**Journal of Plant Sciences**
2017; 5(2): 68-74
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20170502.14
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

# An Efficient Method for High Quality RNA Extraction from *Moringa oleifera*

**Preethi Praba Umesh[1], Mohd Akram Ansari[1, 2], Ganapathi Sridevi[1, *]**

[1]Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai, Tamil Nadu, India
[2]Division of Genetics and Plant Molecular Biology, CSIR-NBRI, Rana Pratap Marg, Lucknow, Uttar Pradesh, India

## Email address:

preethi.praba5@gmail.com (P. P. Umesh), akramansari1988@gmail.com (M. A. Ansari), srideviganapathi@yahoo.com (G. Sridevi)
[*]Corresponding author

## To cite this article:

Preethi Praba Umesh, Mohd Akram Ansari, Ganapathi Sridevi. An Efficient Method for High Quality RNA Extraction from *Moringa oleifera*. *Journal of Plant Sciences.* Vol. 5, No. 2, 2017, pp. 68-74. doi: 10.11648/j.jps.20170502.14

**Received**: February 19, 2017; **Accepted**: February 28, 2017; **Published**: March 28, 2017

**Abstract:** *Moringa oleifera*, the Miracle Tree is rich in all nutrients and minerals. It has prominent distribution of secondary metabolites like polysaccharides, phenols and mucilage, which makes extraction of RNA quite difficult. A high quality and pure RNA is prerequisite for the study of high through put transcriptomics and functional genomics. A protocol for isolation of highly qualitative and quantitative RNA from *M. oleifera* was optimized by comparing five different methods like Trizol, Guanidine hydrochloride, combined Trizol and Guanidine hydrochloride, modified CTAB and hot phenol method. The combined Guanidine hydrochloride and Trizol method gave good yield and pure RNA based on the absorbance $A_{260}$ value 2.0. The model plant *Nicotiana tabacum* served as a positive control in which the Trizol method yielded a good quality and quantity RNA. The present study is a preliminary step for studying the function and expression pattern of the genes. This is the first report on the comparison of different RNA extraction methods in *M. oleifera* to our knowledge.

**Keywords:** *Moringa oleifera*, *Nicotiana tabacum*, RNA Isolation, Trizol, Guanidine Hydrochloride, RT-PCR

## 1. Introduction

*Moringa oleifera* commonly known as the drumstick or ben oil tree is nutritious and the leaves are rich source of vitamin A, B, C and iron. It is locally known as sajna, muringa, mullanggay, etc. It is a cultivated species of monogeneric family, Moringaceae. It is grown mainly in semiarid, tropical and subtropical areas. Being a fast growing tree, it grows best in dry, sandy soil, and tolerates poor soil [9]. It is native to the sub-himalayan tracts of north western India and are spread in eastward India to the lower part of china, South East Asia and the Philippines. They are spread westward from India to Egypt, the horn of Africa, around to the Mediterranean and West Indies in America. *M. oleifera* is first described as a medicinal herb around 2000 BC ago. It is very nutritious and has a variety of potential uses [21]. There exists number of reports highlighting the nutritional qualities of *Moringa*. The fresh leaves of *M. oleifera* contains 7 times more of vitamin C than oranges, 4 times more vitamin A and calcium than carrot and milk, 3 times more amount of potassium of bananas, 2 times protein of yogurt and ¾ times iron of spinach. The leaves are enriched with micronutrients like phosphorus, zinc, copper, magnesium, etc. [7]. The dried leaves are characterized with high nutritional content compared to fresh leaves as it is 10 times more vitamin A of carrots, ½ the vitamin C of oranges, 17 times the calcium of milk, 15 times the potassium of bananas, 9 times the protein of yogurt and 25 times the iron of spinach [6]. The flowers, leaves, roots and bark are used as folk remedies for tumors, abdominal discomfort, boils, conjunctivitis, high blood pressure, hysteria and skin disease [8]. *M. oleifera* is a promising food source especially for its nutrient and mineral rich during the dry season when other foods are typically scarce [5]. *M. oleifera* is not nitrogen fixing tree, but its fruits, flowers and leaves contain 5 to 10% proteins on average [4].

The genome size of *Moringa* was estimated to be 315 Mb and a well annotated high-quality draft genome sequence has been reported [20]. *Moringa* possesses a compact genome and a number of gene families related to heat tolerance, stress tolerance, high protein content, fast-growth, etc. have been

identified. Further, the role of micro RNAs from *Moringa* that might contribute to medicinal properties was studied [19]. There is paucity of information available about the transcriptomics and almost completely absent regarding the functional genomics aspects in *Moringa.* As a result there is a prerequisite for high quality RNA for the functional characterization of those genes and high throughput transcriptomics.

