DNA extraction and quantification of some economically important fruits in Tamil Nadu

ABSTRACT

Humans can benefited greatly from functional foods in order to preserve good health. The research began with the extraction of DNA from the white edible section of *Cocos nucifera* and the flesh of *Borassus flabellifer* fruits using the simple, Edward, PCI, and CTAB methods. To confirm the existence of DNA, the complete isolated DNA was examined using the DPA and Keller-killani tests. Following the identification test, UV spectroscopy was used to quantify the results by taking the absorbance of the samples at 260nm and 280nm and quantified using absorbance ratio procedure. When compared to other procedures, the CTAB approach produces acceptable results at low temperatures. Both the white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* gave high purity DNA on extraction. Further the concentration of DNA obtained from *Cocos nucifera* was 4.94, 4.7, 5.25 and 8.25 µg\ml by simple, Edward PCI and CTAB methods respectively and for *Borassus flabellifer* the concentration DNA isolated was found to be 2.48, 2.14, 4.25, 6.4 µg\ml by simple, Edward PCI and CTAB methods respectively.

Key words:

Borassus flabellifer; Cocos nucifera; DNA isolation; Electrophoresis.

1. INTRODUCTION

Food and health are inextricably linked. The present notion of functional foods arose from a growing knowledge that eating nutritious foods contributes to a healthy diet, as well as research into the mechanisms by which foods affect metabolism and health. The most basic goal of eating is to obtain nutrients for our body and to meet our metabolic requirements [1]. Some foods provide additional benefits in addition to their nutritional value that has a positive impact on an individual's health [2]. The functional food may act as anti-cancer, anti-diabetes, anti-hypertension, anti-inflammation, anti-microbial, anti-viral, anti-parasitic, anti-psychotic, anti-spasmodic, anti-ulcer characteristics in many disease states in animals, and human epidemiology in addition to nutritional supply [3].

Cocos nucifera and *Borassus fabellifer* were known to be functional food not only due to their health benefits and its nutritional values, but also goes way beyond it. [4]. Copra contains approximately 65 to 75 percent oil. The spa is transformed into jaggery, vinegar, and sugar [5, 6]. In India, the coconut water and coconut kernel contain microminerals and nutrients hence it is used in Ayurvedic medicine. Which have anti-bacterial, hypoglycaemic activity [7, 8].

The Palmyra palm is a synonym of *Borassus flabellifer*. The tree is widely exploited in areas where it grows and has a very wide range of applications [9], Such as making paper, absorption of heavy and toxic metals, energy production and soil fertilizing [10,11,12] .

The central information storage system of most animals, plants, and even some viruses is DNA, or deoxyribonucleic acid. The term originates from its structure, which consists of a sugar and phosphate backbone with bases protruding from it. So "deoxyribose" is the sugar part, and "nucleic acid" is the phosphate and bases such as adenine, cytosine, thymine, and guanine, simply A, C, T, and G,. As part of those chromosomes, DNA is arranged structurally into chromosomes, which are subsequently looped around nucleosomes [13].

It is organised functionally into genes, which are pieces of DNA that lead to observable traits. And those characteristics are derived not from the DNA itself, but rather from the RNA derived from the DNA, or, more commonly, from proteins derived from the RNA derived from the DNA. Thus, the central dogma of molecular biology is that genes, which are made of DNA, are converted into messenger RNAs, which are then converted into proteins [14]. In case of functional food that are consumed in raw having the risk of exposed to allergen, in coconut allergens such as 7S and an 11S globulin

protein called Coc n2 and Coco n4. Among them the 7S globulins already been identified as an allergens so that some patients are allergic to coco nuts show. These allergens not only causes simple allergic reaction like skin rashes but also some time causes anaphylaxis shock to some patients those who are sensitive enough for a particular allergens. Hence there is need to identify the allergen and allergenic protein to qualify the food is suitable for consumption or not. In these regards various researches were reported using RT-PCR [15, 16] and UV procedure to quantify the DNA and to determine its purity [17]. Methods lie RT-PCR, fluorescence assay are time consuming and expensive techniques which is not afford by all coconut farmers to test their material in house. In all the previous report they were took leaf as the source for DNA isolation from coconut plant [22]. In case of *Borassus flabellifer* there were no reports were found yet. Fruit is edible portion of both plants which has direct risk of causing allergic reactions.

Hence in our research we reported a development of method to isolate DNA from the edible part of above plants and a method to quantify the DNA isolated and compare the efficiency of method to get high pure DNA.

2. MATERIALS AND METHODS

Plant collections and extraction

Flesh of *Borassus flabellifer* fruits and the white edible section of *Cocos nucifera* fruits were harvested from farms near Thindal, Erode. Plant material was collected and handled exclusively at room temperature for further research.

