Journal of
Plant Science & Research

Open Science Publications

Volume 8, Issue 2 - 2021

www.opensciencepublications.com

# Protocol Establishment-As Well Defined Rule a and Step for Extreme Good DNA Extraction, Qualification, Quantification and *Amplification Efficiency from Leaves and Fruit Peel of Phyllanthus Emblica, Tamarindus Indica, Cambopogon Citrates, Borhevia Diffusa* and *Bryophyllum Pinnatum*- Containing Sour Taste

## Research Article

**Ladani MR and Parabia FM***

*Ashok and Rita Patel Institute of Integrated Biotechnology & Allied Sciences, New Vallabhvidyanaga-388120*

***Corresponding author:** Parabia FM, Ashok and Rita Patel Institute of Integrated Biotechnology & Allied Sciences, New Vallabhvidyanaga-388120 Gujarat, India; Tel: 9879578029; Email: farzin_parabia@yahoo.co.in



**Article Information:** **Submission:** 09/06/2021; **Accepted:** 08/07/2021; **Published:** 10/07/2021

**Abstract**

DNA extraction from plant is crucial step in molecular genetics and for this CTAB protocol is developed which is cationic detergent and CTAB can't be denatured at higher temperature 60°C to 65°C that is one of the advantage of using CTAB in plant DNA extraction. Due to presence of complex polysaccharides like polyglycosides and other secondary metabolites like tannins, alkaloids and polypheols in plant tissues it is difficult to quantify and qualify pure plant DNA though they are also precipitated with isolated plant DNA. Therefore day by day certain modifications are carried out in CTAB protocol depending upon polysaccharide and other polyphenol composition of individual plant tissue material. Present study explains standardization procedure for quantification and qualification of DNA from leaves of *Phyllanthus emblica, Tamarindus indica, Cambopogon citrates, Borhevia diffusa and Bryophyllum pinnatum*. Leaf tissues of these plants contain high amount of polyphenols, secondary metabolites like tannins, flavanoids and alkaloids and complex polysaccharides like polyglycosides and more or less amount of Ascorbic acid and citric acid. DNA extraction from Ascorbic acid or Vitamin C rich tissues is bit difficult due to its lower pH that leads to degradation of DNA. So to deal this issue we standardize one small protocol that don't need use of Rnase enzyme and liquid nitrogen but require frequent pH monitoring during incubation step. DNA isolated from this plant by this protocol is of good quality and quantity and are effective enough to amplify in multiple copies.

**Keywords:** *Phyllanthus Emblica; Tamarindus Indica; Cambopogon Citrates; Borhevia Diffusa; Bryophyllum pinnatum*

## Introduction

Isolation of good quality of DNA is an important step for study of molecular genetics of an organism. A hike came in the development of CTAB protocol for DNA extraction in plants [1]. CTAB is cationic detergent and is adaptable in high salt concentration in plant material. It disrupts cell membrane and release the DNA. Alternatively plant tissues are rich in complex polysaccharides like polyglycosides and secondary metabolites like polyphenols, alkaloids, tannins etc. These metabolites co-precipitate with DNA and cause interference during


ISSN: 2349-2805

isolation procedure. The presence of these compounds shows the difficulty due to long and time-consuming extraction procedures those do not give good quality and quantity of DNA.

There are number of protocols developed for isolation and purification of DNA from polyphenols, polysaccharides and secondary metabolites rich plant materials but degradation of DNA is one of the major problems in some plant species containing low pH in their leaf tissues which generates inaccurate bands or no amplification of DNA in PCR based studies. There are many problems came across while isolating high molecular weight DNA from several plant species which has been accounted for the degradation of DNA due to endonuclease activity, co-isolation of viscous polysaccharides and presence of certain inhibitor compounds with directly or indirectly hinders enzymetic reactions [2]. Polyphenols are powerful oxidizing agents that reduce the quality and yield of DNA [3].

