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# An efficient nucleic acids extraction protocol for *Elettaria cardamomum*

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ABSTRACT

*Elettaria cardamomum* is an economically important spice crop. Genomic analysis of cardamom is often faced with the limitation of inefficient nucleic acid extraction due to its high content of polyphenols and polysaccharides. In this study, a highly efficient DNA and RNA extraction protocol for cryopreserved samples from small cardamom plant was developed with modification in the CTAB and SDS method for DNA and RNA, respectively, with the inclusion of 2% ascorbic acid. DNA isolated by this method is highly suitable for PCR, restriction digestion and RAPD analysis. The RNA extraction method described here represent the presence of plant mRNA, small RNAs and viral RNA and, the isolated RNA proved amenable for RT-PCR and amplification of small and viral RNA. Nucleic acids extraction protocol developed here will be useful to develop genetic marker for cardamom, to clone cardamom genes, small RNAs and cardamom infecting viral genes and to perform gene expression and small RNA analysis.

## 1. Introduction

*Elettaria cardamomum* Maton is a perennial-herbaceous monocotyledonous plant recognized as 'Queen of Spices'. Cardamom is one of the most economically important spice crops which contains high level of polysaccharides and polyphenols (Bhatti et al., 2010). The presence of these secondary metabolites hinders the extraction of good quality DNA and RNA from cardamom tissues by employing routine protocols. Obtaining satisfactory level of quality and quantity of nucleic acids is found to be more challenging for downstream applications such as DNA restriction, amplification, cloning, Next generation sequencing of transcriptomes and Real Time PCR (RT-PCR) analysis.

Available methods for the nucleic acids extraction are applicable for model species and use of these protocols for other non-model species is often limited (Yockteng et al., 2013). The composition of the biomolecules like polysaccharides, polyphenols varies between the species may not allow an optimal nucleic acid yield with single protocol. Even the closely related species may require different isolation protocols. Accordingly, modification of composition as well as quantity of the different constituents in the extraction buffer needs to be standardized for each species (Devi et al., 2013).

Keeping in view the above facts, we have standardised a simple, rapid and cost-efficient nucleic acids extraction protocol for the cardamom plant. The DNA and RNA extracted by employing the worked-out methods are suitable for restriction digestion, RAPD, regular PCR, Real Time PCR and small RNA analysis and amplification of viral genes.

## 2. Material and methods

### 2.1. Sample collection

Healthy and mosaic symptomatic leaves of *Elettaria cardamom* were collected from Coorg district, Karnataka, India. The leaf samples were rinsed briefly with running tap water, followed by distilled water and then stored at −80 °C freezer until further processing.

### 2.2. DNA extraction

The cryopreserved leaf sample (100 mg) of different cardamom plants (A, B & C) was ground into a fine powder with 1 mg of polyvinylpyrrolidone (PVP) salt using pre-chilled mortar-pestle and liquid nitrogen. 1 ml of pre-heated (60 °C) extraction buffer (100 mM Tris HCl (pH 8.0), 50 mM EDTA (pH 8.0), 2 M NaCl, 2% CTAB and 2% ascorbic acid) was added to the powder and ground again. Subsequently, the tubes were incubated at 60 °C for 20 min. The tubes were gently

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inverted for every 5 min and centrifuged at 12,000 rpm for 10 min. After centrifugation, the supernatant was transferred to a microfuge tube and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The contents of the tubes were vigorously mixed and then centrifuged at 12,000 rpm for 10 min. This step was repeated twice. Two volumes of cold isopropanol was added to the aqueous phase and incubated at −20 °C freezer for 1 h. The tubes were then centrifuged at 12,000 rpm for 10 min and then the pellet was washed with 70% ethanol. Pellet was air dried and dissolved in 30 µl of TE buffer containing RNase A. The concentration and purity of DNA extracted from the frozen cardamom sample was quantified using Nanodrop (Thermo Fisher Scientific).

### 2.3. PCR

PCR mix prepared with 1X PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 10 pmols of H3 forward and reverse primers (Table S1), 50 ng of genomic DNA, and 1 U Taq polymerase (Sigma-Aldrich). The reaction mixture was run on a thermal cycler (Bio-Rad) as follows: Step 1-initial denaturation at 94 °C for 3 min, Step 2–94 °C for 1 min, 55 °C for 45 s, 72 °C for 45 s for 30 cycles and Step 3-final extension at 72 °C for 8 min. The PCR product was run on a 1% agarose gel stained with ethidium bromide.

