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EXTRACTION AND COMPARISON OF QUALITY AND PURITY OF DNA OF MEDICINAL PLANTS BY CTAB AND KIT METHOD

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ABSTRACT

Background: Today is a world of bioengineering. Man is playing with DNA. Extraction and isolation of DNA has become as a key part for the researches of modern times. One needs high quality and increased quantity of DNA for performing several genetic techniques. There are several methods through which DNA can be extracted but the quality of the DNA cannot be compromised. **Material methods:** In the current research work extraction of DNA from four medicinal important plants *Cassia fistula*, *Saccharum officinarum*, *Albizia lebbbeck* and *Cymbopogon citratus* was carried out by two methods. One is a manual method that is used for the extraction of DNA from plant source; The CTAB method, and the other is extraction with the help of commercially available kit. **Results:** Results of the work showed that the high quantity of the DNA was obtained by CTAB method while DNA extracted with the kit was of better quality and purity than CTAB method. **Conclusion:** In techniques where high quality DNA is required like genetic engineering, micro arrays, extraction of DNA through kit method should be preferred, while in cases where quantity of DNA matters over its quality CTAB method is better to be used and economic.

Keywords: CTAB, DNA, *Cassia fistula*, *Albizia lebbbeck*, *Saccharum officinarum*, *Cymbopogon citrates*.

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INTRODUCTION

Today is an era of biotechnology. There is rapid advancement in the field of engineering and biotechnology [1]. Scientists are deliberately working on DNA. Pure quality DNA is required in many modern techniques, these include transgenic DNA techniques, hybrid species, fortified foods production, breeding of animals and crops with better yield [2]. All these require extracting DNA from the donor with best quality, which is then incorporated into the recipient's genome to get the products of desired quality [3].

With the deliberate use of allopathic medicines people are suffering from number of side effects. Moreover development of antibiotic resistance is also a big issue faced by the developing world. People are moving towards the therapeutic sources from the natural origin with lesser side effects. Many herbal pharmaceutical industries are also focusing on medicinal plants for the preparation of new herbal formulation for treating ailments [4].

With the advancement in the field of genetic engineering and biotechnology man is now playing with DNA of different species [5].

Medicinal plants are now targeted for genetic engineering so that plants with better therapeutic potential can be produced [6]. Medicines derived from plants parts are considered safer than the drugs from artificial origin. Plant derived medicines like antimicrobial peptides from these plants serve as the natural source, so they are considered better than the synthetic drugs [7]. These medicinal plants are now increasingly used for the treatment of several even complicated diseases like dengue etc [8]. There are several methods through which the DNA is isolated from the whole plant. Some methods result in the yield of better quality DNA while some methods yield DNA with increased quantity [9]. Selection of the appropriate method for extracting DNA is a key point for best outcomes of research. In the present research work extraction of DNA

from four medicinal important plants was carried out with the help of two methods. Comparison of the amount & quantity of DNA was done and evaluated.

MATERIAL METHODS

The work was performed in Molecular Biology Lab, in the Department Biochemistry and Biotechnology, The Women University Multan (WUM), Pakistan.

Collection of Plant Sample

Cymbopogon citrates and *Saccharum officinarum* were purchased from the local market of Bahawalpur. *Albizia lebbek* and *Cassia fistula* were collected from Dring Stadium bahawalpur. Plant's parts were identified by the Department of Botany, **The Women University Multan, Pakistan**. Fresh leaves of each plant were taken, washed with 75% ethanol, dried, cut into pieces and stored. Samples were processed for the extraction of DNA.

Genomic Study

DNA Isolation

DNA can be isolated from any part of the plant. The most convenient part that can be used for DNA extraction is the one that can be easily crushed and ground into a fine paste. So the plant leaves for selected for the extraction of plant DNA. DNA was isolated by CTAB method and by using (thermo scientific GeneJET Plant Genomic DNA Extraction Mini Kit). DNA was quantified by measuring absorbance at 260 and 280 nm (A260/A280) through spectrophotometer. Integrity of DNA was checked by performing gel electrophoresis.

