ejpmr, 2019,6(1), 423-426

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DRIED POWDER OF UNANI AND AYURVEDIC MEDICINES YIELDS REMARKABLE DNA UPON EXTRACTION

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Article Received on 03/11/2018 **Article Revised on 24/11/2018** **Article Accepted on 14/12/2018**

ABSTRACT

Genomic DNA extraction is an important aspect of plant molecular biological research. The objective of the study was to recommend the cheap and efficient genomic DNA extraction method for some kobiraki medicines in Bangladesh. The modified plant genomic DNA extraction methods extracted drupe, pome, hespiridium, pepo and complex fruits. Extraction kit (Qiagen) method were applied with eight different ayurvedic medicine powder such as *Phyllanthus emblica, Withania somnifera, Abroma augusta, Syzygium cumini, Oroxylum indicum, Chrysogenum aurium, Mucuna pruriens, Terminalia bellirica*. Based on the quantity of the extracted genomic DNA tested by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer, quality determined by the ratio of A260 / A280 and the amplifiable quality of DNA determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V, the method explained by Cheng et al and the Genomic DNA extraction kit yielded good quality DNA with satisfactory concentration for all the powder medicine tested. Therefore the modified method of Cheng et al, 1987 could be recommended for the efficient and cost effective DNA extraction from fruit species instead of the commercially available expensive and chemically hazardous DNA easy plant kit method.

KEYWORDS: *Phyllanthus emblica, Withania somnifera, Abroma augusta, Syzygium cumini.*

INTRODUCTION

Kabiraj is a title of honor for people practicing Ayurveda (or customs involving traditional medicines) in India and Bangladesh were also called Kabi (Vaidhya). As such they were given the honorable title of Kabiraj. The descendants of such persons started using Kabiraj or Kaviraj as a surname. The word Kabi for a Vaidhya was used generally in Eastern India (West Bengal, Assam, Bihar, Orissa) and Bangladesh as such these surname is often found in persons originating from these regions. Nowadays, many people are using it as main name.

DNA is an almost universal genetic material, and that genes present in simple viruses, bacteria, plants, and animals are all made of DNA. It was a very long polymer made up of millions of nucleotides.[1] The living cell is an extraordinarily complicated entity producing thousands of different macromolecules and harboring a genome. The methods of molecular biology depend upon an understanding of the properties of biological macromolecules. The systematic comparison of different animal genomics gives a chance of identifying genetic basis for diversity. We are fast entering a golden era of comparative genome analysis.[2] Methods used to isolate the DNA depend on the source, age and size of the sample. Principle behind the separation of DNA which is present in the cells is to make the DNA free from the other cellular components.[3] Isolation of DNA is needed for the genetic analysis, which is used for scientific, medical or forensic purpose. Scientists use DNA in a number of applications, such as introduction of DNA into the cells and animals or plants, or for diagnostic purposes.[4] Many protocol have been used for isolation of plant DNA, but because of chemical heterogeneity of the species many of them could be applied to a limited number of species or even closely related species in some case fail to respond to the same protocol. Plants contain an array of secondary metabolites.

Kobiraji medicines which were used as sample in DNA extraction

Mucuna pruriens (Alkushi)

- Supports a healthy central & peripheral nervous system
- Is a natural source of levodopa (L-dopa)
- Supports physical balance & posture
- Promotes healthy motor skills & coordination
- Improves energy & endurance
- Supports the intellect.

Terminalia bellirica (Bahera)
Fruits are laxative, astringent, anthelmintic and antipyretic; useful in hepatitis, bronchitis, asthma, dyspepsia, piles, diarrhoea, coughs, hoarseness of voice, eye diseases and scorpion-sting; used as a hair tonic.

Oroxylum indicum
The Oroxylum indicum seed is used in the traditional Indian ayurvedic medicine as a remedy for pains in joints or rheumatism

Chrysogenum aurium
Fenugreek seeds are a rich source of trigonelline, lysine and l-tryptophan. The seeds also contain a large amount of saponins and fibers that may account for many of the health benefits of fenugreek. The following are some of the ways in which the fenugreek herb has been used traditionally for treating a variety of conditions.

Fenugreek has been known since ancient times as an herbal galactagogue

Abroma augusta
Botanically, Abroma Augusta is name given to Indian medicinal plant known as Ulat Kambal in Hindi and Devil's Cotton in English. Abroma Augusta has beneficial effects in uterine disorders, dysmenorrhea, arthritic pain, rheumatism, and diabetes. It also relieves headache associated with sinusitis.