### 2.4. Restriction analysis

Genomic DNA (500 ng) was digested overnight with 10 U of *Hind*III restriction enzyme under optimal temperature and buffer, following the manufacturers recommendations (Fermentas, USA). The digested DNA fragments were fractionated on 0.8% agarose.

### 2.5. RAPD

PCR was performed in a thermal cycler, with reaction mix (25 µl) containing 1X PCR buffer with 1.5 mM $MgCl_2$, 0.25 mM dNTP, 50 ng of cardamom genomic DNA, 10 µM RAPD primers: OPAL-12, OPN-02, and OPI-07 (Sigma Pvt Ltd) (Table S1) and 1U Taq polymerase (Sigma) were used. PCR conditions employed for the amplification was as follows: Initial denaturation at 94 °C for 3 min, cyclic conditions with 40 cycles of 94 °C for 1 min, 37 °C for 1 min, 72 °C for 2 min and final extension at 72 °C for 6 min. The PCR product was loaded in 0.8% agarose gel stained with ethidium bromide.

### 2.6. RNA extraction and cDNA synthesis

Mortar, pestle, microfuge tubes and tips used for RNA extraction was rinsed with diethyl pyrocarbonate (DEPC) treated water, dried and autoclaved. 100 mg of cardamom leaf samples of three different plants, with 1 mg of PVP salt was ground into a fine powder using a pre-chilled mortar and pestle with liquid nitrogen. Immediately, 1 ml of pre-heated (60 °C) extraction buffer (100 mM Tris-HCl - pH 8, 50 mM EDTA- pH 8, 2 M NaCl, 2% SDS, 2% ascorbic acid and 1% β- mercaptoethanol) were added and ground further. Samples were incubated at room temperature for 2 min. Equal volume of acid phenol (pH 4.3 saturated with 0.1 M citrate buffer) (Sigma) was added and the contents of the tubes were vigorously mixed and incubated at room temperature for 10 min. The samples were then centrifuged at 12,000 rpm for 10 min at 4 °C. The aqueous phase was transferred to a fresh tube and 0.1 vol of 3 M sodium acetate and two volumes of isopropanol were added. The tubes were then incubated at −20 °C for 1 h and centrifuged at 12,000 rpm for 10 min at 4 °C. Supernatant was discarded and the pellet was washed twice with 70% ethanol. The dried pellets were resuspended in 25 µl of sterile DEPC treated water. RNA was run on 1% agarose gel and stained with ethidium bromide. As a control, RNA extraction from cardamom leaf samples was done with Trizol method.

The concentration and purity of the extracted RNA was quantified by Nano drop. Quality assessment of total RNA was performed using Agilent 2100 Bioanalyzer System with the Plant RNA Nano assay. The obtained data was generated as an electropherogram with the help of 2100 Expert software (Agilent -version B.02.08) and compared with the reference RNA sample. The parameters such as RNA area, RNA concentration, rRNA ratio 25 S/18 S and RNA integrity number (RIN) were recorded.

DNase treatment was performed by treating 1 µg RNA with 1X DNase buffer and 1U/µl of DNase I enzyme (Thermo Fisher Scientific). It was incubated at 37 °C for 30 min. Subsequent to incubation, 1 µl of 0.5 M EDTA was added to terminate the reaction. cDNA synthesis was performed using 1 µg of RNA, 0.5 µg of oligo dT primer, 1 X first-strand reaction buffer, 5 mM DTT, 0.5 mM dNTPs and 1 µl of Superscript II RT (200 U/µl) (Invitrogen) at 42 °C for 1 h.

### 2.7. Amplification of mRNA of Histone 4 gene

PCR mix included 1 X PCR buffer with 1.5 mM $MgCl_2$, 0.25 mM each dNTP, 10 pmols of H4 forward and reverse primers, 50 ng of cDNA, and 1 U Taq polymerase (Table S1). PCR programme was as follows: Step 1-Initial denaturation at 94 °C for 3 min, Step 2–94 °C for 1 min, 55 °C for 45 s, 72 °C for 45 s, Step 2 is repeated for 30 cycles and Step 3-Final extension at 72 °C for 8 min. The PCR product was run on a 1% agarose gel and stained with ethidium bromide.

