

Research Article

Optimization of total RNA extraction protocol from Ziarat Juniper (*Juniperus excelsa* M.Bieb)

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Abstract

Ziarat Juniper (*Juniperus excelsa*) forest, in Balochistan Province, has importance because of the second largest Juniper forest in the world and one of the oldest slow growing Juniper trees of the world having trees as old as 2500 to 3500 years. Total RNA extraction from Ziarat Juniper will be the entrance of transcriptome world to reveal functional genome of this important plant (*Juniperus excelsa*). But its high phenolic compounds and polysaccharides contents cause rapidly bind or co-precipitate with RNA during RNA extraction. So standard protocols for RNA extraction do not produce high quality total RNA. In this study, an optimized total RNA extraction protocol based on used of CTAB (Cetyl Trimethyl Ammonium Bromide) PVP (Polyvinyl Pyrrolidone) and 2-mercaptoethanol, in the extraction buffer and an extraction with organic solvents (Phenol and Chloroform) is described for *Juniperus excelsa* shoot. Agarose gel electrophoresis clearly showed 28S and 18S ribosomal RNA bands and produced RNA with high yield (1.5-2.0 µg/µl) and high quality (1.9-2.0 A260/280 ratio). Thus this protocol for RNA extraction is suitable for molecular analysis of *Juniperus excelsa*.

Keywords: *Juniperus excelsa*; Phenolic compounds; RNA extraction

Introduction

Junipers are coniferous plants in the genus *Juniperus* of the family Cupressaceae [1]. The genus *Juniperus* comprises about 70 species [2] which occur throughout the northern hemisphere of the world [3]. Shrubs and trees in this genus are slow growing [4] and can live more than 1,500 years [5]. Junipers produce a variety of

wood and non-wood forest products of value to human societies [6, 7].

In Pakistan Juniper forests of Balochistan is our national and biological heritage [8] and considered as one of the world's largest, oldest, drought resistant tree species [9]. Balochistan has approximately 141,000 hectares of *Juniperus excelsa* forests, out of which approximately 86,000 hectares of these are found in Ziarat and Loralai districts. The

Ziarat Juniper is dry temperate Juniper forest [10] with evergreen *Juniper excelsa* as dominant species.

Higher trees are rich in secondary metabolites, polysaccharides and polyphenolics. *Juniperus* like many higher plant species, contain abundant polyphenolic and polysaccharide compounds. Daniel and justyna [11] reported $2.40 \pm 0.23\%$ to $3.43 \pm 0.17\%$ phenolic compounds in sprouts of *Juniperus communis* L. and Reza et al., [12] reported 1.85 ± 0.02 mg/g \pm SD phenolic compounds in crude extract of *Juniperus excelsa* fruit.

The isolation of high-quality total RNA is necessary for downstream molecular research. The isolation of total RNA from many plants specially the higher plants is very difficult because they contain high quantities of metabolites, e.g., phenolics and polysaccharides that bind with RNA during extraction. Several studies describe extraction techniques for specific plant tissues that creates problems for the isolation of high-quality RNA [13-20]. Some RNA extraction protocols for plants rich in phenolics and polysaccharides use sodium dodecyl sulfate (SDS), soluble polyvinyl pyrrolidone (PVP) and ethanol precipitation [21] Chomczynski and Sacchi [22] modified an acid guanidinium thiocyanate-phenol-chloroform method and Wan and Wilkins [23] modified the hot borate method. One modified protocol used acetone treatment of freeze-dried and powdered plant materials [24] and another modified the cetyl trimethyl ammonium bromide (CTAB) method [25, 26].

In this study modified protocol to isolate high yield and purified total RNA from *Juniperus excelsa* is described which is suitable for further downstream molecular research.

Materials and Methods

Sample collection

Samples (Shoots) of *Juniperus excelsa* were collected from Sasnamana Valley, Ziarat, Balochistan. The collected samples were stored in air sealed polythene bags.

Reagents and solutions

An extraction buffer consisting of 3% CTAB (w/v), NaCl (2 M), 100 mM Tris-HCl pH 8.0 and 25 mM EDTA pH 8.0 was prepared. After being autoclaved for 20 min, 3% PVP (w/v), 2% (v/v) 2-mercaptoethanol and 2% SDS (w/v) were added to the extraction buffer. In addition, acidic Phenol: chloroform: isoamyl alcohol (25:24:1, v/v/v), chloroform: isoamyl alcohol (24:1, v/v), Ethanol (70%, 100%), Sodium acetate (5M) solution (pH 8.0), DEPC and TAE buffer were the additional solutions used in this optimization protocol.

Total RNA extraction

Glass material and mortar & pestle were treated with 0.1% DEPC-treated water and autoclaved. RNA was extracted by following method.