Syzygium cumini
The major function of Syzygium cumini is that it helps in the treatment of diabetes.

Phyllanthus emblica (Amloki)
1. Source of Vitamin C
2. Recovery from Arthritis
3. Controls Diabetes and High Blood pressure
4. Prevents cancer
5. Anti Inflammatory.

***Withania somnifera* (Ashwagandha)**
Some key examples of the healing effects of Ashwagandha are:
- Protects the immune system
- Helps combat the effects of stress
- Improves learning, memory, and reaction time
- Reduces anxiety and depression without causing drowsiness
- Helps reduce brain-cell degeneration
- Stabilizes blood sugar
- Helps lower cholesterol.

The objective of the current study was to establish a DNA extraction procedure. Eight separate dried powder kobiraji medicine plant species were collected---the powder were chopped in mortar and pestle and transferred for DNA extraction. After extraction of DNA, DNA presence was tested by Agarose gel electrophoresis. With NanoDrop Machine, the DNA content was measured.

MATERIALS

COLLECTION AND PRESERVATION OF Kobiraji medicine
The eight type of powder medicine was collected in the Kawran bazaar by the professional shop keeper, Tejgaon, Dhaka.

HARVESTING OF FRUITS
The powdered medicines were preserved at room temperature until use.

DNA Quantification using Nano Drop
The Thermo Scientific NanoDrop™ 1000 Spectrophotometer measures 1 ul samples with high accuracy and reproducibility. The full spectrum (220 nm-750 nm) spectrophotometer utilizes a patented sample retention technology that employs surface tension alone to hold the sample in place. This eliminates the need for cumbersome cuvettes and other sample containment devices and allows for clean up in seconds. In addition, the NanoDrop 1000 Spectrophotometer has the capability to measure highly concentrated samples without dilution (50X higher concentration than the samples measured by a standard cuvette spectrophotometer).

Reagent
Tris-HCl, EDTA, Triton X-100 and 5µl RNase A (10mg/ml) was added into the sample tube and mixed by vortexing. Tris-HCl, EDTA, Triton X-100 are used for the purpose of breaking open cells. Tris-HCl, EDTA, Triton X-100 are added to break up membrane structures.

Procedure
Tissue Dissociation
50 mg of powder medicine was grinded under liquid nitrogen to a fine powder. It was transferred into a microcentrifuge tube.

Lysis
The mixture was incubated at 65° C for 10 minutes to weaken the cell walls and to lyse.

100 µl lysis buffer was added and mixed by vortexing. The lysis buffer contains sodium hydroxide (NaOH) and the detergent Sodium Dodecyl (lauryl) Sulfate (SDS). SDS is to solubilize the cell membrane. NaOH helps to break down the cell wall, but more importantly it disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA (dsDNA) in the cell, including the genomic DNA SDS also denatures most of the proteins in the cells, which helps with the separation of the proteins.

The closed tube was placed in the ice bath using forceps to hold the tube. The tube was kept in the bath for three minutes to freeze. All cells, the basic structural and

functional unit of life, consist of living material bounded by layers of membranes made of lipids, proteins, and some other compounds. Cell lysis is the first step in the process of DNA purification. The DNA genome contains all the genetic information of an organism, and is protected from the external environment by the cell membrane. In order to release the genetic material for study and analysis, cells must be broken open, or lysed. There are several methods available for cell disruption including physical and chemical techniques. For this DNA extraction, freeze-thaw was used because it is a very common method used to lyse plant tissue cells.

Cell lysis was followed by precipitation of proteins, which traps chromosomal DNA in insoluble fraction and after centrifugation, a filter column was placed in a 2 ml Collection Tube. The mixture from previous step was applied into the Filter Column.The filter column was centrifuged at full speed (13,000 rpm) for 3 minutes. The filter column was discarded and clarified supernatant was carefully transferred in Collection Tube to a new microcentrifuge tube.

2.2.4.3DNA Binding
A GD Column was used as 2 ml Collection Tube.

700µl the mixture (including any precipitate) was applied from previous step into the GD Column.

The solution was centrifuged at full speed (13,00 rpm) for 2 minutes. The flow through was discarded in Collection Tube.