Nucleic acid extraction from plants is diverse and varies among individual plant species. Isolation and purification of RNA from some of the plant species may be problematic due to the presence of highly viscous polysaccharides and secondary metabolites like phenols. The presence of polyphenols, which are powerful oxidizing agents can reduce the yield and purity of nucleic acid [13, 16]. It has also been observed that phenolic compounds are readily oxidized to form covalently linked quinones [13]. Polyvinylpyrrolidone (PVP) plays a role in removing phenolic compounds and secondary metabolites during nucleic acid preparations [2] and it also prevents browning effect caused due to polyphenols. PVP can strongly bind to the polyphenol compounds by its CO-N=group [22]. RNA isolation from plants like *Arabidopsis*, tobacco, tomato, potato or maize is usually achieved by classical phenol/lithium chloride method and/or guanidine based methods [3, 17, 18]. RNA extraction from leaves and stem of spinach was done by lithium chloride method, where the leaves yield good RNA than stem [14]. Extraction methods also depend on the stages of plant tissues. In *Arabidopsis thaliana*, RNA extraction from young leaves was done by two different methods (trizol and phenol:chloroform - lithium chloride method) where the later gave high quality of RNA [1].

Our main objective is to obtain a good quality and quantity of RNA for the functional genomics detailed transcriptomics in *M. oleifera*. We compared five different methods of RNA extraction from the leaves of *M. oleifera* (trizol, guanidine hydrochloride, guanidine hydrochloride with trizol, modified CTAB and hot phenol). The yield and purity of RNA was evaluated using spectrophotometer, integrity by gel electrophoresis and finally confirmed by RT-PCR analyses.

# 2. Materials and Methods

### *2.1. Plant Material*

The leaves of field grown *Moringa oleifera* var. PKM 1 was taken for RNA analyses. Five different methods were deployed for RNA extraction and evaluated.

a. TRIzol method

RNA extraction by Trizol method was performed as per manufacturer's instruction (TRI reagent SIGMA-ALDRICH, USA). One gram of plant tissue (leaf) was ground into fine powder with the help of liquid nitrogen and transferred into 15 ml polypropylene tubes. The powder is then resuspended in 5 ml of trizol reagent and mixed by vortexing for 30 sec followed by the addition of $1/5^{th}$ volume of chloroform. The tube was vortexed approximately for 20 sec and incubated in room temperature for 15 min. The contents were centrifuged at 12,000 rpm for 15 min at 4 °C in order to separate DNA and proteins. The supernatant with RNA phase was transferred into a fresh tube and added equal amount of ice cold isopropanol. The tubes were kept at room temperature for 10 min for precipitation and centrifuged at 12,000 rpm for 30 min at 4 °C. After the precipitation of RNA the supernatant was discarded and the pellet was washed with 75% ethanol. Finally the pellet was vacuum dried and dissolved in 20 µl of sterile DEPC water (preheated at 65 °C) and stored at -80 °C until use.

b. Guanidine hydrochloride with TRIzol method

One gram of leaf tissue was homogenized preliminarily using liquid nitrogen and later with 5 ml of guanidine hydrochloride buffer [6.5 M guanidine hydrochloride, 100 mM Tris-Cl (pH 8.0), 100 mM sodium acetate (pH 5.5), and 0.1 M β- mercaptoethanol (added after sterilization)]. Then the slurry was transferred to the centrifuge tube, mixed vigorously and incubated at room temperature for 10 min undisturbed. The samples were centrifuged at 12,000 rpm for 10 min at 4 °C. To the supernatant 5 ml of trizol reagent and 2 ml of chloroform were added. The tube was incubated at room temperature for 2 min, and centrifuged at 12,000 rpm for 10 min at 4 °C. The aqueous phase was taken carefully and transferred to fresh tube. Ice cold isopropanol was added in 1:1 ratio to the above and incubated in ice for 30 min followed by centrifugation at 12,000 rpm for 20 min at 4 °C. The supernatant was discarded and the pellet was washed using 5 ml of 75% ethanol, centrifuged at 12,000 rpm for 5 min at 4 °C. The pellet was vacuum dried and dissolved in 20 µl of sterile DEPC water (preheated at 65 °C) and stored at -80 °C until use.