DNA Extraction by Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit

Plant leaves samples were ground into a fine powder. 100mg of this powdered sample was mixed with 350 µl of Lysis buffer A, vortexed for 15 seconds and added 50µl of Lysis buffer B with 20µl of RNase A. Incubated at 65 degree for 10 minutes with intermittent mixing. Afterwards 130µl of precipitation solution was added. Mixture was centrifuged for 5 minute at 14000 rpm. Then 400µl binding solution 400µl of ethanol was added to the supernatant. Mixed and passed the solution through spin column three times and centrifuged in between for 1 minute at 8000 rpm. Solution was then washed with the help of 500µl of wash buffer. After that 50µl of elution buffer was added, incubated at 37°C for 5 minutes. Centrifuged at 12000rpm for 1 min. Repeated the elution step followed by centrifugation. Stored the elute at -20 °C.

DNA Extraction by CTAB Method

1g of powdered plant leaf sample was further ground into a fine paste in preheated CTAB buffer. Then added 1% β-mercaptoethanol and incubated at 60°C for 20 minutes. Sample was then centrifuged

for 10min. at 13,000 rpm. Top layer was mixed with equal amount of 24:1 chloroform: isoamyl alcohol. Centrifugation was carried out again for 3min. at 13,000rpm. Added 0.1 volume 3M Sodium Acetate (pH 5.5) and vortexed. Ice cold 70% ethanol was then added to allow the DNA to precipitate. Mixture was left overnight at -20 °C. Centrifuged for 10min. at 13,000 rpm Discarded the supernatant. Washed the pellet in 500µl cold 70% EtOH. Dried the pellet in the 37 °C incubator for 10 min. Added 50µl TE Buffer and stored in 4°C.

DNA Quantification

Quantification of DNA was done by spectrophotometer at the wavelength of 260 nm. Concentration of DNA was checked by using the formula.

Concentration of DNA = (OD260) x (50 ng/ul) x (dilution factor).

Where dilution factor is 1000.

Purity of DNA

Purity of DNA extracted by CTAB and kit methods were checked by the given formula. Absorbance was checked at 280 nm and 260 nm

Purity of DNA = OD260/OD280.

RESULTS AND DISCUSSION

DNA Isolation

DNA was isolated from leaves of plant by Cetyl Trimethyl Ammonium Bromide (CTAB) method (**Fig. 1**) the bands isolated from CTAB method were so bright which showed the increased quantity of DNA in the band. DNA isolated from Thermo scientific Plant Genomic DNA Extraction Mini Kit shown in (**Fig. 2**) were clear but not so bright indicating the lesser quantity of DNA.

Quantification of DNA

Out of four medicinal plants *Saccharum officinarum* extracted through CTAB method showed maximum concentration of 119300 ng/ul. *Cymbopogon citratus* extracted through CTAB method showed concentration of 110950 ng/ul, Shown in (**Table 1**). While the same plant's DNA extracted from kit showed lesser concentrations of 52050 ng/ul and 68000 ng/ul for *Saccharum officinarum* and *Cymbopogon citratus* respectively (**Table 2**).

Purity of DNA

Purity of DNA is checked by measuring the absorbance of DNA at 260 & 280 nm. The ratio of about 1.8 is considered as pure DNA. DNA extracted by CTAB method showed the purity of 0.82, 1.07, 0.887 and 1.26 for *Cassia fistula*, *Albizia lebbek*, *Cymbopogon citratus*, *Saccharum officinarum* respectively (**Table 1**). While the DNA extracted by kit showed the purity of 1.23, 1.34, 0.98 and 1.46 for *Cassia fistula*, *Albizia lebbek*, *Cymbopogon citratus*, *Saccharum officinarum* respectively (**Table 2**). Results showed that the

DNA extracted by kit method are more pure than CTAB method. There are several methods, which are used for the isolation of DNA from different plant parts [10]. However, all of these methods are not completely perfect. Some methods produce either insufficient or poor quality of DNA. Extraction of DNA from different plant's parts with good quality is often required and demand of time [11], many commercial kits are also available to extract genomic DNA from plant tissues with sufficient quality, but the yield of DNA produced from commercial kits is often low. Moreover, the cost can be prohibitive for small laboratories. Some research works require high quantity of DNA while in some researches the quality of DNA matters.

Several studies on the isolation and characterization of DNA from plant has been carried out. In one of the DNA barcoding and phylogenetic analysis of *Saccharum officinarum* was done [12]. We also carried out the extraction of DNA from different medicinal plants through different methods.