Lot many protocols have been developed to isolate high quality DNA from numerous plant species [4-9]. Degradation of DNA was reported as the major problem during isolation [10]. But the problem regarding good quality of DNA remains as it is. Earlier an efficient protocol was developed for isolation of good quality of DNA from leaves of *Phyllanthus emblica* without degradation of DNA by stabilizing pH at different steps of isolation. But still quality was the problem. More additionally this DNA was used for other downstream applications like DNA fingerprinting and chloroplast genome analysis from different germplasm of *Phyllanthus emblica* [11].

In the present study we established modified easiest and quick protocol for isolation of good quality and quantity of DNA from the leaves of *Phyllanthus emblica*, *Tamarindus indica and Cambopogon*citrates which contains more or less amount of ascorbic acid in their leaf tissue them gives them sour taste. Leaves of T.*indica*contain highest amount of vitamin C compared to any other plant species without the use of Phenol, liquid nitrogen and other enzymes like Rnase and protenase K. The isolated DNA was also PCR amplified by chloroplast gene.

## Materials and Methods

### Materials

Leaves fruits samples of *Phyllanthus emblica*and leaves of *Tamarindus indica*and *Cambopogon citrates*were collected from the botanical garden of Anand Agriculture University, Gujarat and Store at -20$^{0}$C before using.

### Preparation of collected sample

Fresh leaves were collected and then after washed properly with sterilized distilled water, properly air dried at room temperature and then quickly stored at avoid -20$^{\circ}$C to avoid nuclease contamination.

### Genomic DNA Isolation:

Total genomic DNA was extracted by means of certain modifications in CTAB protocol.

### Preparation of Reagents:

**1.**CTAB extraction buffer:

20mM EDTA, 100mM Tris-HCl, 1.14M NaCl, 3%w/v CTAB, pH 8.0

**2.**0.1N NaOH

**3.**0.2% β-mercaptoethanol

**4.**0.1%w/v PVP

0.1gram PVP dissolved in 100ml of Distilled water.

**5.**Chloroform: isoamylalcohol (24:1)

48ml of chloroform and 2ml of isoamylalcohol mixed properly in 50 ml of distilled water make final volume of 100ml.

**6.**5M potassium acetate

**7.**3M sodium acetate

**8.**Ice cold Isopropanol 9.1x TE buffer:

9. 10mMTris-HCL, 1mM EDTA(pH 8.0m).

**10.** 10xTBE stock buffer(100ml)

10.8gram tris base, 5.5gram boric acid and 4ml of 0.5M EDTA and make final volume of 100ml by distilled water.

**11.** 1x TBE buffer(100ml)

Add 10ml of 10x TBE buffer in 90 ml of distilled water to make final volume of 100ml.

**12.** Ethyl Bromide Dye (EtBr) stock

5µgms are dissolved in 1ml of distilled water.

**13.** 0.7% Agarose

0.7grams of agarose powder dissolved in 100ml of distilled water.

14. 1x TE bubber:

10mMTris-HCL, 1mM EDTA(pH 8.0m)

## Protocol

0.2 gram of leaves were cut into small pieces and ground thoroughly into prechilled mortle and pestle without the use of liquid nitrogen under extraction buffer.β-mercatopethanol and PVP were added during the process of grinding.

Tubes were incubated at 65$^{\circ}$C for 30-35 minutes. pH was measured during 5-10 minutes interval and adjusted with the help of 0.1N NaOH with gentle mixing of samples with regular time intervals.

Then tubes were centrifuged at 8000rpm for 10 minutes at room temperature. Supernatant was collected in a new tube and 250µl of 5M potassium acetate was added. Tubes were incubated at -20C for 1hr. Then tubes were centrifuged at 8000rpm for 10 minutes at room temperature.

Supernatant was collected in a new tube and equal amount of Chloroform: isoamylalcohol (24:1) was added and centrifuged at 8000rpm for 10 minutes. Supernatant was collected in another tube and again repeated Chloroform: Isoamyalcohol step.1/10th volume of 3M Na-acetate was added in the final supernatant and half the volume of isopropanol was added to precipitate the DNA.

In the last step tubes were centrifuged at 10000rpm for 15 minutes. Pellet was dissolved in 50µl 1xTE buffer. There was intermittent pH monitoring during each step.


