

SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE ESTIMATION OF DNA ISOLATED FROM VARIOUS PARTS OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Plants are attracting more attention among contemporary pharmacy scientists because some human diseases resulting from antibiotic resistance have gained worldwide concern. A number of methods are available and are being developed for the isolation of nucleic acids from plants. *Catharanthus* is in the Apocynaceae family, well known for being rich in alkaloids. Vinca alkaloids are used widely in the treatment of both childhood and adult cancers. Due to biochemical organization they target the α / β tubulin subunit of heterodimers, and they act to inhibit cell division by disrupting microtubule dynamics. The different parts of *Vinca rosea* were studied for their nucleic acid content by using spectrophotometric analysis. In order to measure DNA content of the Leaves, Roots and Flowers of Vinca, Spectrophotometry serves various advantages i.e non-destructive and allows the sample to be recovered for further analysis or manipulation. Spectrophotometry uses the fact that there is a relationship between the absorption of ultraviolet light by DNA/RNA and its concentration in a sample. This article deals with a modern approaches to develop a simple, efficient, reliable and cost-effective method for isolation, separation and estimation of total genomic DNA from various parts of the same species.

Keywords: *Catharanthus*, *V.rosea*, Genomic DNA extraction & Spectrophotometric

INTRODUCTION

The World Health Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines. *Catharanthus roseus* (Vinca rosea) a traditionally used medicinal plant, belongs to the family Apocynaceae, is an erected procumbent herb or under shrub containing latex. It is an evergreen shrub or herbaceous plant growing to 1 m tall. The leaves are oval to oblong, 2.5–9 cm long and 1–3.5 cm broad, glossy green, hairless, with a pale midrib and a short petiole 1–1.8 cm long; they are arranged in opposite pairs. The flowers are white to dark pink with a darker red centre, having a basal tube 2.5–3 cm long and a corolla 2–5 cm diameter with five petal-like lobes^{2,3}. The fruit is a pair of follicles 2–4 cm long and 3 mm broad. *Catharanthus roseus* has been found to contain as many as 130 constituents with an indole or dihydroindole structure. The principal component is vindoline (up to 0.5%); other compounds are serpentine, catharanthine, ajmalicine (raubasine), akuammine, lochnerine, lochnericine and tetrahydroalstonine. Ajmalicine and serpentine are essentially present in the roots, whereas catharanthine and vindoline accumulate in aerial parts. The aerial parts contain 0.2–1% alkaloid^{4,5}. This plant has possesses known antibacterial, antimicrobial, antifungal, antioxidant, anticancer and antiviral activities⁶.

DNA is polymer found in all living cells. DNA contains all genetic information needed for controlling cellular growth and development. Many protocols have been used in plant DNA isolation, but because of the chemical heterogeneity of the species many of them could be applied to a limited number of species or even closely related species in some cases fail to respond to the same protocol⁷. Plants, especially medicinal plants contain an array of secondary metabolites. The compounds which make them interesting for molecular biology studies and hence, for DNA isolation, themselves interfere with the DNA isolation procedure. The objective of many bioassay methods is to selectively quantitate a single biomolecule, such as a particular enzyme or antibody, or to determine the presence or absence of a known DNA sequence in an unknown sample⁸. The present study deals with modern approaches to develop a simple, efficient, reliable and cost-effective method for isolation and estimation of total genomic DNA from various parts of the same species.

MATERIALS AND METHODS

Plant material

To facilitate better homogenization leaves, roots and flowers were used for the experimental study. For comparing DNA concentrations plant material was collected from the same plant. The plant material was sterilized with distilled water and external moisture from the leaves, roots & flowers were allowed to dry.

Reagents and chemicals

The following chemicals and reagents were used: lysate buffer (autoclaved) [1.4 M Sodium Chloride, 20 mM EDTA, 0.02 M Sod. Citrate, 2% CTAB and 100 mM Tris-HCl pH8]. Ethanol, Diphenylamine and Glacial acetic acid. All chemicals were obtained from Shyam brothers, 27- Sindhi market, Bhopal (M.P.).