### 2.8. Amplification of small RNA

The total RNA was treated with Poly (A) Polymerase to synthesize cDNA for low molecular weight RNA. cDNA was synthesized from 1 µg of RNA using 1X Poly A buffer, 0.25 mM dNTP mix, 0.25 mM ATP, 10 pmols oligodT primer, 100 U of Reverse transcriptase (New England Biolabs) and 2 U of Poly (A) Polymerase (New England Biolabs). Sample was incubated at 42 °C for 1 h and then enzyme was inactivated at 95 °C for 5 min.

In order to detect U87 and 5.8S rRNA, the PCR mixture was set with 10 pmols of Rno-U87-F and Rno-U87-R primers (Table S1) and 5.8 S rRNA (forward and reverse primer (Table S1)) primers, respectively. PCR was done at 95 °C for 3 min, 40 cycles of 95 °C for 10 s, 60 °C for 15 s, 72 °C for 10 s and a final extension at 72 °C for 5 min. Amplification of U87 and 5.8S rRNA were checked on 10% PAGE and 1.5% agarose gel, respectively. After electrophoresis, the gel was stained with ethidium bromide and then visualized through UV transilluminator.

### 2.9. Amplification of Potyviral RNA

Total RNA was extracted from 100 mg of *Cardamom mosaic virus* (CdMV) infected cardamom leaf samples. Viruses of Macluravirus contains poly A tail at their 3′ end of genome (Kondo and Fujita, 2012) and cDNA of CdMV was synthesized from the total RNA with oligo dT primer as described previously. CdMV genome specific primers, CI-6K2 forward and CI-6K2 reverse were used to amplify the viral gene fusion, CI-6K2 (Table S1). PCR was programmed with an initial denaturation for 3 min at 94 °C and 30 cycles of 94 °C for 1 min, 55 °C for 45 s 72 °C for 2 min and a final extension of 72 °C for 8 min.

### 2.10. Real-time PCR analysis

RT-PCR was performed for cDNA using 5.8 S rRNA primers. For PCR, 1 µg of cDNA was mixed with 12.5 µl of 2 X SYBR green master mix and 10 pmoles of 5.8 S rRNA forward and 5.8 S rRNA reverse primers (Table S1) in a final volume of 25 µl. The reactions were performed in triplicates with ABI (Applied Biosystems) 7500 Real time PCR system. A dissociation curve for the amplicon was analyzed to verify the selectivity of each amplification reaction and the dissociation curve was acquired by heating the amplicon from 60 °C to 95 °C.

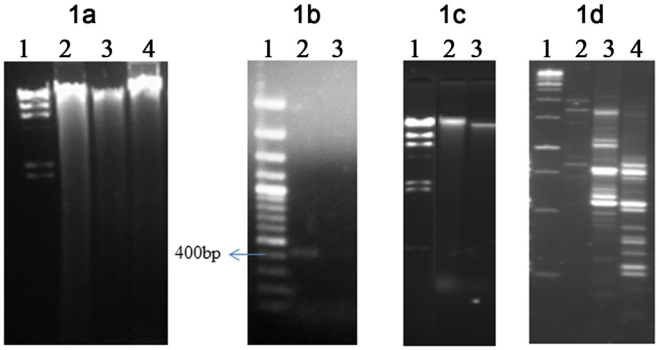


**Fig. 1.** A. Electrophoretic analysis of genomic DNA extracted from leaf sample of three different *E. cardamomum* plants. Lane 1: λ DNA/*Hind*III marker (Thermo Fisher Scientific), DNA extracted from plant A (lane 2), B (lane 3) and C (lane 4). DNA extraction from 3 different cardamom plants showed intact DNA without any degradation. B. Amplification of histone 3 gene from the DNA of *E. cardamomum*. Lane 1: 100 bp ladder (Thermo Fisher Scientific), lane 2: PCR product of histone 3 gene, lane 3: No template control. C. Restriction analysis of total genomic DNA. Lane 1: λ *Hind*III marker (Thermo Fisher Scientific), lane 2: Undigested DNA of sample C, lane 3: DNA of sample C digested by *Hind*III restriction enzyme. Due to the restriction digestion, DNA band in the lane 3 has showed lower mobility than lane 2. D. RAPD analysis with DNA of *E. cardamomum*. Lane 1: 1Kb marker (Thermo Fisher Scientific), Amplification with OPAL-12 (lane 2), OPN-02 (lane 3) and OPI-07 (lane 4) primers. Cardamom plant has showed varied amplification pattern for OPAL12, OPN02 and OPI07 primers.