**Citation:** Ladani MR, Parabia FM. Protocol Establishment-As Well Defined Rule a and Step for Extreme Good DNA Extraction, Qualification, Quantification and Amplification Efficiency from Leaves and Fruit Peel of *Phyllanthus Emblica, Tamarindus Indica, Cambopogon Citrates, Borhevia Diffusa* and *Bryophyllum Pinnatum*- Containing Sour Taste. J Plant Sci Res. 2021;8(2): 208

Above protocol was repeated two times for better results. Then above protocol was also tried one time for extraction of DNA from surface peel of P.*emblica* fruits which are highly acidic.

### Quality of Isolated DNA

Quality of isolated DNA by this method from leaves of P.*emlica*, T.*indica*, C.*citrates*, B.*pinnatum*and B.*diffusa* and fruit peels from P.*emblica* was checked two times and loading10 µl of sample in each time on 0.7%agarose gel. This 10µl volume contained 7µl of diluted sample and 3µl of EtBrdye(5µgm/ml).

### Quantification of DNA

The yield of isolated DNA was determined by taking nanodrop at 260nm and 280 nm for measurement of 260/230 and 260/280 ratio to check purity of isolated DNA.

### Amplification of isolated DNA

The isolated genomic DNA was further analyzed for its analytical applications by performing polymerase chain reaction to just check quality of isolated DNA from chloroplast gene rbcL (650bp) and ITS gene(450bp) with an initial denaturation 95°C for 3minutes.

It was followed by 35cycles of 1min denaturation at 95°C, 30s annealing at 54°C and 1min extension at 72°C, with a final extension of 10minute at 72°C.96 well thermal cycler (Applied Biosystems).

PCR reaction mixture containing of mastermix 20µl (Contains: 10X Taq buffer, 2mM MgCl2,0.4mM dNTP mix and 3 unit Taq DNA polymerase (Blakbio).

## Results

### Quality of Isolated DNA

DNA extracted by this method didn't contain contamination of RNA, polyphenols, complex polysaccharides like glycosides and ascorbic acid without giving treatment of Rnase. In this way DNA from Fresh leaves of *Phyllanthus emblica, Tamarindus indica and Cambopogon citrates, Bryophyllum pinnatum* and*Borhevia diffusa* and fruits peels for P.*emplica* containing low pH was extracted.

Contamination of polyphenol was removed by use of PVP (polyvinylpyrolidon) and contamination of polysaccharides was removed by addition of salt(1.4M NaCl) containing extraction buffer and pH was adjusted with the help of 0.1N NaOH.

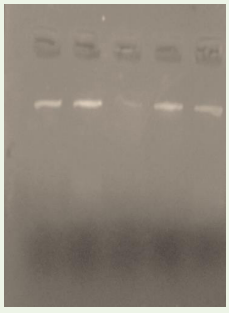

**Figure 1:** shows 0.7% Agarose gel of isolated DNA from Chilled leaves of from *P. emblica*,*T.* indica, C.*citrates, B.pinnatum, B.diffusa* from above protocol 1st time.

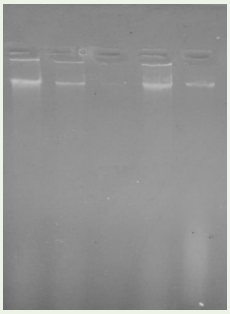

**Figure 2:** shows 0.7% Agarose gel of isolated DNA from Chilled leaves of from *P. emblica,T. indica, C.citratus, B.pinnatum, B.diffusa from* above protocol 2nd time.

In the present study we maintained pH 8.0 at the time of incubation as well as at the time of grinding by adding 0.1N NaOH during 10 to 15 minutes time interval at the time of incubation and observed a good quality of DNA while running it on 0.7% Agarose gel.

Good quality of DNA was observed in gel shown in Fighre1-3 isolated from leaves of P. *emblica*, T. *indica*, C.*citrates*, B.*pinnatum*, B.*diffusa* two times (Figures 1,2) and from Fruit peels of P.*emblica* one time (Figure-3).