Simple method

The white edible section of the *Cocos nucifera* fruit and the flesh of the *Borassus flabellifer* fruit were extracted separately and moistened and macerated in the Eppendof tube at room temperature for 15 seconds without buffer. The material was vortexed for 5 sec after that about 400 µl of extraction buffer was added. This mixture was then left at room temperature for at least 45 minutes until all of the samples have been extracted. The extracts were separated for 1 minute at room temperature (13,000 rpm) before

being transferred to a new Eppendof tube. The supernatant was mixed with 300 µl isopropanol and left to settle at room temperature for 2 minutes. After centrifuging at (13,000 rpm) for 5 minutes, the pellet was vacuum dried and dissolved in 100 µl 1xTE. After then, the isolated DNA was stored at 4°C until next use [18].

Edward method

Separately, 50 mg of the white edible part of *Cocos nucifera* and Flesh of *Borassus flabellifer* fruit were weighed and ground with 350 µl of extraction buffer. To make it homogeneous enough to put into a micro centrifuge tube, more buffer was added until it reaches a final volume of 1100 µl. The homogenates were harvested at room temperature. In a new micro centrifuge tube, add an equal volume of isopropanol to the supernatant and gently mix by inversion. For 5 min, immerse the mixture in an ice bath. The micro centrifuge was then spun for 4 min at 13,000 rpm. After draining the supernatant, wash the DNA pellet with 500µ l of 70% ethanol (v/v). Centrifuge at room temperature (13,000 rpm) for 2 min in a micro centrifuge. Blot away any excess ethanol by inverting / placing the pellet on a clean paper towel. Allow the pellet to air dry before dissolving the extracted plant DNA in 50 µl of water and storing it at 4°C for immediate use or -20 °C for long-term storage [14].

Phenol-Chloroform-isoamylalcohol extraction method:

Tissue digestion was accomplished by introducing 50 mg of white edible component of *Cocos nucifera* to a micro centrifuge tube containing the flesh of *Borassus flabellifer* fruits. Per sample, add 400 µl of extraction buffer. Incubate at 50°C for 2 h, or until the sample is clear, after adding 10 µl of proteinase k. Add an equivalent amount of PCI to the digested DNA to be purified in a tube. After 5 min of moderate mixing and 10 min of microcentrifugation at 10,000 rpm at room temperature. Spin the DNA solution for a longer time if it is thick or contains a lot of protein. Carefully decant the DNA-containing supernatant into a new tube. Chloroform and isoamylalcohol are mixed in 24:1 proportions, 2 min of gentle mixing followed by 1 min at room temperature centrifugation (10,000 rpm) [19].

Using a 1000 µl micropipette, remove the top aqueous phase containing DNA and transfer it to a new tube. Gently mix and place it in a deep freezer overnight then spin for 20 min at maximum speed in a fixed angle microcentrifuge to remove the supernatant. The supernatant can simply be poured off if the pellet is larger in size. Separate the ethanol supernatant with a micropipette for small pellets and carefully add 1ml of 70% ethanol at room temperature. Gently invert the microcentrifuge tube. Use 95 percent ethanol at this stage if the DNA molecules being separated are very small. The supernatant is removed,

and the pellet is dried at room temperature before being dissolved in 20-40 µl of TE buffer at pH 8 and stored at 4°C [11].

CTAB Extraction method:

In a sterile Eppendorf tube, 400 µl of CTAB extraction buffer was added to 3 % Poly vinyl pyrrolidine, which was heated to 65°C. 2-mercaptoethanol (10 µl) was added to this combination right before the commencement of the extraction procedure in two tubes. The white edible component of *Cocos nucifera* and the flesh of *Borassus flabellifer* fruit were crushed into powder separately in two micro centrifuge tubes with buffer and reagents. Ground frozen tissue was incubated with a poly vinyl pyrrolidine / 2 mercaptoethanol / CTAB extraction solution that is heated (65°C). Inversion was used to gently mix the mixture on a regular basis. Add Chloroform isoamyl alcohol to the homogenate and mixed by gentle inversion, then centrifuged at room temperature (10,000 rpm) for 5 min. Pipette out the supernatant and add to a CTAB/NaCl solution (0.1 volumes at 65°C) and gently mixed by inversion. [20].

After mixing and centrifuging, the upper phase was recovered. At 65°C, one volume of CTAB precipitation solution was added to the recovered supernatant. A precipitate formed at this point. Pellet was recovered by decanting the upper phase. The samples were dissolved in 500 µl of high salt TE buffer (white edible part of *Cocos nucifera* and fruit flesh of *Borassus flabellifer*). The DNA was precipitated by adding 300 µl of ice cold isopropanol, gently mixing it in, and centrifuging it for 15 minutes at 1000 rpm. To recover the DNA pellet, the upper liquid was decanted and washed in 80 % and 100 % ethanol. After drying, the pellet was resuspended in TE buffer and stored at - 20°C [21].

