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Dried Powder of Unani and Ayurvedic Medicines Yields Remarkable DNA upon Extraction

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Abstract

Genomic DNA extraction is an important step in plant molecular biological research. The objective of the study was to recommend the customized genomic DNA extraction method for some kobiraji medicines in Bangladesh. The customized plant genomic DNA extraction method extracted eight different ayurvedic medicine powders such as *Phyllanthus emblica, Withania somnifera, Abroma augusta, Syzygium cumini, Oroxylum indicum, Chrysogenum aurium, Mucuna pruriens and Terminalia bellirica.* The quantity of the extracted genomic DNA was measured and tested at 260 nm using Nanodrop® ND-1000 spectrophotometer and the quality of DNA was determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60 V. Genomic DNA extraction gave original and real DNA with amplifiable and usable amount for all the powder medicine tested. Sufficient amount of DNA was obtained from the powder medicines, but no result found in electrophoresis as the DNA size was too large.

Keywords:* *Genomic DNA extraction; Electrophoresis; Plant

Introduction

Kabiraj are people who practice Ayurveda and Unani medicine in India and Bangladesh. They are also called Vaidhya. DNA is an almost universal genetic material and those 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 [1,2]. 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 [4]. Plants contain an array of secondary metabolites.

Kobiraji medicines which were used as sample in DNA extraction

Mucunapruriens (Alkushi)

- Supports a healthy central and peripheral nervous system.

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

***Terminalia bellirica (Bahera)* fruits:** Laxative, astringent, anthelmintic and antipyretic.

***Oroxylum indicum* seed:** The Oroxylumindicum seed is used in the traditional Indian ayurvedic medicine pains in joints or rheumatism.

***Chrysogenum aurium* seed:** Fenugreek has been known since ancient times as an herbal galactagogue.

Abroma augusta: Used in uterine disorders, dysmenorrhea, arthritic pain, rheumatism and diabetes.

Syzygium cumini: **The major function of Syzygiumcumini 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):** Used in anxiety, depression, stress. Lowers cholesterol and sugar in blood.

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 [5].

Materials and Methods

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 [6].

Harvesting of fruits

The powdered medicines were preserved at room temperature until use.

Reagent

Tris-HCl, EDTA, Triton X-100 and 5 µl RNase A (10 mg/ml) was added into the sample tube and mixed by vortexing. Tris-HCl, EDTA and Triton X-100 are used for the purpose of breaking open cells. Tris-HCl, EDTA and Triton X-100 are added to break up membrane structures [7].

Procedure

Tissue dissociation: 50 mg of powder medicine was grinded.

Lysis: The mixture was incubated at 65°C for 10 min to weaken the cell walls and to lyse. 100 µl lysis buffer was added and mixed by vortexing. The closed tube was placed in the ice. For cell lysis, a filter column was placed in a 2 ml Collection Tube. The filter column was centrifuged. The filter column was discarded and clarified supernatant was carefully transferred [8].

DNA binding: A GD Column was used as 2 ml Collection Tube.
700 µl was taken and was centrifuged. 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 [9].

Washing: 400 µl of wash buffer was added into the Gd Column. Again, it was centrifuged. The flow through was discarded. 600 µl of Wash Buffer was added into the Gd Column. It was centrifuged. The flow through was discarded and returned into the 2 ml Collection tube. It was centrifuged again for 3 min at full speed to dry the Column matrix.

DNA elution: The elution volume was reduced, the elution step was repeated. The composition of Elution Buffer was: 10 mM Tris-Cl, pH 8.5.

Results and Discussion

Quantity of extracted fruits

DNA extraction was according to Geneaid's ISO-certified quality management system (TABLES 1-5) [10,11].

TABLE 1. **Quantity of Extracted fruits using NanoDrop machine.**

Plant species	DNA yield
Phyllanthusemblica (Amloki) Sample 1	240 ng/µl
Phyllanthusemblica (Amloki) Sample 2	181 ng/µl
Phyllanthusemblica(Amloki) Sample 3	76.2 ng/µl
Phyllanthusemblica (Amloki) Sample 4	87.3 ng/µl
Withaniasomnifera (Ashwagandha) Sample 1	88.1 ng/µl
Withaniasomnifera (Ashwagandha) Sample 2	53.6 ng/µl
Withaniasomnifera (Ashwagandha) Sample 3	18.5 ng/µl
Withaniasomnifera (Ashwagandha) Sample 4	33.6 ng/µl

TABLE 2. **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

TABLE 3. **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

TABLE 4. **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

TABLE 5. **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

Sufficient amount of DNA was obtained from the powder medicines, but no result found in electrophoresis as the DNA size was too large (TABLES 6 and 7).

TABLE 6. **No results found in electrophoresis as the DNA size was too large.**

Scientific name of Alkushi (*Mucunapruriens*)	**Nanogram/microlitre**
Sample-1	106.5
Sample-2	574
Sample-3	45.1
Sample-4	623.9

TABLE 7. **No results found in electrophoresis as the DNA size was too large.**

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

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*.

Conclusion

The customized plant genomic DNA extraction method extracted eight different ayurvedic medicine powder such as *Phyllanthus emblica*, *Withania somnifera*, *Abroma augusta*, *Syzygium cumini*, *Oroxylum indicum*, *Chrysogenum aurium*, *Mucuna pruriens*, *Terminalia bellirica*. The quantity of the extracted genomic DNA was measured and tested at 260 nm using Nanodrop® ND-1000 spectrophotometer and the quality of DNA was determined by the horizontal agarose gel electrophoresis using 1% agarose in TBE buffer at constant voltage of 60 V. Genomic DNA extraction gave original and real DNA with amplifiable and usable amount for all the powder medicine tested. Sufficient amount of DNA was obtained from the powder medicines, but no result found in electrophoresis as the DNA size was too large.

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