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QUANTITATIVE ESTIMATION OF DNA ISOLATED FROM LEAVES AND STEMS OF *COLEUS AROMATICUS*

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ABSTRACT
Medicinal plants play a vital role to preserve human health. The genus, *Coleus* consists of herbs, that are widespread in all over India and represents highly valuable plant species having therapeutic and neutraceutical importance. Genetic variation is essential for long term survival of species and it is a critical feature in conservation. For efficient conservation and management, the genetic composition of the species in different geographic locations needs to be assessed. 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**.** The different parts of *Coleus aromaticus* were studied for their nucleic acid content by using spectrophotometric analysis. In order to measure DNA content of the Leaves and stems of *C.aromaticus*, 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 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: *Coleus aromaticus*, Genomic DNA extraction and Spectrophotometric

INTRODUCTION

Natural products are the source of synthetic and traditional herbal medicines [1]. They are still the primary health care system in some parts of the world [2]. In India, local impirical knowledge about medicinal properties of plants is the basis for their uses in home remedies [3]. The genus *Coleus* was first described by De Loureiro (1970). The name *Coleus* is derived from the Greek word *Koleos*, which means sheath around the style[4]. There are about 150 plants belonging to the mint herb family. Today, there are more than 500 varieties of coleus in cultivation all over the world. *Coleus* plants are very colorful and can be grown in-door as well as outdoors. Medicinal plants have curative properties due to the presence of various complex chemical substances of different chemical nature, which are found as secondary plant metabolites in one or more parts of these plants[5]. *Coleus aromaticus Benth. syn. C. amboinicus Lour, Plectranthus amboinicus (Lour.) Spreng.* English: Country borage, Indian borage; Sanskrit: Karpuravalli, Sugandhavalakam; Hindi: Patharchur; Bengali: Paterchur; Malyali: Panikkurkka, kannikkurkka; Tamil: Karpuravalli. It is found throughout the tropics and cultivated in homestead gardens. It is a large succulent aromatic perennial herb with hispidly villous or tomentose fleshy stem. Leaves are simple, opposite, broadly ovate, crenate and fleshy. Flowers are pale purplish in dense whorls at distant intervals in a long slender raceme. Fruits are orbicular or ovoid nutlets. The leaves are useful in cephalagia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea, cholera, halitosis, convulsions, epilepsy, cough, asthma, hiccough, bronchitis, strangury, hepatopathy and malarial fever[6]. 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 protocol [7].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 sample[8]. The aim of 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 species.

MATERIALS AND METHODS

Plant material: To facilitate better homogenization leaves and stems 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 & stem 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 Sodium Citrate, 2% CTAB and 100 mM Tris-HCl pH8]. Ethanol, diphenylamine, glacial acetic acid and all other 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 sodium 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 volumes CTAB Solution (5%w/v CTAB and 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% saline [9].

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 acid [10].

Diphenylamine (DPA) Test: Sample with DPA reagent [1 gm DPA + 50 ml glacial acetic acid + 2.5ml conc.H_2SO_4].Placed above mixture in boiling water bath for few min. A blue colour observed confirm the presence of DNA[11].

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^{o}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 illuminator (Jyoti Scientific Ltd.) to visualize the fluorescent bands of ethidium bromide-stained DNA separation [12].

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 and made up the volume to 10ml with distilled water. Aliquots of solutions prepared 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 A_{260}/A_{280} nm values were determined[13].

RESULTS AND DISCUSSION

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 *C.aromaticus* 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 ±1200 bp. In leaves and stem explants portion of plant A_{260}/A_{280} ratio of ranges 1.6 to 1.9 (Average about 1.8) indicating the level of purity of DNA (Table 2 & Table 3). The DNA obtained was unshared, showing little or no RNA contamination[14].

For a good and clean preparation of nucleic acid, the $A_{260/280}$ ratio, which represent protein contamination, should be between 1.8 to 2.0 while the $A_{260/230}$ ratio, which represent carbohydrate contamination, should be more than 2.0[15].The quantization of the obtained DNA from leaves and stem were found to be 3.7 &0.40 µg/ml respectively(Table 4). Poor stem DNA quantity could be due to certain reasons like mixing of RNA or protein, improper expression of transcription factor or secondary metabolite interferences. *C.aromaticus* plant which is the source of natural products or bioactive substances produced a large amount of secondary metabolites and substances of medicinal importance. 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 metabolites[16].This indicated that the isolated DNA was amenable to further processing in cloning experiments as well as DNA fingerprinting. The isolated DNA can be amplifying for producing molecular marker. Molecular markers have been shown to be useful for genetic variation of plant species. Several different PCR- based techniques have been developed during the last decade, each with specific advantages and disadvantages. The randomly amplified polymorphic DNA (RAPD) markers technique is quick, easy and requires no prior sequence information; it detects nucleotide sequence polymorphisms using single primer of arbitrary nucleotide sequence[17]. RAPD markers have been extensively used for DNA fingerprinting.