## 3. Results

### 3.1. DNA extraction, restriction analysis and PCR

Genomic DNA extracted from the frozen leaf samples of different *E. cardamomum* plants with PVP, ascorbic acid and sodium chloride showed a DNA band with very less degradation and without any detectable protein contamination (Fig. 1A). The yield of DNA extracted from three different plants was as follows: A-7.5 µg/gm, B-6.1 µg/gm, C-7.2 µg/gm. The average DNA yield from various cardamom leaf tissues was 6.9 µg per gram of leaf tissue. The purity of the DNA was confirmed with $A_{260/280}$ and $A_{260/230}$ ratio showed the value of 1.68 and 1.77 for sample A, 1.89 and 1.91 for B and 1.83 and 2.77 for C, respectively. DNA of sample C has high yield and purity was taken for further molecular analysis. The authenticity of the DNA was checked by using the isolated DNA in PCR amplification with primers specific to the histone 3 gene has shown the amplification of 408 bp (Fig. 1B). The genomic DNA of *E. cardamomum* leaf tissue was subjected to restriction digestion with *Hind*III enzyme showed lower mobility than undigested DNA (Fig. 1C). RAPD analysis was performed with different RAPD primers, such as, OPAL12, OPN02 and OPI07. Variable amplification pattern was observed with these primers (Fig. 1D).

### 3.2. RNA extraction

The total RNA extraction with the worked-out methodology of the present study has shown distinct low molecular weight RNA bands in addition to 28S rRNA and 18S rRNA bands when compared to Trizol method (Fig. 2). The yield of the RNA from plant A, B and C were 235, 424 and 657 ng/gm. The purity of the RNA was confirmed with $A_{260/280}$ ratio showed the value of 1.82, 1.97 and 1.86 for plant A, B and C, respectively and plants A, B and C showed the value 1.87, 1.92 and 2.02 for $A_{260/230}$. The average RNA yield from various cardamom leaf tissues was 4.3 µg per gram of tissue. The RNA sample was run on 2100 Bioanalyzer System and representative electropherograms is shown for sample B and reference samples in Fig. 3. The high quality and purity of extracted RNA was determined by RIN value of 6.1 (Fig. 3A and B)

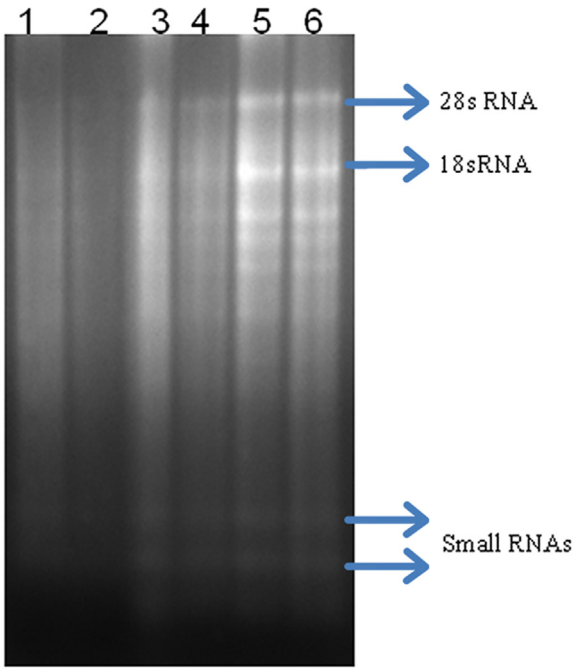


**Fig. 2.** Electrophoretic analysis of total RNA extracted from *E. cardamomum*. RNA extracted from plant A (lanes 1) B (lane 2) and C (lane 3) by Trizol method and proposed method from plant A (lane 4), B (lane 5) and C (6). RNA extracted from the proposed method (lane 4, 5 and 6) showed intact 28S and 18S rRNA, and smaller RNAs.

(RIN = 1 is totally degraded; RIN = 10 is intact). The ratio of 25S and 18S rRNA peak was recorded as 1.7 and 1 for reference and cardamom samples, respectively (Fig. 3A and B).

The extracted total RNA includes both higher and lower molecular weight RNAs. The presence of mRNA in the total RNA was confirmed by performing the PCR for histone gene 4 (Fig. 4A). The low molecular weight RNA bands observed on the gel may indicate the presence of other small RNAs. To evaluate the presence of low molecular weight RNAs, total RNA of sample B was treated with Poly (A) Polymerase and then cDNA was synthesized using oligo dT primer (Balcells et al., 2011). PCR was performed with U87 small cajal body-specific RNA (SCARNA) primers and 5.8S rRNA primers have shown the amplification of 100 bp (Fig. 4B and C). The amplification of U87 and 5.8S rRNA confirmed the presence of small RNAs in the extracted total RNA.