DNA isolation protocol

The plant material was cut into small pieces of about 2–3 mm sq. [1.4 M NaCl, 20 mM EDTA, 0.02 M sod. Citrate, 2% CTAB and 100 mM Tris-HCl pH8]. Above tissues separately suspended into prepared lysate buffer & homogenized in blender. The mixture was centrifuged at 5000 rpm for 10 minutes and the aqueous phase was transferred to a new tube containing 0.2 volume CTAB Solution (5% (w/v) CTAB, 0.7 M NaCl). They were mixed together and added 0.01% of pepsin enzyme solution. Again centrifuged and collected the aqueous phase to a new tube. When the supernatant had become clear, DNA was precipitated using double volumes of 95% cold ethanol. The test tubes were left for 5 min and observed the white webby mucus like interference formation which was separated by using micropipette into another test tube. This was best stored in PBS (pH=7.4) or 0.9% saline⁹.

Qualitative estimation of Nucleic acid

Killer – Killani Test

Sample with 1 ml of glacial acetic acid containing one drop of 1% ferric chloride solution. Under lay the mixture with 1 ml of concentrated sulphuric acid from the side wall of tube, a brown ring at the interface indicates a deoxy-sugar (Pentose sugar) characteristic of every nucleic acid¹⁰.

Diphenylamine (DPA) Test

Sample with DPA reagent [1 gm DPA + 50 ml glacial acetic acid + 2.5ml conc.H₂SO₄].Placed above mixture in boiling water bath for few min. A blue colour observed confirm the presence of DNA¹¹.

Gel Electrophoresis

1.2% (w/v) agarose was dissolved in 1X TAE buffer (40 mM Tris-Acetate, 1 mM EDTA) by heating in microwave oven for about 2 minutes. It was then cooled to about 50°C before 1 mg/ml Ethidium bromide (EtBr) was added. EtBr was included in the gel matrix to enable fluorescent visualization of the DNA fragments under UV light. The warm gel solution was poured onto casting tray to solidify. The DNA samples were mixed with 2 µl loading dye (50% (v/v) glycerol, 1 mM EDTA, 0.4% (w/v) bromophenol blue, 0.4% (w/v) xylene cyanol) and loaded into the sample wells. Agarose gel was submerged in electrophoresis buffer (TAE buffer) in a horizontal electrophoresis apparatus. The gel was run at 80 volt for about 45 minutes. When electrophoresis was done, the gel was placed on a UV transilluminator (Jyoti Scientific Ltd.) to visualize the fluorescent bands of ethidium bromide-stained DNA separation¹².

Quantitative estimation of DNA

100 mg of calf thymus DNA (oxford Lab. Reagent) was dissolved in 100ml distilled water (1mg/ml Primary stock solution) then pipette out 1ml Primary stock solution of and make up the volume up to 10ml with distilled water. Now prepared different dilution ranging from 20-100µg/ml. The absorbance was measured at 260 nm by using UV-Spectrophotometer (Shimadzu 1700). In this method, the absorbance of the unknown sample in a 1-cm cuvette was measured at 260 and 280 nm. The A₂₆₀/A₂₈₀ nm values were determined¹³.

RESULTS AND DISCUSSION

The differences in quality and quantity of isolated DNA observed in gel electrophoresis. DNA samples are subjected to agarose gel electrophoresis and subsequently stained with ethidium bromide. The dye intercalates into the DNA double helix, and the intensity of fluorescence induced by UV light is proportional to the amount of DNA in the corresponding lane. Comparison to a dilution series of standards, e.g., λ-DNA, gives an estimate of the amount of DNA in an unknown sample. Modified CTAB method gave good quality of DNA. This method was determined to be the best method for *Catharanthus roseus* DNA isolation. This is because, it could be clearly seen from the gel electrophoresis (figure 4) that the DNA band obtained from the modified procedure yield the highest quantity of DNA. There was no smear of protein interference for the DNA obtained using CTAB. The size of isolated DNA was about ±23000 bp. In all three explants portion of plant A₂₆₀/A₂₈₀ ratio of ranges 1.6 to 1.9 (Average about 1.8) indicating the level of purity of

