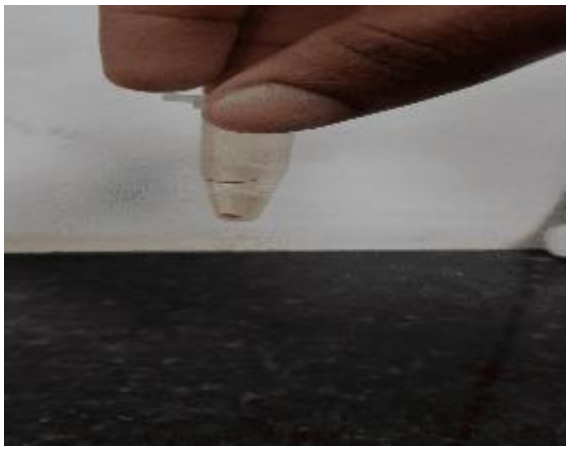

Figure 4 Pellet formation

4. Results and discussion

4.1. Characterization

4.1.1. Agarose gel electrophoresis

Agarose gel electrophoresis is a common laboratory technique used to separate and analyze DNA fragments and other biomolecules based on their size. It is widely employed in molecular biology, genetics, and biochemistry research. The process involves the use of a gel made from agarose; a polysaccharide derived from seaweed. Agarose forms a porous matrix when mixed with a buffer solution and allowed to solidify. This gel matrix creates a molecular sieve through which the biomolecules can migrate under the influence of an electric field.

4.2. Gel electrophoresis method

- Make 500ml of TAE buffer (1X) and 1 gram of agarose with 100ml of TAE buffer.
- For agarose gel preparation: To 1 gram of agarose add 100ml of TAE buffer and heat the agarose mixture until the mixture dissolve.
- Dilute the ethidium bromide in 10ml of distilled water.
- Cool the Agarose mixture at room temperature and add 1-2 drops of ETBR.
- Mix it well.
- Clean the gel electrophoresis tray and comb.
- Cover the tray with tape and check for any leakage.
- Pour the agarose mixture into the gel tray without making bubbles.
- Place the comb into the tray to let the gel solidify.
- Slowly remove the comb.
- Place the gel tray into the electrophoresis chamber and fill the chamber with TAE until it is completely submerged.
- Take 8µl of sample from the re-suspended DNA pellet.
- And add 7µl of loading dye to it. (Make sure the mixture of DNA sample and loading dye do not exceed the level of well).

Connect the power cord to the electrophoretic power supply according to the convention (red: anode, black cathode) NOTE: make sure the wells are on the cathode side because the DNA is negatively charged, and it moves towards the positive charge (anode).

Electrophoresed at 50/100V till the front dye (bromophenol blue) travel 3/4 th of the gel (Do not let the dye run out of gel).

- Wait for 30 to 40mins for the run.
- Visualize the gel under UV-trans illuminator or Gel documentation system.
- Calculate the molecular size of the DNA fragments.