c. Modified CTAB method

The isolation of RNA was performed using CTAB method with some modifications. One gram of plant tissue was ground with liquid nitrogen into fine powder. Approximately, 200 mg of leaf powder was taken in a 2 ml eppendorf tube followed by addition of 1 ml of 2% CTAB buffer [2% CTAB, 100 mM Tris-Cl (pH 8.0), 1.4 M NaCl, 2% PVP, 20 mM EDTA (pH 8.0), β- mercaptoethanol (added after sterilization)], mixed for 30 sec and incubated at 65 °C for 30 min. The contents of the tubes were inverted 4-5 times in between the incubation. After incubation, 800 µl of chloroform was added and mixed properly. Then the samples were centrifuged at 10,000 rpm for 10 min at 4 °C. To the aqueous phase, 800 µl of TE saturated phenol was added, mixed for 30 sec and centrifuged at 10,000 rpm for 10 min at 4 °C. Again to the supernatant, added equal volume of chloroform: isoamyl alcohol (24:1) mixed for 30 sec and centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant along with 1/3 volume of 8 M $LiCl_2$ was kept at 4 °C overnight for precipitation of RNA. The samples were then centrifuged at 10,000 rpm for 20 min 4 °C and the pellet was washed with 95% ethanol and then with 75% ethanol by centrifuging at 10,000 rpm for 5 min at 4 °C. The pellet was air dried and dissolved in 20 µl of sterile DEPC water (preheated at 65 °C) and stored at -80 °C until use.

d. Hot phenol method

RNA extraction by hot phenol method was performed according to the method of Pawlowski et al. [15]. One gram of leaf tissue was ground into fine powder using liquid nitrogen and transferred to the eppendorf tubes. Added 500 µl of preheated (90 °C) RNA extraction buffer (100 mM lithium chloride, 1% SDS, 100 mM Tris-Cl, pH 9.0, 100 mM EDTA) to the powder mixed and incubated at 65 °C water bath for 30 min. The contents of the tubes were inverted 3-4 times once in every 10 min. Added 500 µl of Tris EDTA saturated phenol, mixed and centrifuged at 12,000 rpm for 10 min at room temperature. The upper liquid layer was transferred to the fresh tube, to which equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed and centrifuged at 10,000 rpm for 5 min at room temperature (performed twice). The upper liquid layer was transferred to a fresh tube and 8 M $LiCl_2$ was added to achieve a final concentration to 2 M $LiCl_2$ followed by overnight precipitation at 4 °C. The tubes were centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant was discarded. The pellet was washed with 75% ethanol, and centrifuged at 12,000 rpm for 5 min at 4 °C. The pellet was dissolved in 300 µl of 0.3 M sodium acetate (pH 5.2). Added equal volume of chloroform: isoamyl alcohol (24:1) mixed gently and centrifuged at 10,000 rpm for 5 min at 4 °C. The upper aqueous layer was measured and added 95% ethanol, mixed gently and centrifuged at 12,000 rpm for 10 min at 4 °C. Finally, the pellet was again washed with 75% ethanol and centrifuged at 12,000 rpm for 5 min at 4 °C. The pellet was vacuum dried and dissolved in 20 µl of sterile DEPC water (preheated at 65 °C) and stored at -80 °C until use.

e. Guanidine hydrochloride method

One gram of plant leaf tissue is homogenized in a guanidine hydrochloride - buffer containing 6.5 M guanidine hydrochloride, 100 mM Tris-Cl (pH 8.0), 100 mM sodium acetate (pH 5.5), and 0.1 M β-mercaptoethanol (added after sterilization) as described by Logemann et al. [12]. The slurry was transferred to the centrifuge tube, mixed vigorously and incubated at room temperature for 10 min undisturbed. The samples were centrifuged at 12,000 rpm for 10 min at 4 °C. To the supernatant, 2 ml of chloroform was added. The tube was incubated at room temperature for 2 min, and then centrifuged at 12,000 rpm for 10 min at 4 °C. The aqueous phase was taken carefully and transferred to fresh tube. Ice cold isopropanol was added in 1:1 ratio to the above and incubated in ice for 30 min for precipitation of RNA, followed by centrifugation at 12,000 rpm for 20 min at 4 °C. The supernatant was discarded and the pellet was washed using 5 ml of 75% ethanol, centrifuged at 12,000 rpm for 5 min at 4 °C. The pellet was air dried and dissolved in 20 µl of sterile DEPC water (preheated at 65 °C) and stored at -80 °C until use.