Almost 100 mg frozen tissue per sample was ground in 1 ml extraction buffer (3 % CTAB (w/v), NaCl (2 M), 100mM Tris-HCl pH 8.0, 25mM EDTA pH 8.0, 3% PVP (w/v), 2% (v/v) 2-mercaptoethanol and 2% SDS (w/v). The mixture was vigorously mixed and incubated at room temperature for 10 min with 2-3 times vortex and centrifuged for 15 min at 13,000 rpm at 4°C. The supernatant was transferred to a new 1.5ml Eppendorf tube and extracted with equal volume of chloroform: isoamyl alcohol (24:1, v/v) thoroughly mixed and centrifuged for 15 min at 13,000 rpm at 4°C. The supernatant was again transferred to a new tube and equal volume of acidic phenol/chloroform/isoamyl alcohol (PCI) (25:24:1, v/v/v) was added mixed thoroughly and Centrifuge at 13000rpm for 20 min at 4°C. The upper aqueous phase was transferred to a new tube. RNA was

precipitated with 20ul of 5M sodium acetate and twice volume of 100% ethanol and the mixture was left overnight at -20°C. After overnight incubation at -20°C, RNA was collected by centrifugation at 13,000 rpm for 20 min at 4°C. The pellet was washed with 70% ethanol by centrifuging at 13,000 rpm for 10 min at 4°C. Ethanol was discard and pellet was allow to air dry. After air drying the RNA was dissolved in 80ul DEPC treated water and stored at -20°C.

Quantitative and Qualitative analysis

Quantitative analysis of RNA was performed by measuring optical density at 260 nm and 280 nm using Genova Nano spectrophotometer.

Polysaccharide contamination was determined by maximum absorbance measurement at 230 nm. Ratio measurements at wavelengths 230, 260 and 280 indicated degree of RNA purity. The absorption ratio $A_{260}/230$ indicated polysaccharide/polyphenolic contaminants and $A_{260}/280$ indicated protein contaminates [25, 26]. Total RNA were loaded on a 1% agarose gel, stained with ethidium bromide (EtBr) [27] electrophoresed to separate RNA, and visualized under UV light to assess the integrity of ribosomal bands.

Results and Discussion

In this study a modified method was applied to the isolation of RNA from *Juniperus excelsa* containing high amounts of polysaccharides and phenolic compounds. Similar study was done by Chun *et al.*, [28] in fruit trees and Evi *et al.*, [29] in different plant tissues that contain large amounts of polysaccharides and polyphenol compounds. Lee, *et al* [30] developed an optimized RNA isolation protocol for peach fruits based on several protocols developed for isolating RNA from grape berries, pine trees, and woody plants *Cinnamomum tenuipilum* [31-33]. The isolated RNA was of high quality as it showed a reading in between 1.8 to 2.1 after calculating the ratio of absorbance 260/280 nm.

The ethidium bromide fluorescence of the two ribosomal RNAs (28S and 18S) indicated that the RNA was not degraded and was of good quality (Fig. 1). This modified protocol made it possible to extract high quality RNA from *Juniperus excelsa*, which can be used in future molecular characterization and further downstream applications.-

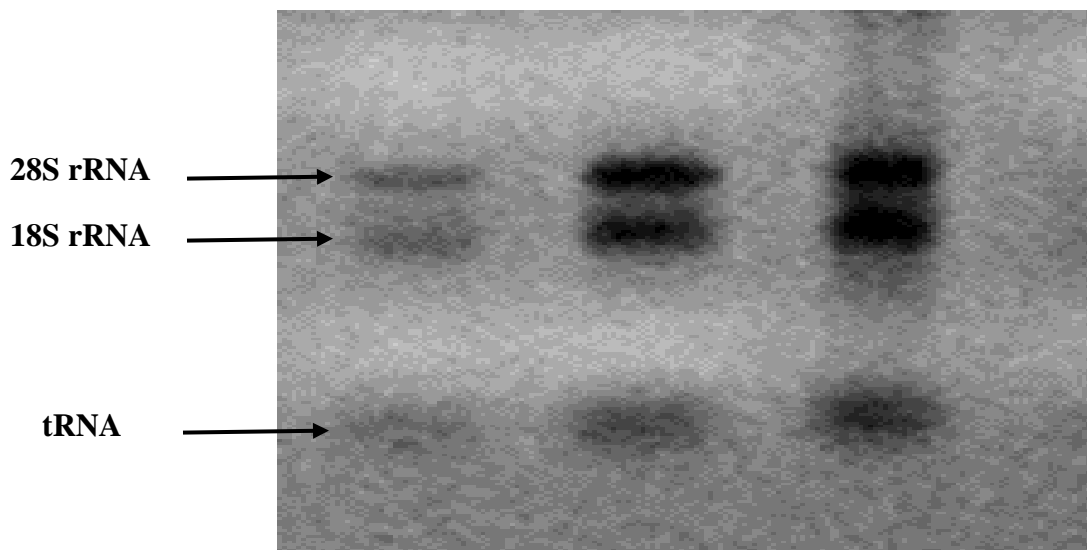


Figure-1 Total RNA was extracted from the shoots of Juniper tree (*Juniperus excelsa*) and run on 1% agarose gel, stained with ethidium bromide, showing two intact rRNA bands

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