In order to confirm whether the worked-out method is also suitable to extract viral RNA, the total RNA was extracted from the CdMV infected cardamom plant and then cDNA synthesized with oligo dT primer. PCR was performed with cDNA for the CI-6K2 genes of CdMV have shown the expected amplification of 1.7 kb (Fig. 4D).

### 3.3. Real-time PCR analysis

The influence of RNA purity on gene expression was analyzed using real-time RT–PCR. To evaluate the quality of RNA isolated for downstream practices, cDNA was synthesized with random hexamer and PCR amplification efficiency for RNA sample was assessed using RT-PCR. Gene expression profiles obtained from RNA sample exhibited high degree of quality with RNA specific 5.8S rRNA primers. These primers generate an amplicon of 100 bp (Fig. 4C). In the RT-PCR analysis, as expected, a distinct and highly significant correlation between RIN and threshold cycle (Ct) measure was observed. The Ct values ranged from 28.13 to 28.74 for triplicate sample (Fig. 5A) and the standard deviation (SD) value of 0.316 for the sample B, indicating good reliability of Ct values and RT-PCR assay was stable and reliable. Melting curve analysis was carried out to check the specificity of the amplification,

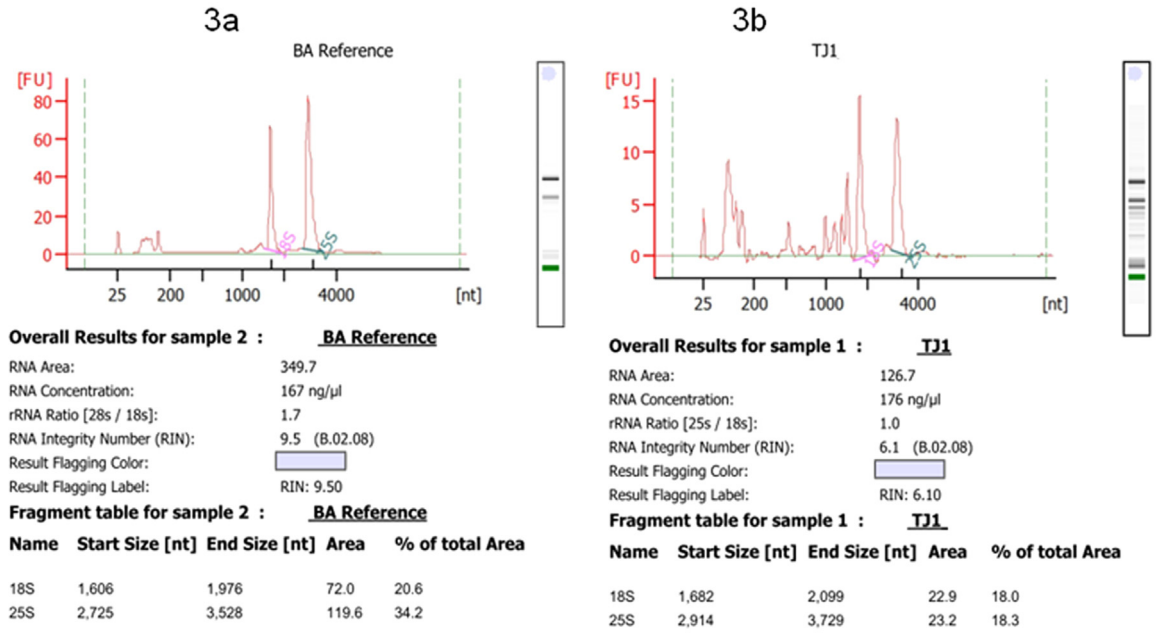


**Fig. 3.** Electropherogram summary of reference RNA (A) and total RNA isolated from cardamom (B).

showed single peak, which determines the specificity of the reaction (Fig. 5B). These results indicated that RNA isolated by the worked-out methodology was of high quality and suitable for real time PCR.