### *2.2. RNA Estimation*

The total RNA was estimated by measuring the optical density at 260 nm and 280 nm in UV spectrophotometer. The RNA sample, 2 µl was mixed in 998 µl of DEPC water, estimated and calculated by using the formula: [1 OD at 260 nm is equal to 40 ng of RNA] [17].

Concentration of RNA = $40*A_{260}*500$

The total RNA was loaded on 1.2% formaldehyde agarose gel and visualized under UV light to determine the integrity of RNA.

### *2.3. DNase Treatment*

Total RNA (30 µg) was taken and the reaction volume made up to 50 µl with 1 µl of DNase enzyme, 40 µM Tris (pH 7.5) 6 mM $MgCl_2$ and DEPC water. The tubes were gently tapped and incubated at 37 °C for 30 min. After incubation, 200 µl RNase free DEPC water was added. To that equal volume of phenol: chloroform (24:1) was added and centrifuged at 12,000 rpm for 5 min at 4 °C (repeated twice). The supernatant was measured, added 0.1 volume of 3 M sodium acetate and 2.5 volume 95% ethanol. The samples were stored at -70 °C for 30 min and centrifuged at 4 °C, 12,000 rpm for 10 min. The supernatant was discarded and the pellet was washed by 75% ethanol. The pellet was vacuum dried and dissolved in 20 µl of sterile DEPC water (preheated at 65 °C) and stored at -80 °C until use.

### *2.4. RT-PCR Analysis*

Total RNA sample (*M. oleifera*) after treatment with DNase I enzyme was subjected to RT-PCR following the instruction manual of two step RT-PCR kit (TaKaRa, Kyoto, Japan). The cDNA was synthesized following the manufacturer's instruction. After the cDNA synthesis, 5 µl of sample was taken for PCR using actin primers: forward primer 5' GCTATTCAGGCTGTCCTTTCCTTGATATG 3' and reverse primer 5' CCGATATCAACATCACACTTCATAATG 3'. The PCR conditions include initial denaturation at 98 °C for 30 sec, followed by 30 cycles [98 °C - 10 sec, 50 °C - 30 sec, 72 °C - 1 min] and final extension at 72 °C for 5 min. Total RNA from *Nicotiana tabacum* was used as positive control along with appropriate PCR controls. The PCR products after amplification were electrophoresed on 1.2% agarose gel.

## 3. Results and Discussion

A reliable quality of RNA is essential for downstream applications such as RT-PCR, cDNA library construction, gene expression studies, high through put transcriptomics etc. The quality and quantity of RNA differs based on the method of the isolation. Therefore the present study was attempted to isolate a high quality and quantity RNA which can be used in high through put transcriptomics.

A comparison study of total RNA extraction from the leaves of *Moringa oleifera* was done by five different methods: TRIzol method, guanidine hydrochloride method, combination of guanidine hydrochloride and TRIzol method, CTAB method with some modifications and hot phenol method. There were lot of hindrances in obtaining good quality of RNA from *M. oleifera* due to presence of high mucilage content and other polysaccharides. The leaves of

tissue culture grown wild type *Nicotiana tabacum* (Wisconsin38) served as a positive control for the comparison of different methods of RNA extraction.

The total RNA extracted by five different methods was analyzed for their yield, purity and integrity by three different steps. The quantity of total RNA was determined by measuring the absorbance in UV Spectrophotometer at 260 nm. The quantity of total RNA was comparable in both trizol method and hot phenol method (292.8 and 291.2 µg/g fresh weight). The yield of above two methods was higher compared to the other three methods. Further the combined guanidine hydrochloride and trizol method gave 212.0 µg/g FW followed by modified CTAB method and guanidine hydrochloride method (Table 1). The isolation of RNA was difficult in plants containing polysaccharide, polyphenols and mucilage. Plants like *Hibiscus tiliaceus* [23], *Cinnamomum tenuipilum* [24] contains high polysaccharides, mucilages and other secondary metabolites. RNA isolation from these plants was performed by modified CTAB method. *C. tenuipilum* is highly viscous and RNA extraction was successful by trizol kit based method, Guanidine thiocyanate method and Phenol/SDS method. The yield of RNA in *C. tenuipilum* was 20 µg/g but when tried with CTAB method with some modification in centrifugation step the yield was 60-100 µg/g. Similar results with increased RNA yield (113.6 µg/g) was obtained in our modified CTAB method of extraction too (Table 1).the addition of 2% PVP and 2% PVPP together also contributed for increased quality and quantity of RNA in the above method while it is lesser in trizol, hot phenol and combined trizol and GnHCl methods [23].