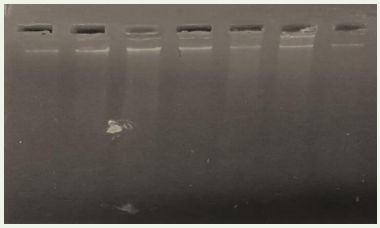

**Figure 3:** all lanes of figure-3 shows 0.7% Agarose gel of isolated DNA from highly acidic fruit peel of *P. emblica*.

### Quantity of isolated DNA

Quantity of 1st time isolated DNA from leaves 260/280 Ratio: It is crucial to measure 260/280 nm ratio of isolated DNA especially when DNA isolation is under intermittent pH monitoring. Because if there will be mall change in pH that will lead to variations in 260/280 ratio. Generally standard values of this ratio for good quality DNA is 1.8-1.9.However high 260/280 ratio is not much problematic. But if ration is greater than 1.9 then it is problematic and it indicates contamination of RNA and then in that case needs treatment of Rnase during isolation. Very much high value sometimes indicates contamination of protein.

In Figure 4 Bar graph indicates that that 260/280 ratio of all quantified plant. DNA samples are not more than 1.9.So ratio is not so much higher than standard values. So 1st time quantified. DNA from this protocol is RNA free and is not quantified in acidic pH that means during isolation pH was maintained at 8.

### 260/230 Ratio

Generally standard 260/230 ratio of quantified DNA is in between 2-2.5.Sometimes these values can reach upto 3.But values more than



**Citation:** Ladani MR, Parabia FM. Protocol Establishment-As Well Defined Rule a and Step for Extreme Good DNA Extraction, Qualification, Quantification and Amplification Efficiency from Leaves and Fruit Peel of *Phyllanthus Emblica, Tamarindus Indica, Cambopogon Citrates, Borhevia Diffusa* and *Bryophyllum Pinnatum*- Containing Sour Taste. J Plant Sci Res. 2021;8(2): 208

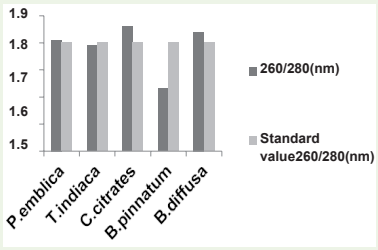


**Figure 4:** shows comparative 260/280nm ratio of1st time Quantified DNA of *P. emblica,T. indica, C.citrates, B. pinnatum, B.diffusa*.Horizonal Bar shows name ofsample plants and vertical bar shows values of 280/260 in nm.

3 will be problematic. So unusual values indicated contamination of reagents used during extraction procedure like phenol, chloroform and other polysaccharides present in leaf tissue sample. Such unusual value showing quantified DNA is difficult to amplify. So 1st time quantified DNA from this protocol shows nearer to standard 260/230 values that is not more than 3 that means Isolated Quantified DNA is Phenol free, chloroform free and also free from other polysaccharides.

In Figure-5 Bar graph indicates that 260/230 ratio of all quantified plant DNA samples are not more than 3.So ratio is not so much higher than standard values. So 1st time quantified DNA from this protocol is chloroform free, phenol free (if used) free and polysaccharides free. Because presence of polysaccharides contain high 260/230 ratio which is generally higher than 3.Such type of DNA needs further step of purification either manually or with help of kit.

## Quantity of 2nd time isolated DNA from leaves 260/280 Ratio

260/280 ratio was measured of 2nd time isolated DNA by this protocol for better standardization.

In Figure 6 Bar graph indicates that 260/280 ratio of all quantified plant DNA samples are not more than 1.9.So ratio is not so much higher than standard values. So again 2nd time quantified DNA from this protocol is RNA free and is was not quantified in acidic pH that means during isolation pH was maintained at 8.

In Figure7 Bar graph indicates that 260/230 ratio of all quantified plant DNA samples are not more than 3.So ratio is not so much higher than standard values. So 2nd time quantified DNA from this protocol is chloroform free, phenol free (if used) free and polysaccharides free. Because presence of polysaccharides contain high 260/230 ratio which is generally higher than 3.Such type of DNA needs further step of purification either manually of with help of kit.