CONCLUSION

In conclusion, these results show that leaves can be an alternative source for total genomic DNA from medicinal and succulent plants that contain high quantities of secondary metabolites. Leaves 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, mtDNA, and cpDNA. Phylogenetic variations were also determined in *coleus* species by DNA typing. 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

Test	Observation	Inference
Killer –Killani Test	A brown ring at the interface	Indicates a deoxy sugar (Pentose sugar)
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.

Type of the tissue	Absorbance at 260nm	Absorbance at 280nm	$A_{260/280}$	Inference
Leaves	0.21	0.116	1.8	The DNA obtained was unshared, showing little or no RNA contamination
Stem	0.024	0.0126	1.9	The DNA obtained was unshared, showing little or no RNA contamination

Table 3: Comparison of DNA quantity obtained following the present plant DNA isolation protocol with other routine methods(Carbohydrate contamination).

Type of the tissue	Absorbance at 260nm	Absorbance at 230nm	$A_{260/230}$	Inference
Leaves	0.21	0.15	1.4	The DNA obtained was unshared, showing little or no carbohydrate contamination
Stem	0.024	0.017	1.4	The DNA obtained was unshared, showing little or no carbohydrate contamination

Table 4: Quantitative estimation of DNA

Type of the tissue	Absorbance at 270nm	Statistical Analysis	Concentration (µg/ml)
Leaves	0.23	Correlation coefficient = 0.998	3.7
Stem	0.025	Straight Line equation y= 0.061x	0.40

Fig1: ***Coleus aromaticus***

Fig 2: Addition of chilled ethanol

Fig 2: Precipitation of DNA

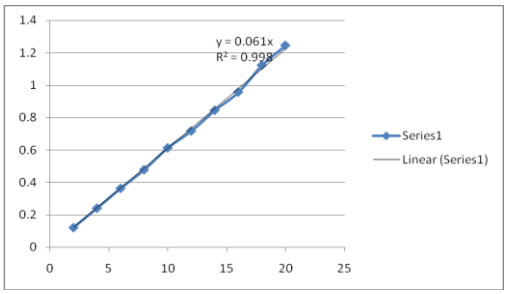

Fig 3: Standard curve of DNA

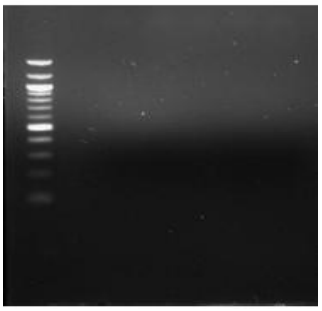

Fig :5 DNA isolated resolved on agarose gel.

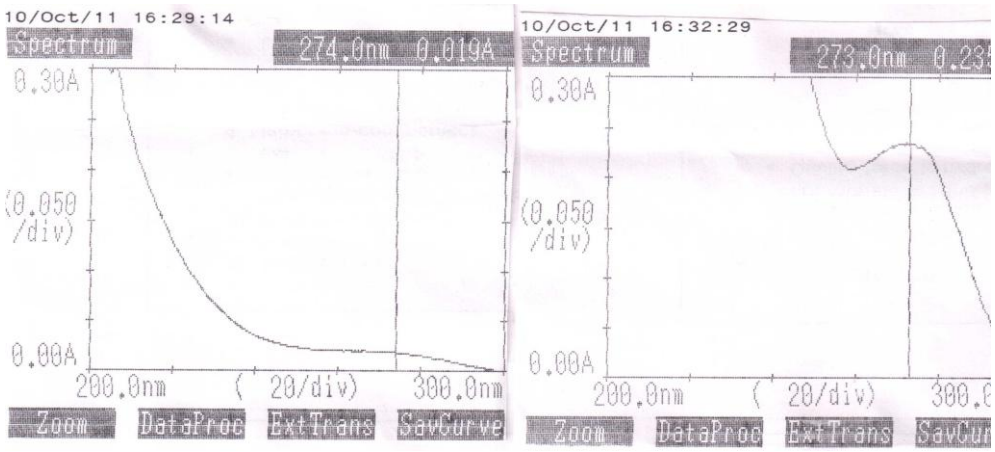

Fig:4 UV scanning of Stem DNA **UV scanning of Leaves DNA**

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