DNA (Table 2). The DNA obtained was unshared, showing little or no RNA contamination¹⁴. UV absorbance ratio for protein contamination (A₂₆₀/280) and carbohydrate contamination (A₂₆₀/230) . For a good and clean preparation of nucleic acid, the A₂₆₀/280 ratio, which represent protein contamination, should be between 1.8 to 2.0 while the A₂₆₀/230 ratio, which represent carbohydrate contamination, should be more than 2.0¹⁵. The quantization of the obtained DNA from leaves, roots and flower were found to be 59.18, 4.27 & 44.26 µg/ml respectively (Table 3). Poor root DNA quantity could be due to certain reasons like mixing of RNA or protein, improper expression of transcription factor or secondary metabolite interferences. *Catharanthus roseus* plant which is the sources of natural products or bioactive substances produced a large amount of secondary metabolites and substances of medicinal important. The cells of the plant are known to contain high concentrations of polysaccharides in addition to the active metabolites, complicating the problem of DNA isolation. Thus, problems are encountered arising from the presence of polyphenols, polysaccharides and other secondary metabolites¹⁶. This indicated that the isolated DNA was amenable to further processing in cloning experiments as well as DNA fingerprinting

CONCLUSION

In conclusion, these results show that leaves and young flower petals can be an alternative source for total genomic DNA from medicinal and succulent plants that contain high quantities of secondary metabolites. Leaves and Flower petals from succulent plants were easier to crush and grind under liquid nitrogen as well as lyses in buffer than succulent tissues. The isolated genomic DNA was of high molecular weight and the amount increased proportionally as the amount of petals tissue increases. This technique measures the total amount of nucleic acids in a sample (including DNA, RNA, oligonucleotides, and mononucleotides). It is therefore only useful for pure DNA preparations of a reasonably high concentration. This technique allows, at the same time DNA quantization, estimation of the extent of contamination by RNA, evaluation of DNA quality and integrity (i.e., the extent of degradation). DNA fingerprinting has used to elucidate genetic relationships at various taxonomic levels and also helpful in phylogeographic studies which can be based on information from nuclear DNA, mt-DNA, and cp-DNA. This protocol will be used in future to isolate genomic DNA from tested and other related plant species for downstream molecular biology studies and can probably be extended also to other angiosperm species.

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Table 1: Qualitative estimation of DNA

S. No.	Test	Observation	Inference
1.	Killer –Killani Test	A brown ring at the interface	Indicates a deoxy sugar (Pentose sugar)
2.	DPA Test	Blue colour observed	Presence of DNA

Table 2: Comparison of DNA quantity obtained following the present plant DNA isolation protocol with other routine methods.

S. No.	Type of the tissue	Absorbance at 260nm	Absorbance at 280nm	A ₂₆₀ /A ₂₈₀	Inference
1.	Leaves	3.61	1.81	1.9	The DNA obtained was unshared, showing little or no RNA contamination
2.	Roots	0.261	0.14	1.8	The DNA obtained was unshared, showing little or no RNA contamination
3.	Flowers	2.7	1.68	1.6	The DNA obtained was unshared, showing little or no RNA contamination

Table 3: Quantitative estimation of DNA

S. No.	Type of the tissue	Absorbance at 260nm	Statistical Analysis	Concentration (µg/ml)
1.	Leaves	3.61	Correlation coefficient	59.18
2.	Roots	0.261	R ² = 0.998	4.27
3.	Flowers	2.7	Straight Line equation y= 0.061x	44.26

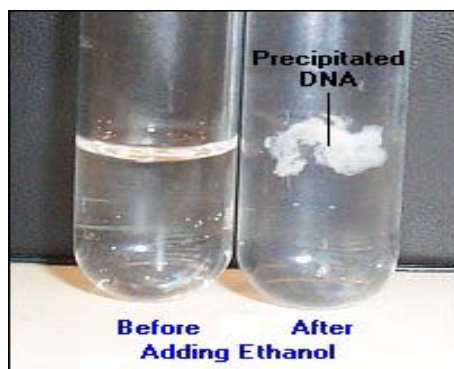


Fig. 2: precipitation of DNA

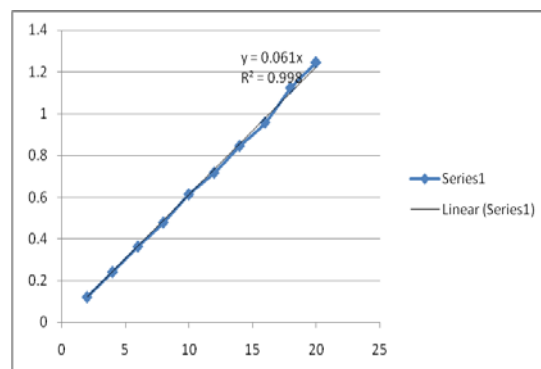


Fig. 3: Standard curve of DNA



Fig. 4: DNA isolated from different parts of same plant species resolved on 0.8% agarose gel