The quality of RNA was analyzed spectrophotometrically by evaluating the $A_{260}/A_{280}$ ratio. The value range of 1.8 - 2.0 indicates the purity of isolated RNA. The combined GnHCl and trizol, hot phenol, modified CTAB and trizol methods gave a similar absorbance value as 1.6 - 1.7. *N. tabacum* served as a positive control and gave highest quality of RNA in trizol method compared to other methods of extraction (Table 1).

The RNA integrity was analyzed electrophoretically by loading 5 µg of total RNA on to a 1.2% denaturing agarose gel and electrophoresed. The RNA was visualized by staining with ethidium bromide and observed under UV transilluminator. All the four methods except guanidine hydrochloride method showed good quality of bands which revealed that the integrity of RNA is high in all the four methods in terms of purity and quantity (Fig. 1).

DNase treatment was done to get rid of the trace amounts of DNA. The purest RNA was achieved by combined guanidine hydrochloride with trizol method with absorbance value 2.0. The trizol method gave high quantity of total RNA (20 µg) with low purity. Both modified CTAB method and hot phenol method yielded high amount of RNA (18 µg and 16 µg) and similar purity in accordance with trizol method. The least quality and quantity of RNA was achieved in guanidine hydrochloride method whose purity was 1.3 only (Table 2). One possible reason for poor quality or no bands visualized in the guanidine hydrochloride method (Fig. 1) may be due to the least purity of RNA with a ratio of 1 (Table 2).

Finally the cDNA was synthesized from the total RNA and RT-PCR was performed using the tobacco actin primer that yielded an amplified product of 500 bp in size. The RT-PCR analysis with actin primer gave intact 500 bp amplification (Fig. 2) in all the methods except hot phenol method. RT-PCR performed with trizol method showed less amplification intensity compared to the other methods. The other methods such as modified CTAB method, and trizol method did have comparable yield after DNase treatment (Table 2). On the other hand the RT-PCR results were not comparable to the combined method (Fig. 2). Trizol method was proven to be the best method for the model plant, *N. tabacum*. This method provided highest yield, pure and well integrated RNA compared to all other methods.

RNA isolation protocols in each plant have to be optimized [10]. A high quality and quantity of RNA is mandatory for the construction of cDNA library from *M. oleifera* to study the transcriptomics. The RNA extracted by combined guanidine hydrochloride and trizol method gave high quantity and quality RNA for *M. oleifera*. The thiocyanate salt of guanidine is one of the constituent in TRIzol and contributes for better cell disruption and denaturation of proteins. The presence of phenol along with thiocyanate salt and hydrochloric acid resulted in high quality as well as higher quantity of pure RNA from *Moringa*.

Guanidine hydrochloride method resulted in poor quantity and quality of RNA (Table 1 and 2), even though amplification did occur with actin primers in RT-PCR. Earlier reports revealed that the total RNA isolation with guanidine hydrochloride method resulted in good yield [11, 12]. On the contrary, guanidine hydrochloride method in our study resulted in poor quantity and quality of RNA (Table 1 and 2), even though amplification did occur with actin primers in RT-PCR.

The hot phenol method of extraction did possess hindrances due to the presence of high content of phenols and viscosity. Earlier reports revealed that polysaccharides makes nucleic acid viscous and the use of PVP in RNA extraction buffer would be helpful to remove polyphenols [16]. The spectrophotometer estimation of hot phenol method showed good quantity and quality RNA compared to all the other four methods (Table 1). The RNA isolated by this method showed smear like pattern in 1.2% formaldehyde agarose gel (Fig. 1), which indicates the degradation of RNA. Further, this was confirmed by RT-PCR analyses, as the respective lane did not show any amplification (Fig. 2). Therefore the above results indicate that hot phenol method may not be a suitable method for RNA extraction from *M. oleifera*.