In Figure 8 Bar graph indicates that 260/280 ratio of quantified plant isolated DNA samples from highly acidic fruit peel of P.*emlica* replicates, These values are not more than 3.5, In some replicates (vertical Bar-1, 2, 3, 7) values are nearer to standard value that is 1.8.So it is good indication of RNA free DNA and rest of replicates indicates more or less contamination of protein. But we successfully isolated good quantity of DNA from acidic fruit peel also.

In Figure 9 Bar graph indicates that 260/230 ratio of quantified plant isolated DNA samples from highly acidic fruit peel of

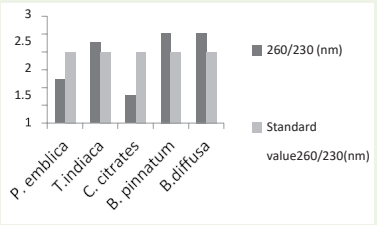


**Figure 7:** shows comparative 260/230nmratio of 2nd time Quantified DNA of *P. emblica,T. indica, C.citrates, B. pinnatum, B.diffusa*. Horizonal Bar shows name of sample plants and vertical bar shows values of 280/230 in nm.

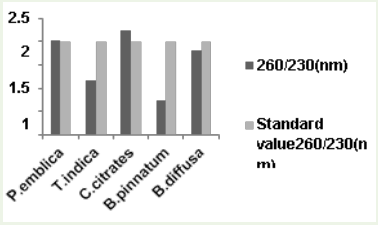


**Figure 5:** shows comparative 260/230nm ratio of 1st time Quantified DNA of *P. emblica,T. indica, C.citrates, B. pinnatum, B.diffusa*.Horizonal Bar shows name of sample plants and vertical bar shows values of 280/230 in nm.

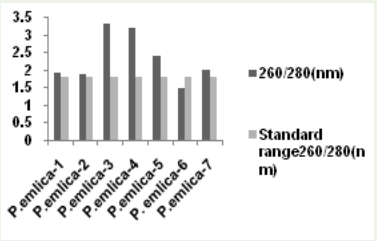


**Figure 8:** shows comparative 260/280nm ratio ofone time Quantified DNA from fruit peels of *P. emblica.* Horizontal bar shows all replicates of *P.emblica* fruit.

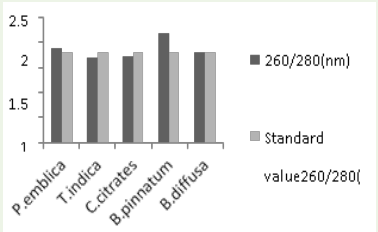


**Figure 6:** shows comparative 260/280nm ratio of 2nd time Quantified DNA of *P. emblica,T. indica, C.citrates*.

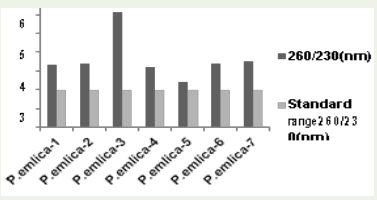


**Figure 9:** shows comparative 260/230nm ratio of one time Quantified DNA from fruit peels of *P. emblica*.Horizontal bar shows all replicates of *P.emblica* fruit peels.



**Citation:** Ladani MR, Parabia FM. Protocol Establishment-As Well Defined Rule a and Step for Extreme Good DNA Extraction, Qualification, Quantification and Amplification Efficiency from Leaves and Fruit Peel of *Phyllanthus Emblica, Tamarindus Indica, Cambopogon Citrates, Borhevia Diffusa* and *Bryophyllum Pinnatum*- Containing Sour Taste. J Plant Sci Res. 2021;8(2): 208

P.*emlica*replicates. Among all seven replicates in some replicates value reach upto 6.5 where as in some replicates it is nearer to standard value that is 2 (vertical bar 4 and 5).Sometimes high 260/230 ratio is not due to contamination of reagents and other handling errors but it is due to presence of complex polysaccharides like polyglycosides. We check this quantified DNA for PCR amplification which is well isolated manually, but for further downstream processing like DNA fingerprinting use of column is required.