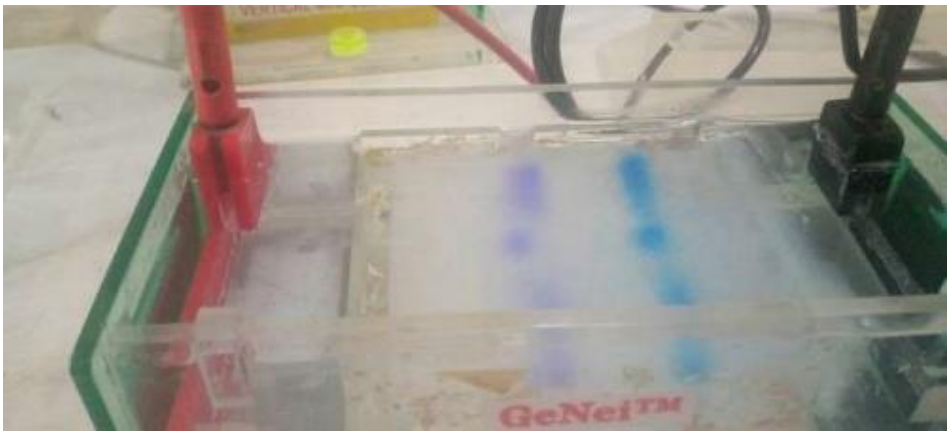

Figure 5 Gel electrophoresis set-up

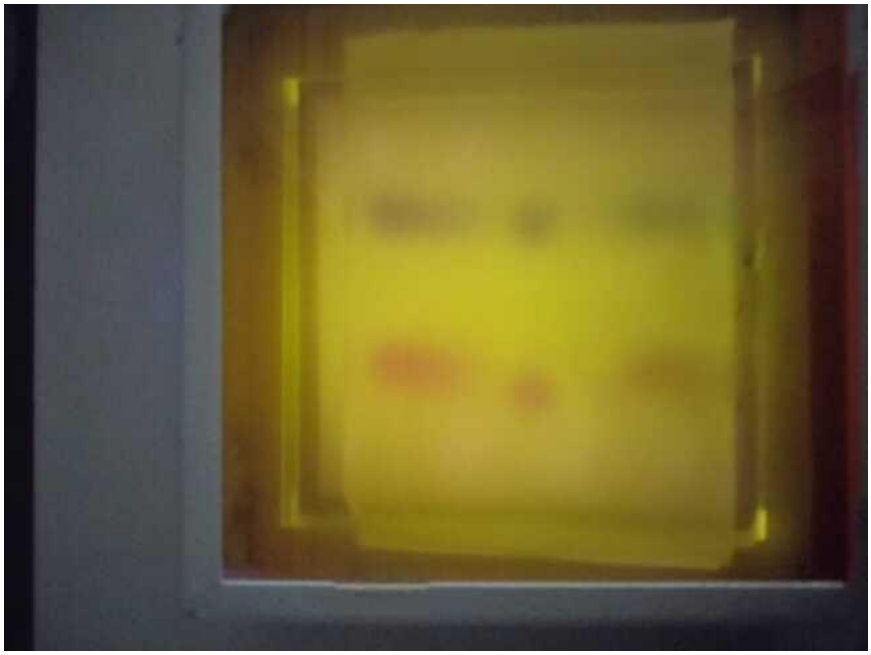

Figure 6 Gel electrophoresis characterization results

4.3. UV visible spectrophotometer for DNA quantitation

- Take the DNA sample and dilute the DNA sample with TE buffer [13].
- Set up the UV spectrophotometer.
- Clean the cuvette with distilled water.
- Fill the cuvette with TE buffer.
- Place the cuvette into the spectrophotometer.
- Click the MENU and select BIO METHOD option [15].
- Then select DNA QUANTITATION and select the wavelength 1 (260nm) and click START.
- The concentration of DNA, protein and absorbance are shown.
- The concentration of DNA in our sample was 374.00.
- Report the result.

Figure 7 UV spectrophotometer setup

Figure 8 UV spectrophotometer result

Abbreviations

- DNA – Deoxyribonucleic acid.
- TE Buffer – TRIS-EDTA Buffer.
- EDTA - Ethylenediaminetetraacetic acid.
- MAPs - Medicinal & Aromatic Plants.
- PCR - Polymerase chain reaction.
- UV-Vis Spectrophotometer - Ultraviolet–Visible Spectrophotometer.
- CTAB - Cetyltrimethylammonium Bromide.
- RFLP - Restriction Fragment Length Polymorphism.
- HCl – Hydrochloric acid.
- H_2O – Water.
- Tris-HCl - TRIS hydrochloride.
- pH – Power of Hydrogen.
- TAE Buffer - Tris-acetate-EDTA Buffer.
- NaCl – Sodium chloride.

SI units

- mM **–** Millimolar.
- g – Gram.
- Mol-mole.
- mg – Milligram.
- mg/ml - Milligram per Milliliter.
- mm – Micrometer.
- Rpm - Revolutions per minute.
- Nm – Nanometer.

5. Conclusion

The present investigation explains extraction of DNA from medicinally important plant species called *Phyllanthus emblica*. Extraction of DNA was done using certified protocol with some modification. By subjecting our DNA samples to gel electrophoresis, we were able to visualize the presence of intact DNA fragments and assess the overall purity of our extractions. Furthermore, UV spectrophotometry played a vital role in quantifying the concentration of DNA in our sample. This quantitative data obtained from UV spectrophotometry informed our optimization process, enabling us to adjust extraction protocols and ensure optimal yields of high-quality DNA. Together, gel electrophoresis and UV spectrophotometry complemented our efforts in DNA extraction optimization, providing valuable insights into the integrity, quantity, and purity of DNA obtained from Phyllanthus emblica. Our optimization efforts not only enhance the yield and purity of extracted DNA but also minimize the use of resources and time, making the technique more sustainable and cost-effective.