***Table 1.*** *Qualitative and Quantitative analysis of total RNA extracted by different methods.*

| S. No | RNA Extraction method | $A_{260}/A_{280}$ | Amount of RNA µg/µl | Concentration of RNA µg/g FW |
|---|---|---|---|---|
| 1 | *N. tabacum* (TRIzol) | 1.6379±0.15 | 9.86±0.0 | 735.8 |
| 2 | *M. oleifera* (TRIzol) | 1.5907±0.03 | 2.47±0.52 | 291.2 |
| 3 | *N. tabacum* (GnHcl + TRIzol) | 1.8293±0.0 | 1.2±0.0 | 221.6 |
| 4 | *M. oleifera* (GnHcl + TRIzol) | 1.3196±0.0 | 0.620±0.0 | 212.0 |
| 5 | *N. tabacum* (M-CTAB) | 1.6277±0.03 | 2.77±0.26 | 81.6 |
| 6 | *M. oleifera* (M-CTAB) | 1.6084±0.01 | 3.0±0.22 | 113.6 |
| 7 | *N. tabacum* (hot phenol) | 1.3922±0.26 | 0.180±0.0 | 132.8 |
| 8 | *M. oleifera* (hot phenol) | 1.6926±0.02 | 1.42±1.30 | 292.8 |
| 9 | *N. tabacum* (GnHcl) | 1.6916±0.07 | 1.66±0.36 | 96.0 |
| 10 | *M. oleifera* (GnHcl) | 1.7293±0.10 | 3.6±0.008 | 49.6 |

The results are the average of three replicates ± SD. SD: Standard Deviation. FW: Fresh weight of leaves. GnHcl: Guanidine hydrochloride; M-CTAB: Modified CTAB

***Table 2.*** *Purity and yield of total RNA after DNase treatment extracted in five different methods.*

| S. No | Methods of RNA Extraction | $A_{260}/A_{280}$ | Amount of RNA µg/µl | Amount of RNA after DNase Treatment |
|---|---|---|---|---|
| 1 | *N. tabacum* (TRIzol) | 1.4196 | 0.380 | 7.60 µg |
| 2 | *M. oleifera* (TRIzol) | 1.5350 | 1.020 | 20.4 µg |
| 3 | *N. tabacum* (GnHcl + TRIzol) | 1.5795 | 0.680 | 13.6 µg |
| 4 | *M. oleifera* (GnHcl + TRIzol) | 2.0647 | 0.620 | 12.4 µg |
| 5 | *N. tabacum* (M-CTAB) | 1.7727 | 0.200 | 4.0 µg |
| 6 | *M. oleifera* (M-CTAB) | 1.5397 | 0.900 | 18.0 µg |
| 7 | *N. tabacum* (hot phenol) | 1.5905 | 0.500 | 10.0 µg |
| 8 | *M. oleifera* (hot phenol) | 1.5871 | 0.840 | 16.8 µg |
| 9 | *N. tabacum* (GnHcl) | 1.5039 | 1.88 | 3.76 µg |
| 10 | *M. oleifera* (GnHcl) | 1.3784 | 0.120 | 2.40 µg |