## Amplification of isolated DNA

Just to check amplification efficiency of well isolated and well quantified DNA that was PCR(Thermo)amplified by chloroplast rbcL gene(650bp).PCR amplification was carried out just one time and amplified DNA was run on 2% agarose gel.

In Figure 10 lane-1 shows 100bp Standard DNA marker and from lane-2 to lane-6 shows PCR amplified DNA of P. *emblica*,T. *indica*, C.*citrates*, B. *pinnatum*, B.*diffusa*. In all lanes 3µl of amplified DNA of all samples as well as marker was loaded.

Amplification efficacy of well isolated and well quantified one time from fruit peels of P.*emblica* was also checked and only two replicates containing good 260/280 and 260/230 ratio were selected for PCR(Thermo) amplification and they are amplified by ITS (450bp)gene just to check amplification efficiency.

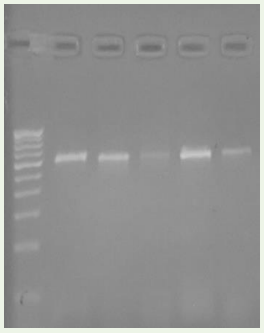

**Figure 10:** shows 2% agarose gel of PCR amplified DNA with *rbcL*(650bp) from *P.emblica*, *T.*indica, C. *citrates,B. pinnatum and B.diffusa.Lane-1 shows 100bp*.

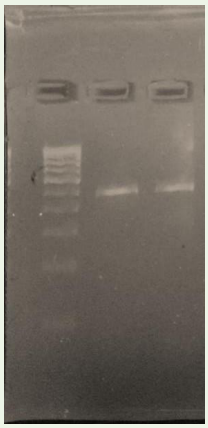

**Figure 11:** shows 2% agarose gel of PCR amplified DNA with ITS(650bp) gene from fruit peel of P.emblica..Lane-1 shows 100bp marker DNA.

## Discussion

It is difficult to extract genomic DNA from plants containing high amount of polyphenol and polysaccharides. These polysaccharides produce complex form of DNA which becomes resistant from certain modifying enzymes like Restriction endonucleases, DNA polymerase (Taq polymerases) and DNA ligase etc[16].

High quality and quantity of DNA are necessary for successful PCR amplification and other downstream applications like DNA fingerprinting. There are various protocols available for isolation of good high quality of DNA from plant tissues [12-19].But none of them were applicable to plants containing high acidic pH.

Earlier DNA was isolated from the leaves of *Phyllanthus emblica*but still quality and quantity was a problem [20].They isolated DNA from three different maturity levels of leaves from different varieties of *Phyllanthus emblica*by adjusting pH at different steps of isolation and pH was adjusted in between 7.0-7.66.

In present study by this standardized protocol quantification values of isolated DNA shown in Table 1 both 1st time and 2nd time indicates good results that is nearer to standard value that is 1.8 and free from contamination of Rnase. pH-8 is adjusted very much carefully.260/280 ratio nearer to 1.8 indicates that DNA is not degraded and it is well quantified.

**Table 1:** shows Quantification values in form of260/280 ratio of 1st time as well as 2nd time isolated DNA

| Samples | 260/280 ratio (nm) | Standard 260/280 value |
|---|---|---|
| *P.emlica-1st time* | 1.81 | 1.8 |
| *P.emblica-2nd time* | 1.88 | 1.8 |
| *T.indica-1st time* | 1.79 | 1.8 |
| *T.indica-2nd time* | 1.70 | 1.8 |
| *C.citrates-1st time* | 1.86 | 1.8 |
| *C.citrates-2nd time* | 1.72 | 1.8 |
| *B.pinnatum-1st time* | 1.63 | 1.8 |
| *B.pinnatum-2nd time* | 2.18 | 1.8 |
| *B.diffusa-1st time* | 1.84 | 1.8 |
| *B.diffusa-2nd time* | 1.81 | 1.8 |