Spin column-based nucleic acid purification is a solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions.

2.2.4.4 Washing
400µl of wash buffer was added into the GdColumn. Again it was centrifuged at 10,000 xg (13,00 rpm) for 30 seconds. The flow through was discarded and placed back in the Collection Tube.600µl of Wash Buffer was added into the GdColumn. It was centrifuged at 10,000 xg (\13,00 rpm) for 30 econds. The flow through was discarded and returned into the 2ml Collection tube. It was centrifuged again for 3 minutes at full speed to dry the Column matrix.

DNA Elution
Standard elution volumn is 100 µl. If less sample volumn is used. The elution volume was reduced (30-50 µl) to increase DNA concentration. If higher DNA yield is required, the elution step was repeated to increase DNA recovery and the total elution volume to about 200 µl.

The dried GD Column was transferred into a clean 1.5 ml microcentrifuge tube. 100 µl of preheated Elution Buffer was added onto the center of the column matrix. For 3.5 minutes it was kept standing until Elution Buffer absorbed by the matrix.

It was centrifuged at 13,000 rpm for 30 seconds to elute purified DNA. The composition of Elution Buffer is:10mM Tris-Cl, pH 8.5.

3. RESULTS AND DISCUSSION

3.1 Quantity of Extracted Fruits

The quality of the Genomic DNA Mini Kit (Plant) is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Genomic DNA is extracted from 20ml fresh fruit. The purified genomic DNA (>10 µg) is quantified with a spectrophotometer and checked by electrophoresis.

Table 1: Quantity of Extracted fruits using Nano Drop Machine.

Plant Speacies	DNA Yield
Phyllanthus emblica **(Amloki)**Sample1	240ng/µl
Phyllanthus emblica **(Amloki)**Sample2	181ng/µl
Phyllanthus emblica **(Amloki)**Sample 3	76.2ng/µl
Phyllanthus emblica **(Amloki)**Sample 4	87.3ng/µl
Withania somnifera (Ashwagandha) Sample 1	88.1ng/µl
Withania somnifera (Ashwagandha) Sample 2	53.6ng/µl
Withania somnifera (Ashwagandha) Sample 3	18.5ng/µl
Withania somnifera (Ashwagandha) Sample 4	33.6ng/µl

DNA Extraction Result from root of *Abroma augusta*

	ng/µl
Sample-1	18.9
Sample-2	20.3
Sample-3	31.8
Sample-4	54.8

DNA Extraction Result from seed of *Syzygium cumini*

	ng/µl
Sample-1	40.6
Sample-2	71.1
Sample-3	51.2
Sample-4	78.1

DNA Extraction Result from root of *Oroxylum indicum*

	ng/µl
Sample-1	43
Sample-2	21.3
Sample-3	62.2
Sample-4	72.1

DNA Extraction Result from seed of *Chrysogenum aurium*

	ng/µl
Sample-1	78.4
Sample-2	28.7
Sample-3	20.8
Sample-4	52.2

RESULT

Sufficient amount of DNA was obtained from the powder medicines but no result found in electrophoresis as the DNA size was too large

Scientific name of alkushi (*Mucuna pruriens*)	Nanogram/microliter
Sample-1	106.5
Sample-2	574
Sample-3	45.1
Sample-4	623.9

Scientific name of bohera (*Terminalia bellirica*)	Nanogram/microliter
Sample-1	111
Sample-2	128.4
Sample-3	165.4
Sample-4	41.9

DISCUSSION

Extraction kit (Qiagen) method were applied with eight different ayurvedic medicine powder such as *Phyllanthus emblica*, *Withania somnifera*, *Abroma augusta*, *Syzygium cumini*, *Oroxylum indicum*, *Chrysogenum aurium*, *Mucuna pruriens*, *Terminalia bellirica*. Based on the quantity of the extracted genomic DNA tested by measuring the absorbance at 260 nm using Nanodrop® ND-1000 spectrophotometer, quality determined by the ratio of A260 / A280 and the amplifiable quality of DNA determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60V, the method explained by Cheng et al and the Genomic DNA extraction kit yielded good quality DNA with satisfactory concentration for all the powder medicine tested. Therefore the modified method of Cheng et al, 1987 could be recommended for the efficient and cost effective DNA extraction from fruit species instead of the commercially available expensive and chemically hazardous DNA easy plant kit method.

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