QUALITATIVE ESTIMATION OF DNA:

Diphenylamine (DPA) Test:

The diphenylamine was mixed with sample, and the aforesaid mixture was placed in a boiling water bath for a few minutes. The presence of DNA is confirmed by the formation of blue colour [22].

Killer – Killani Test:

Mix the small part of DNA isolates together 1ml of glacial acetic acid with one drop of 1% ferric chloride solution and 1 ml of concentrated sulphuric acid from the side wall of tube. Brown ring at interface indicates a deoxy-sugar (Pentose sugar) characteristic of every nucleic acid [23].

DNA QUANTIFICATION USING UV SPECTROSCOPY METHOD:

All the isolated DNA from Simple, Edward, PCI and CTAB methods were subjected to UV spectrophotometer (model UV- 1800) measurement to determine the concentration of DNA samples [24]. To estimate the isolated DNA absorbance ratio procedure was followed by taking absorbance at 260 and 280 nm [25].

DNA Concentration (*μg/ml*) = ($O.D_{260}$) x (dilution factor) x [50*μg* DNA/*ml*/ (1 $O.D_{260}$ unit)]

DNA QUALIFICATION USING GEL ELECTROPOREHSIS

By heating for about 2 minutes in a microwave oven, 1.2 % w/v agarose was dissolved in TAE buffer. It was cooled to around 50°C before being mixed into the gel matrix at a concentration of 1mg/ml to allow fluorescent visualisation of the DNA fragments under UV light. 6μL of DNA were mixed with 2μL of loading dye before being loaded onto sample cells [26]. Then the gel was run at 80 volts [27].

3.RESULTS

QUALITATIVE ESTIMATION OF DNA

As the DNA isolated from white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* fruit using various methods of extraction**,** the entire extracted portion were performed with identification test in order to confirm that the isolated portion was DNA. TABLE: 1 shows the interferences for both the Keller-Killani test and Diphenylamine test (DPA).

TABLE: 1 ESTIMATION OF DNA

S.No	METHODS	REMARK	INTERFERENCE
1.	**Keller-killani test:**	Brown ring formed between two layer	Presence of de-oxy sugar.
2.	**Diphenylamine test:**	Blue colour obtained	DNA present.

QUANTIFICATION OF DNA USING UV SPECTROSCOPY

The entire DNA that has been isolated from white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* fruit using various extraction methods like Simple, Edward, PCI and CTAB methods, and then followed by the exposure of isolated DNA towards UV in order to quantify the amount of DNA that was isolated and the results were presented in the TABLE: 2 and TABLE: 3

TABLE: 2 COMPARING THE ABSORBTION VALUES OF DNA OBTAINED BY USING VARIOUS METHODS FROM WHITE EDIBLE PART OF *COCOS NUCIFERA*

S.no	Methods	Absorbance at 260nm	Absorbance at 280nm	Absorbance ratio (260/280)
1.	Simple	0.988	0.678	1.45
2.	Edward	0.949	1.036	0.91
3.	Phenol-chloroform	1.050	0.581	1.81
4.	CTAB At room temperature	1.65	0.90	1.83
	At high temperature	1.75	1.25	1.46
	At low temperature	1.95	1.02	1.91

TABLE: 3 COMPARING THE ABSORPTION VALUES OF DNA OBTAINED BY USING VARIOUS METHODS FROM FLESH OF *BORASSUS FLABELLIFER FRUIT*

S.no	Methods	Absorbance at 260nm	Absorbance at 280nm	Absorbance ratio (260/280)
1.	Simple	0.497	0.398	1.24
2.	Edward	0.428	0.605	0.70
3.	Phenol-chloroform	0.851	0.490	1.71
4.	CTAB At room temperature	1.280	0.671	1.85
	At high temperature	1.050	0.801	1.31
	At low temperature	2.12	1.13	1.87

From the results obtained from the absorbance ration the DNA concentration was calculated and the results were presented in the TABLE: 4 and TABLE: 5

TABLE: 4 CHARACTERIZATION OF EXTRACTED DNA USING VARIOUS METHODS FROM WHITE EDIBLE PART OF *COCOS NUCIFERA*

S.no	Methods	O.D $_{(260)}$value	Concentration of DNA (µg/ml)	Absorbance ratio (260/280)
1.	Simple	0.988	4.94 µg/ml	1.45
2.	Edward	0.949	4.70 µg/ml	0.91
3.	Phenol-chloroform	1.05	5.25 µg/ml	1.81
4.	CTAB At room temperature	1.65	8.75 µg/ml	1.83
	At high temperature	1.75	8.25 µg/ml	1.46
	At low temperature	1.95	9.71 µg/ml	1.91