## 4. Discussion

*E. cardamomum* grows in the evergreen forests of Indian sub-continent at the altitude of 700–1500 m. As a routine procedure, cardamom leaf samples are collected from the field, transported to the laboratory and stored at −80 °C freezer. Degradation of nucleic acids might occur during its extraction, due to overnight non-cryogenic transportation (Almakarem et al., 2012) and polyphenol secretion from the cardamom cells (Bhatti et al., 2010). Optimizing the composition in the nucleic acid extraction buffer is known to play a vital role in eliminating the interference of secondary metabolites, such as phenolic compounds, alkaloids and terpenoids in the extraction process. Despite, good quality of DNA and RNA being extracted from various tissues from the members of the order Zingiberales using PVP (Devi et al., 2013; Deepa et al., 2014), combination of PVP, sodium metabisulphite and β- mercaptoethanol were found to be required for the extraction of high quality of DNA from large cardamom species, *viz., Amomum subulatum* Roxb which is also known to contain high level of polyphenols and polysaccharides similar to that of *E. cardamomum* (Mathew et al., 2014). Extraction of DNA and RNA from cardamom with PVP alone did not give good yield (data not shown). Hence, in the present study, along with PVP, 2% ascorbic acid was used in the extraction buffer as an antioxidant to prevent the oxidation of polyphenol during the extraction of DNA and RNA from the tissues of cardamom plant. The antioxidant property of ascorbic acid is helpful to extract nucleic acids form polyphenol rich samples (Borse et al., 2011). Furthermore, addition of 2 M sodium chloride in the extraction buffer would remove polysaccharides, upon co-precipitation with DNA, during the extraction

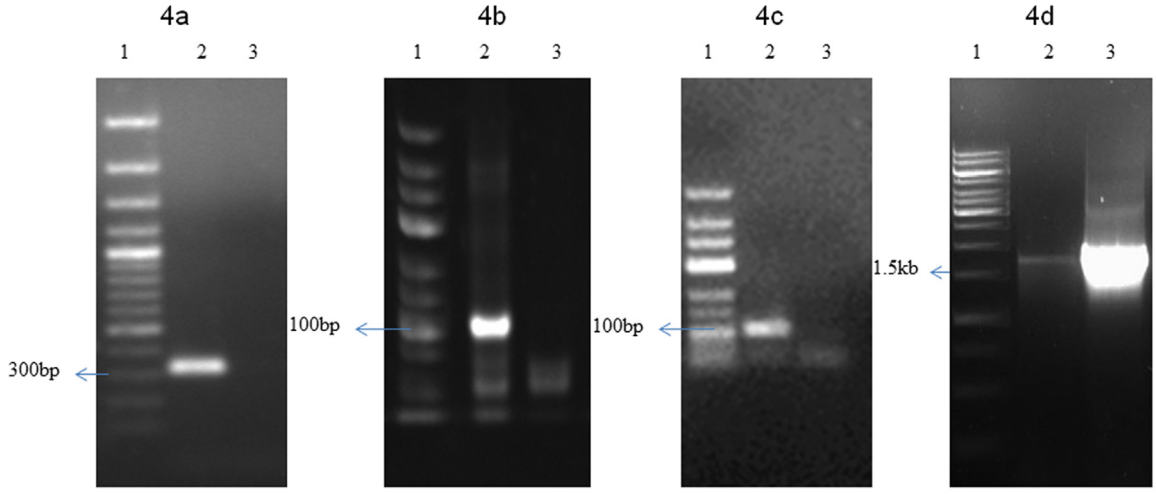


**Fig. 4.** A. Amplification of histone 4 gene from leaf sample B of *E. cardamomum* plant cDNA. Lane 1: 100 bp ladder (Thermo Fisher Scientific), lane 2: PCR product of histone 4 gene, lane 3: No template control. B. Amplification of U87 small RNA from *E. cardamomum* cDNA on 10% SDS PAGE. Lane 1: Low range marker (Thermo Fisher Scientific), lane 2: amplification of U87 small RNA and lane 3: No template control. C. Amplification of 5.8S rRNA from *E. cardamomum* cDNA. Lane 1: Low range DNA marker (Thermo Fisher Scientific), lane 2: amplification of 5.8S rRNA (100 bp) and lane 3: No template control. D. Amplification of viral genes from CdMV infected *E. cardamomum* cDNA. Lane 1: 1Kb marker (Thermo Fisher Scientific), lane 2: amplification of viral genes (CI-6K2 fusion) and lane 3: Positive control.