GnHCl: Guanidine Hydrochloride; M-CTAB: Modified CTAB

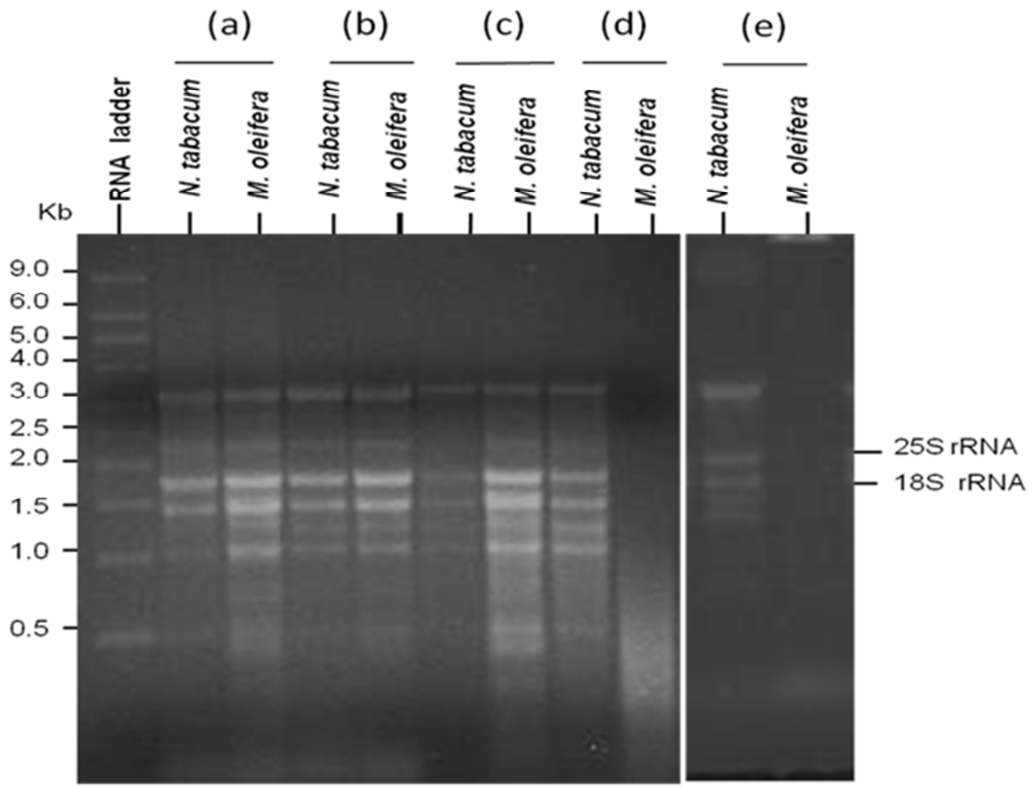


***Figure 1.*** *Agarose gel electrophoresis of RNA extracted by five methods. Electrophoretic pattern of total RNA extracted from Moringa oleifera leaves in different methods on 1.2% formaldehyde agarose gel and stained with ethidium bromide. Nicotiana tabacum (Positive control for all methods of extraction), (a): trizol method, (b): guanidine hydrochloride with trizol method, (c): modified CTAB method, (d): hot phenol method, (e): guanidine hydrochloride method.*

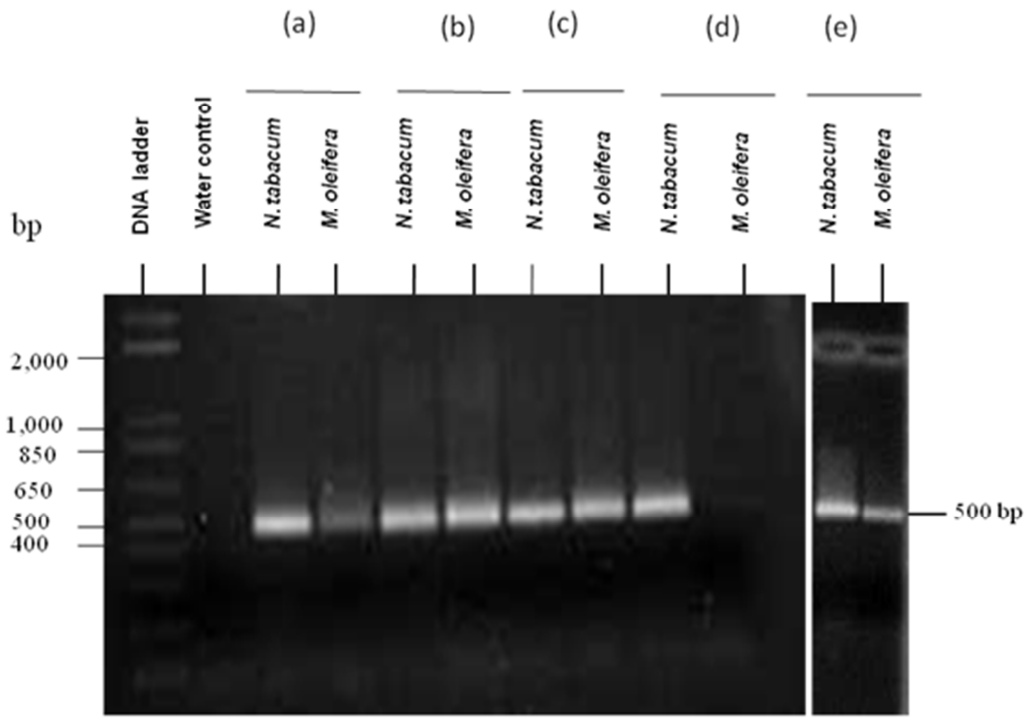


***Figure 2.*** *RT-PCR amplification using* Nicotiana tabacum *actin primer. The amplification was performed with 100 ng of RNA in all the five methods. Nicotiana tabacum, served as a positive control in the comparison study of five different methods also amplified. (a): trizol method, (b): guanidine hydrochloride with trizol method, (c): modified CTAB method, (d): hot phenol method, (e): guanidine hydrochloride method.*

## 4. Conclusion

A comparative study of RNA extraction by five different methods was performed. The guanidine hydrochloride combined with trizol gave pure and high amount of RNA. This method of RNA extraction is more suitable for any other plant like *Moringa* that possesses high mucilage, phenols and polysaccharides.