- D DNA molecular weight marker
- C Plant DNA isolated from leaves
- B Plant DNA isolated from roots
- A Plant DNA isolated from flowers

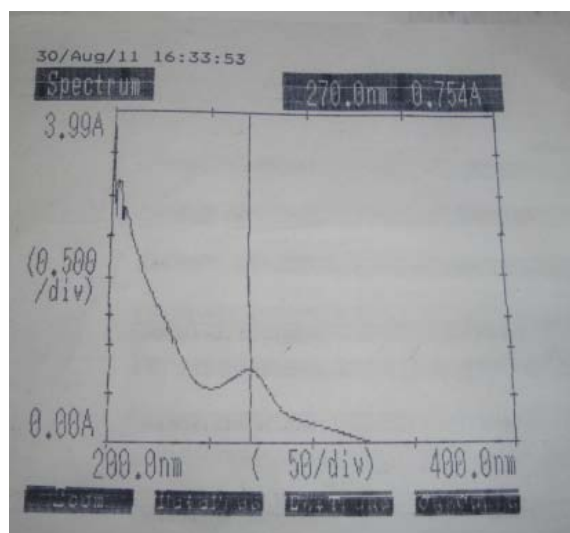


Fig. 5: UV Scan of the sample

REFERENCES

1. Rout SP, Choudary AK, Kar DM, Das L, Jain A. Plants in traditional medicinal system - Future source of new drugs.2009; IJPPS. 1(1):2.
2. Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethanopharmacology.2008; 115: 173 – 183.
3. Marcone A, Ragozzino E, Seemuller S. Dodder transmission of alder yellows phytoplasma to the experimental host *Catharanthus roseus* (periwinkle).Forest Pathology. 1997; 27 (6): 347–350.
4. Gordon H, Svoboda NN, Marvin G. Alkaloids of *Vinca rosea* Linn. (*Catharanthus roseus* G. Don.) V. Preparation and characterization of alkaloids. Journal of Pharmaceutical Sciences.2006; 48(11): 659–666.
5. Bruneton J, translated by Caroline K. Hatton, *Phytochemistry Medicinal Plants*. 2nd Edn.New York: Lavoiser Publication;1999.
6. Namvaran AA, Nouri MHK,Tavakkoli F.Study of Matricaria recutita and Vincristine effect on PTZ induced seizure threshold on mice.IJMS.2011;5(5):1.
7. Weishing K, Nybom H,Wolff K, Meyer W. DNA isolation and purification. In: DNA fingerprinting in plants and fungi. 2 edn. Florida :CRC Press Boca Raton;1995.
8. Alexander RR and Griffiths JM. Basic Biochemical Methods. 1 edn.Nwe York:Wiley-Liss; 1993.
9. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA ,Struhl K. Current Protocols in Molecular Biology. New York City: John Wiley & Sons Inc; 1994.
10. Harborne J B. Phytochemical Methods. 2 Edn . London: Chapman & Hall;1991.
11. Gendimenico GJ, Bouquin PL, Trampusch KM.Diphenylamine-colorimetric method for DNA assay: A shortened procedure by incubating samples at 50°C. Analytical Biochemistry. 1988;173:45-48.
12. Moyo M, Amoo SO, Bairu MW, Finnie JF, Van Staden J. Optimising DNA isolation for medicinal plants. South African Journal of Botany.2008; 74: 771-775.
13. Doyle J J, Doyle J L. Isolation of plant DNA from fresh tissue. Focus.1990;12:13-15.
14. Dellaporta S L,Wood J,Hicks J B. A plant DNA miniprep: Version II. Plant .Mol. Biol. Repr. 1983;1:19-21.
15. Sambrook J, Russel DW . Molecular cloning: a Laboratory Manual. Third Edn.New York: Cold Spring Harbor Laboratory Press New; 2001.
16. Khanuja suman P S, Shasany A K, Darokar M P, Kumar Sushil. Rapid Isolation of DNA from Dry and Fresh Samples of Plants Producing Large Amounts of Secondary Metabolites and Essential Oils. Plant Molecular Biology Reporter. 1999; 17:1–7.