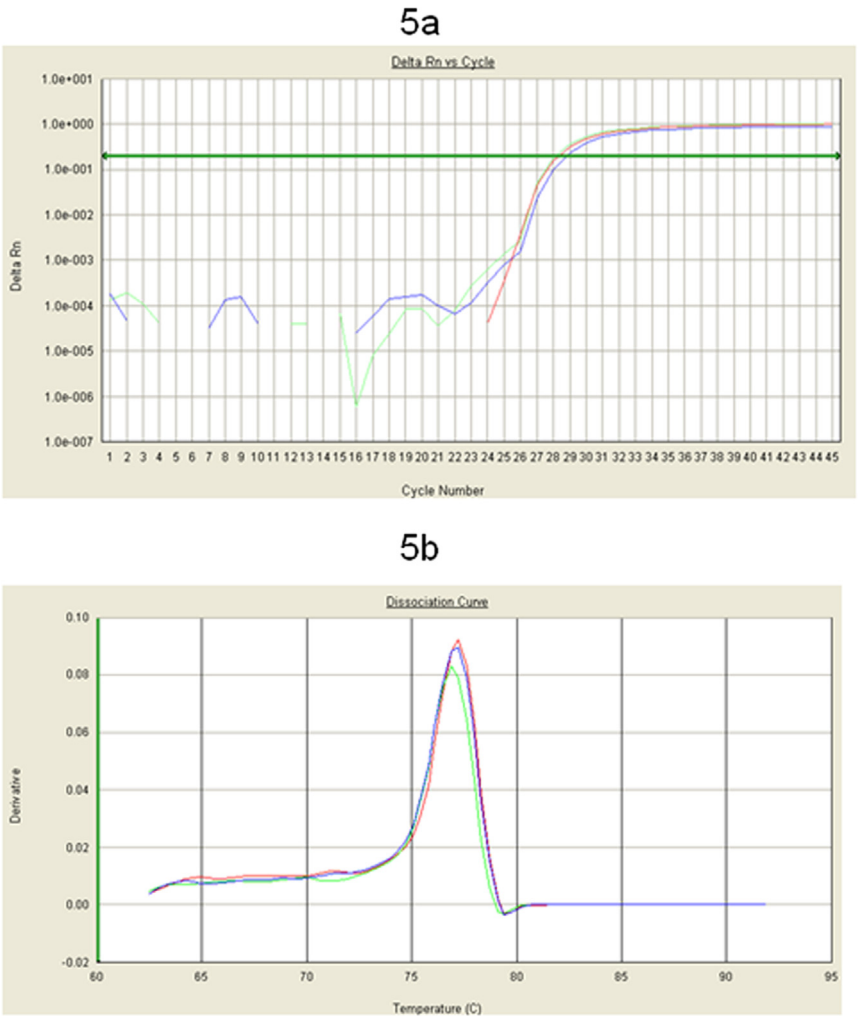


**Fig. 5.** Amplification plot (A) and dissociation curve (B) from Real time PCR analysis. Green, red and purple colours indicate the amplification for triplicates of a sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Ribeiro and Lovato, 2007). In the present study, the quality of DNA extracted from various tissues of cardamom plant by this method was confirmed by PCR, restriction and RAPD analysis (Fig. 1B, C and D). PCR analysis has shown 409 bp amplification with specific primers for histone 3 gene (Fig. 1B). The purity of the DNA sample was confirmed through digestion with restriction enzyme *Hind*III (Fig. 1C). RAPD analysis with three different RAPD primers, also showed the efficiency of the extracted DNA by this method. Observation of different molecular weight amplified fragments in Fig. 1D represents the variability in amplification but specific to primers. Hence, *E. cardamomum* genomic DNA extracted by this method can be used to amplify the gene and to develop genetic marker through RAPD analysis.

Generally, RNA extraction for most of the bio-sources is being done with Trizol, but in case of cryo-preserved cardamom leaves, Trizol method yielded poor quality RNA (Fig. 2, lanes 1–3). The reported protocols involving a combination of commercial kits and conventional CTAB is highly suitable to extract RNA from fresh sample of cardamom (Nadiya et al., 2015) but not for cryopreserved leaf sample (data not shown). In the present study, RNA extraction buffer containing PVP, ascorbic acid and β-mercaptoethanol yielded good quality of RNA. Presence of β-mercaptoethanol in RNA extraction buffer is known to inhibit RNase and consequently minimizes oxidation of the sample (Gonzalez-Mendoza et al., 2008). An earlier report showed the employment of acid phenol for the extraction of RNA from various tissues of cardamom plant in order to eliminate contamination by DNA (Ghawana et al., 2011).