**Table 2:** shows Quantification values in form of260/230 ratio of 1st time as well as 2nd time isolated DNA.

| Samples | 260/230 ratio (nm) | Standard 260/280 value |
|---|---|---|
| P.*emlica*-1st time | 2.03 | 2 |
| P.*emblica*-2nd time | 1.22 | 2 |
| T.*indica*-1st time | 1.15 | 2 |
| T.*indica*-2nd time | 2.265 | 2 |
| C.*citrates*-1st time | 2.237 | 2 |
| C.*citrates*-2nd time | 0.773 | 2 |
| B.*pinnatum*-1st time | 0.723 | 2 |
| B.*pinnatum*-2nd time | 2.527 | 2 |
| B.*diffusa*-1st time | 1.81 | 2 |
| B.*diffusa*-2nd time | 2.527 | 2 |



**Citation:** Ladani MR, Parabia FM. Protocol Establishment-As Well Defined Rule a and Step for Extreme Good DNA Extraction, Qualification, Quantification and Amplification Efficiency from Leaves and Fruit Peel of *Phyllanthus Emblica, Tamarindus Indica, Cambopogon Citrates, Borhevia Diffusa* and *Bryophyllum Pinnatum*- Containing Sour Taste. J Plant Sci Res. 2021;8(2): 208

**Table 3:** shows Quantification values in form of 260/280 ratio of one time isolated DNA from fruit peels of*P.emblica*.

| Samples | 260/230 ratio (nm) | Standard 260/280 value |
|---|---|---|
| *P.emlica-1st time* | 2.03 | 2 |
| *P.emblica-2nd time* | 1.22 | 2 |
| *T.indica-1st time* | 1.15 | 2 |
| *T.indica-2nd time* | 2.265 | 2 |
| *C.citrates-1st time* | 2.237 | 2 |
| *C.citrates-2nd time* | 0.773 | 2 |
| *B.pinnatum-1st time* | 0.723 | 2 |
| *B.pinnatum-2nd time* | 2.527 | 2 |
| *B.diffusa-1st time* | 1.81 | 2 |
| *B.diffusa-2nd time* | 2.527 | 2 |

**Table 4:** shows Quantification values in form of 260/230 ratio of one time isolated DNA from fruit peels of*P.emblica*.

| ***P.emblica* replicates** | **260/230 ratio (nm)** | **Standard 260/230 value** |
|---|---|---|
| *P.emlica-1* | 3.344 | 2 |
| *P.emlica-2* | 3.417 | 2 |
| *P.emlica-3* | 6.118 | 2 |
| *P.emlica-4* | 3.207 | 2 |
| *P.emlica-5* | 2.426 | 2 |

## Conclusion

In the present research work modified CTAB protocol was successfully established and worked on DNA extraction from pre chilled leaves of *Phyllanthus emblica, Tamarindus indica, Cambopogon citrates, Borhevia diffusa and Bryophyllum pinnatum.* Leaf tissues of this plant contain more or less amount of ascorbic acid and due to present of vitamin C reach contents it was difficult to isolate good quality of DNA and quantify them. Problem is solved by this protocol without use of liquid nitrogen, Rnase enzyme and protenase K enzyme but this protocol require intermittent pH monitoring at 8 at every step and especially during step of incubation of 30 minutes at 60°C.So this protocol is suitable for plant leaves as well as fruit peels which are acidic in nature and bit sour in taste and it uncover difficulties of qualification, quantification and amplification of DNA from such type of tissue materials.

## Acknowledgement

Author is thankful to God and Dr Farzin M Parabia for his constant support.Author is also thankful to all teaching and non teaching members of ARIBAS college and beloved friends and sisters, brothers and parents for their love and support.

Author is also thankful to all teaching and non teaching members of ARIBAS college and beloved friends for their love and support.