Compliance with ethical standards

Acknowledgments

I am deeply thankful to my parents and friends for their love and support during this process. Without their encouragement and motivation, I would not have been able to complete this work.

Disclosure of conflict of interest

No conflicts of interest to be disclosed.

Statement of ethical approval

The present research work does not contain any studies performed on animals/ humans subjects by any of the authors.

Reference

[1] Vural, Hasibe Cingilli. Genomic DNA isolation from aromatic and medicinal plants growing in Turkey.*Scientific Research Essays* 4.2009; 59-64.

[2] Dasaroju, Swetha, and Krishna Mohan Gottumukkala. Current trends in the research of Emblica officinalis (Amla): A pharmacological perspective. *Int J Pharm Sci Rev Res* 24.2;2014;150-9.

[3] Rogers, S. O., & Bendich, A. J. Extraction of DNA from plant tissues. Plant Molecular Biology Manual.1989;73–83.

[4] Kumar, A., & Singh, K. Isolation of High-Quality RNA from Phyllanthus emblica and Its Evaluation by Downstream Applications. Molecular Biotechnology.2012; 52(3), 269–275.

[5] Jina Heikrujam, Rajkumar Kishor and Pranab Behari Mazumd. The Chemistry Behind Plant DNA Isolation Protocols. Biochemical Analysis Tools methods for biomolecules studies.2020.

[6] Nishiguchi, M. K., Doukakis, P., Egan, M., Kizirian, D., Phillips, A., Prendini, L., Giribet, G. DNA Isolation Procedures. Techniques in Molecular Systematics and Evolution. 2002; 249–287.

[7] Afshar-Mohammadian, M., Rezadoost, M. H., & Fallah, S. F. Comparative analysis and innovation of a simple and rapid method for high-quality RNA and DNA and extraction of kiwi fruit Methods.2018; 5, 352–361.

[8] Chabi Sika, K., Kefela, T., Adoukonou- Sagbadja, H., Ahoton, L., Saidou, A., BabaMoussa, L., … Gachomo, E. W. A simple and efficient genomic DNA extraction protocol for large scale genetic analyses of plant biological systems. Plant Gene.2015; 1, 43–45.

[9] Tamari, F., Hinkley, C. S., & Ramprashad, N. A Comparison of DNA Extraction Methods using Petunia hybrid a Tissues. Journal of Biomolecular Techniques.2013; JBT, jbt.13– 2403–001

[10] Sangeetha Nagarajan, Mercy Stephen, Kavitha Murugan, Rahul Raveendran Nair, Thilaga Sethuraman, Parameswari Alagar, Doss Ganesh. Improved protocol for isolation of genomic DNA from leaf tissues of Phyllanthus emblica Gaertn. Iranian journal of biotechnology.2011; vol. 9, no. 4.

[11] Azmat, M. A., Khan, I. A., Cheema, H. M. N., Raj wana, I. A., Khan, A. S., & Khan, A. A. Extraction of DNA suitable for PCR applications from mature leaves of Mangifera indica L. Journal of Zhejiang University SCIENCE B.2012; 13(4), 239–243.

[12] Yamamoto, T., Kimura, T., Hayashi, T., & Ban, Y. DNA Profiling of Fresh and Processed Fruits in Pear. Breeding Scienc.2006; 56(2), 165-171.

[13] Lazar, I. M., Ifrim, I. L., Grosu, L., & Lazar, G. Modifications Induced by UV- Vis Irradiation on DNA Extract of Kiwifruit Investigated by Spectroscopic and Statistic Methods. Spectroscopy Letter. 2013;46(2),81-86. 46(2),81–86.

[14] Toshiya Yamamoto, Tetsuya Kimura, Tateki Hayashi, Yoshiyuki Ban. DNA Profiling of Fresh and Processed Fruits in Pear. Breeding science.2006; 年 56 巻 2 号p. 165-171.

[15] Minhas-Khan A, Ghafar-Zadeh M, Shaffaf T, Forouhi S, Scime A, Magierowski S, Ghafar-Zadeh E. UV-Vis Spectrophotometric Analysis of DNA Retrieval for DNA Storage Applications. Actuators.2021; 10(10):246.