High quality RNA is a critical determinant for the success of many downstream processes. The classical method involving 28S/18S ratio of 2.0 to measure the RNA integrity was observed to be a weak parameter. The quality in terms of total RNA integrity was evaluated by analyzing the 28S and 18S rRNA bands on agarose gel (Fig. 2). The agarose gel image for RNA isolated through the Trizol was poor (Fig. 2) and method employed in the present study showed a clean and intensified 28S and 18S RNA bands without any degradation (Fig. 2). The Agilent 2100 Bioanalyzer and its unique RNA Integrity Number (RIN) algorithm have become the hallmark for quality analysis of total RNA. In the present study, extracted RNA was subsequently subjected to Bioanalyzer quality control analysis on the Agilent 2100 Bioanalyzer. The electropherograms generated by Bioanalyzer profiling of isolated RNA showed RIN value of 6.1 (Fig. 3A) which indicated the adequate integrity of the RNA. RNA was found to be intact and showed the prominent peak heights for the 25 S and 18 S rRNA (Fig. 3A). Moreover, for plant RNA samples, the RIN values above 6.0 can be considered as intact and accepted for highly sensitive downstream applications (Schroeder et al., 2006). RNA integrity is a significant factor which greatly influences the amplification efficiency in RT-PCR. RT-PCR was performed for 5.8S rRNA and the coefficient of variation for 5.8S rRNA gene was found to be 0.316; representing significant reliability of Ct values which confirm the reliability of RT-PCR assay (Fig. 5A).

RNA extracted by the proposed protocol contains higher and lower

molecular weight RNA molecules (Fig. 2). Thus the protocol is not only suitable to amplify the mRNA but also to amplify small RNAs (Fig. 4B). Amplification of low molecular weight RNAs, such as, U87 and rRNA (Galiveti et al., 2010) suggest that this method is highly useful to enrich the small RNA from the total RNA. Enrichment of plant miRNA could help to decipher the role of small RNA in the plant-viral interactions. For instance, in *Turnip mosaic virus* (TuMV), P1 and HC-Pro proteins have their amalgamated effect on suppression of gene silencing to overcome the plant defense by interacting with host miRNA (Kasschau et al., 2003).

Another advantage of the proposed RNA extraction method is its suitability to clone the viral RNAs. CdMV is a RNA virus of *Potyviridae*, causes 'katte' disease (Thomas and Usha, 2001). The genome length of CdMV is 8.3 kb. The proposed method is applicable to amplify the CI-6K2 fusion of CdMV which is positioned towards the 5′ end of CdMV genome (Fig. 4D).

Existing methods to extract DNA and RNA from cardamom plant were time consuming, expensive and not suitable for extraction of genomic DNA from cryopreserved leaves. Babu et al. (2012) reported the very lengthy DNA extraction protocol with overnight gradient centrifugation for cardamom plant. Existing methods to extract RNA from cardamom is suitable to extract mRNA alone from the fresh cardamom leaves. The proposed DNA and RNA extraction methods can be preferred for its simplicity, rapidity and for better yield from cryopreserved leaf samples. Also, the methodology does not involve complicated steps, long ultra centrifugations steps and also it can be performed within 4 h. Further, these methods will lead to develop genetic marker and to study phylogeny of different cardamom species. This method is useful to clone cardamom mRNA, small RNA and CdMV viral RNA to characterize the genes of cardamom and to understand the host-virus interactions. The proposed method is also amenable for RT-PCR.

## 5. Conclusion

The proposed nucleic acid extraction methods with the inclusion of 2% ascorbic acid is suitable for extracting DNA and mRNA and small RNA molecules from tissues that contain high level of polysaccharides and polyphenols from *E. cardamomum*. Further these methods are useful to clone cardamom mRNA, small RNA and viral RNA and to perform RT-PCR. The extracted nucleic acids are amenable to PCR and other downstream enzymatic process without the adverse effect of high polyphenol and polysaccharide. The proposed protocols are also cost effective and less time consuming methods to extract DNA and RNA from *E. cardamomum*.

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## Author's contributions

S.N.P, E.S and T.J conceptualized the research problem, S.N.P and E.S done the data curation, formal analysis and wrote the original manuscript. T.J and M.K validated the data, reviewed and edited the manuscript.

## Conflict of interest

All authors declare no conflicts of interest.

## Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2018.11.026.

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