## References

1. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA(1980). Nucleic Acids Res 8: 4321-4325.
2. Weishing K, Nybom H, Wolff K, Meyer (1995) DNA isolation and purification. In:DNA fingerprinting in plants and fungi, CRC Press, Boca Raton Florida 44-59.
3. Porebski S, Bailey LG, Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep15: 8-15.
4. Deshmukh VP, Thakare PV, Chaudhari US, Gawande PA (2007) A Simple method for isolation of genomic DNA from fresh and dry leaves of Terminalia arjuna (Roxb.)Wight and Argot. E J Biotech. 10: 468-472.
5. Chakraborti D, Sarkar A, Gupta S, Das S (2006) Small and large scale genomic DNA isolation protocol for chickpea (Cicer arietinum L.), suitable for molecular marker and transgenic analyses. African J Biotec 5: 585-589.
6. Fu X, Deng S, Su G, Zeng Q, Shi S (2004). Isolating high quality RNA from mangroves without liquid nitrogen.Plant Mol Biol Re 22: 197-197.
7. Aras S, Duran A, Yenilmez G (2003) Isolation of DNA for RAPD analysis from dry leaf material of some Hesperis L. specimens. Plant Mol Biol Rep 21: 461-461.
8. Mogg RJ and Bond JM (2003) A cheap, reliable and rapid method of extracting high-quality DNA from plants. Mol. Ecol. Notes 3: 666-668.
9. Mace ES, Buhariwalla HK, Crouch JH (2003) A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plants Mol Biol Rep. 21: 459-459.
10. Sharma R, Mahla HK, Mohapatra T, Bhargava SC, Sharma MM (2003) Isolating plant genomic DNA without liquid nitrogen.Plant Mol Biol Rep 21: 43-50.
11. Nagarajan S, Steephen M, Murugan K, Nair RR, Sethuraman T (2011). Improved protocol for isolation of genomic DNA from leaf tissues of Phyllanthus emblica Gaertn. Iranian Journal of Biotechnology 9: 4.
12. Saghai Maroof MA, Soliman K, Torgensen RA and Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley:Mendelian inheritance, choromosomal location,and population dynamics. Proc Natl Acad Sci USA 81: 8014-8018.
13. Pirttilä AM, Hirsikorpi M, Kämäräinen T, Jaakola L, et al. (2001). DNA isolation methods for medicinal and aromatic plants. Plant Mol Biol Rep 19: 273.
14. Scott KD, Playford J (1996) DNA extraction technique for PCR in rain forest plant species.Biotechniques 20: 974- 979.
15. Sharma KK, Lavanya M and Anjaiah V (2000) A method for isolation and purification of peanut genomic DNA suitable for analytical applications. Plant Mol Biol Rep 18: 393.
16. Pirttilä AM, Hirsikorpi M, Kämäräinen T, Jaakola L, et al. (2001) DNA isolation methods for medicinal and aromatic plants. Plant MolBiol Rep 19: 273.
17. Drábková L, Kirschner J and Vlcek C (2002) Comparison of seven DNA extraction and amplification protocols in historical herbarium specimens of Juncaceae Plant MolBiol Rep 20: 161-175.
18. Mogg RJ and Bond JM (2003) A cheap reliable and rapid method of extracting high-quality DNA from plants. Mol. Ecol. Notes 3: 666-668.
19. Haymes KM, Ibrahim IA, Mischke S, Scott DL, et al.(2004) Rapid isolation of DNA from chocolate and date palm tree crops. JAgrFood Chem 52: 5456-5462.
20. Nagarajan S, Steephen M, Murugan K, Nair RR, Sethuraman T (2011) Improved protocol for isolation of genomic DNA from leaf tissues of Phyllanthus emblica Gaertn. Iranian Journal of Biotechnology 9: 4.



**Citation:** Ladani MR, Parabia FM. Protocol Establishment-As Well Defined Rule a and Step for Extreme Good DNA Extraction, Qualification, Quantification and Amplification Efficiency from Leaves and Fruit Peel of *Phyllanthus Emblica, Tamarindus Indica, Cambopogon Citrates, Borhevia Diffusa* and *Bryophyllum Pinnatum*- Containing Sour Taste. J Plant Sci Res. 2021;8(2): 208