CONCLUSION

Whenever best quality of DNA is required modern kit methods should be preferred, because in most of the researches high quality DNA is very much required for the exact results. While in other researches where increase quantity of DNA is needed manual method like CTAB are to be preferred.

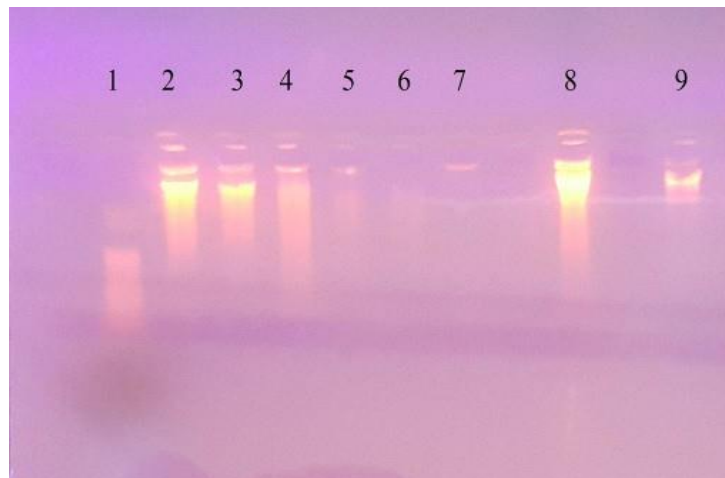


Figure 1: Gel electrophoresis of DNA isolated by CTAB method. Lane 1 shows the 100bp DNA ladder. Lane 2 shows the band of *Albizzia lebeck*, Lane 3 and 5 shows bands of *Cassia fistula*, lane 4 of *Cymbopogon citratus*, 8 and 9 shows the bands of *Saccharum officinarum*.

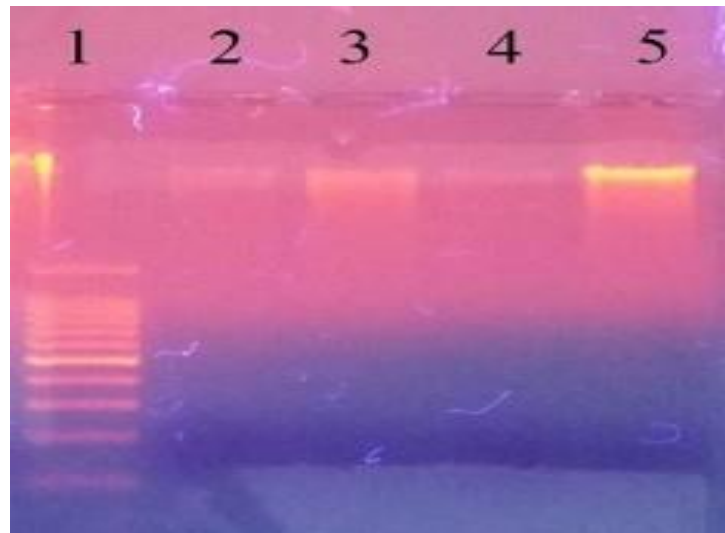


Figure 2: Gel electrophoresis of DNA isolated by Genome kit. Lane 1; 1kb DNA ladder. Lane 2; bands of *Saccharum officinarum*, lane 3; *Cassia fistula* and lane 4; *Cymbopogon citratus*. Lane 5; *Albizzia lebeck*

Table 1: Concentration and purity of DNA of plants through CTAB.

Sr no.	Plant Sample	OD260	OD280	Conc. ng/ul	Purity
1	<i>Cassia fistula</i>	1.4590	1.7590	73050	0.81
2	<i>Albizia lebbek</i>	1.630	1.5140	81401	1.08
3	<i>Cymbopogon citratus</i>	2.2180	2.5020	110951	0.8867
4	<i>Saccharum officinarum</i>	2.3870	1.8940	119300	1.2611

Table 2: Concentration and purity of DNA of plants through Kit.

Sr no.	Plant Sample	OD260	OD280	Conc. ng/ul	Purity
1	<i>Cassia fistula</i>	1.22	0.99	61000	1.23
2	<i>Albizia lebbek</i>	1.556	1.161	77800	1.34
3	<i>Saccharum officinarum</i>	1.041	0.71	52050	1.46
4	<i>Cymbopogon citratus</i>	1.363	1.390	68150	0.98

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