## Acknowledgements

The authors thank the Department of Science and technology, New Delhi, India for providing INSPIRE fellowship to Miss. U. Preethi Praba. We thank Dr. K. Veluthambi, Department of Plant Biotechnology, School of Biotechnology, Madurai Kamaraj University, Madurai-21, TamilNadu, India for extending his facilities to carry out the above research work. We thank Dr. C. Parameswari, TamilNadu Agricultural University, Madurai for providing us the seeds of *Moringa oleifera.*

## References

[1] Box MS, Coustham V, Dean C, Mylne JS. 2011. Protocol: A simple phenol based for 96- well extraction of high quality RNA from Arabidopsis. Plant Method 7:7.

[2] Chan KL, Ho CL, Namasivayam P, Napis S. 2007. A simple and rapid method for RNA isolation from plant tissues with high phenolic compounds and polysaccharides. Protoc Exch 184.

[3] De Vries S, Hoge H, Bisseling T. 1993. in Plant Molecular Biology manual, edited by SB Gelvin, RA Schiport and DPS Verma. Kluwer Academic Publisher, Dordretch 1-13.

[4] Forestry/ Fuelwood Research and Development Project (F/FRDP). 1992. Growing multipurpose trees on small farms. Bangkok. Thailand. Winrock international. (http://agrss.sleman.hawaii.edu/onfarm/tree/tree0012.html).

[5] Fuglie JL. 1999. Natured nutrition for Tropics. Training Manual Church world service, Dakar The multiple attributes of Moringa 172.

[6] Fuglie JL. 2001. The Miracle tree: Moringa oleifera: Natured nutrition for Tropics. Training Manual Church world service, Dakar, Senegal.

[7] Gopalan C, Rama sastri BV, Balasubramanian SC. 1971. Nutritive value of Indian foods.

[8] Hartwell JL. 1967-1971. Plants used against cancer. A Survey. L. Loydia, 30034.

[9] http://WWW.Moringa.rubiconhealth.Org.

[10] Jones S, Iannetta M, Woodhead M, Davies V, McNicol J, Taylor A. 1997. The isolation of RNA from raspberry (Rubus idaeus) fruit. Mol Biotechnol 8:219-221.

[11] Li B, Wang B, Tang K, Wei J. 2006. A simple and convenient approach for isolating RNA from highly viscous plant tissue rich in polysacharrides. Colloid Surf B: Biointerfaces 49:101-5.

[12] Logemann J, Schell J, Willmitzer L. 1987. Improved method for the isolation of RNA from plant tissues. Ann Biochem 163:16-20.

[13] Loomis WD. 1974. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. Method Enzymol 31:528-544.

[14] Manuel GC, Francisco MC. 1999. RNA isolation from plant tissues: a practical experience for biological undergraduates. Biochem Educ 27:110-113.

[15] Pawlowski K, Kunze R, Vries S De, Bisseling T. 1994. Isolation of total, poly(A) and polysomal RNA from plant tissues in S. B. Gelvin and R. A. Schilperoort (ed.) Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordretch.

[16] Porbeski S, Bailey LG, Baum BR. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol component. Plant Mol Biol Rep 15:8-25.

[17] Sambrook J, EF Fritsch, T Maniatis 1989. Molecular cloning: A laboratory manual. Cold spring harbor Laboratory Press Vol 1 and 3.

[18] Soni R, Murray J AH. 1994. Isolation of intact DNA and RNA from plant tissues. Ann Biochem 218:474.

[19] Pirro S, Minutolo A, Galgani A, Potesta M, Colizzi V, Montesano C. 2016. Bioinformatics prediction and experimental validation of micromas involved in cross-kingdom interaction. J comput biol 23:1-14.

[20] Tian Y, Zeng Y, Zhang J, Yang C, Yan L, Wang X, Shi C, Xei J, Dai T, Peng L, Zeng Y, Huan, Xu A, Huang Y, Zhang J, Max, Dong Y, Hao S, Sheng J. 2015. High quality reference genome of drumstick tree (*Moringa oleifera*), A potential perennial crop. Sci CHINA LIFE Sci 58:627-638.

[21] Verma SC, Banerji R, Misra G, Nigam SK. 1976. Nutritional value of Moringa. Curr Sci 45:769-770.

[22] Wang CS, Vodkin L. 1994. Extraction of RNA from tissues containing high levels of procyanidins that bind RNA. Plant Mol Biol Rep 12:132-145.

[23] Yang G, Zhou R, Tang T, Shi S. 2008. Simple and efficient isolation of high quality total RNA from *Hibiscus tiliaceus*, a mangrove associate and its relatives. Prep Biochem Biotechnol 38:25-264.

[24] Zeng Y, Yang T. 2002. RNA Isolation from highly viscous samples rich in polyphenols and polysaccharides. Plant Mol Biol Rep 20:417a–417e.