TABLE: 5 CHARACTERIZATION OF EXTRACTED DNA USING VARIOUS METHODS FROM FLESH OF *BORASSUS FLABLLIFER* FRUIT

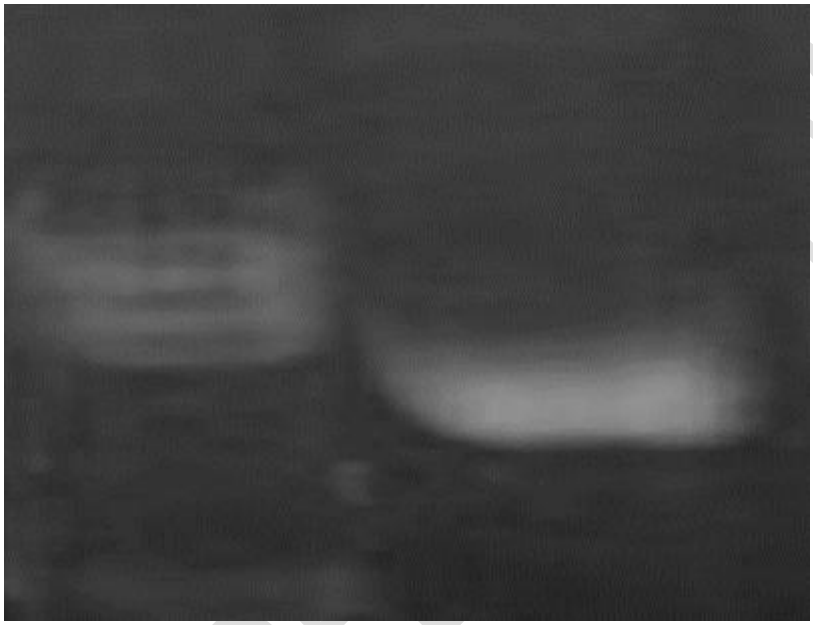
S.no	Methods	O.D $_{(260)}$value	Concentration of DNA (µg/ml)	Absorbance ratio (260/280)
1.	Simple	0.497	2.48 µg/ml	1.24
2.	Edward	0.428	2.14 µg/ml	0.707
3.	Phenol-chloroform	0.851	4.25 µg/ml	1.70
4.	CTAB At room temperature	1.28	6.4 µg/ml	1.85
	At high temperature	1.05	5.25 µg/ml	1.31
	At low temperature	2.12	10.6 µg/ml	1.87

QUALIFICATION BY GEL ELECTROPHORESIS

After the isolation of DNA from white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* fruit using different methods of extraction, highly varies in molecular weight. Gel

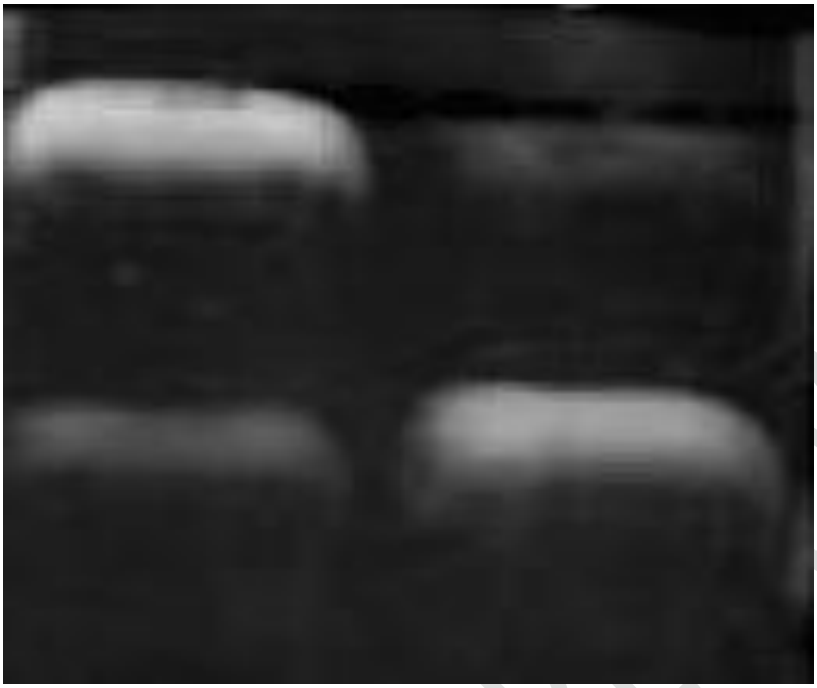
electrophoresis was performed in order to determine the purity level, by observing the trait of the migration of DNA that has been loaded in the wells of the gel with the aid of TAE buffer at the basic pH.

Fig:1, Fig:2, Fig:3 shows the image of agarose gel of isolated DNA using Simple, PCI and CTAB method of extraction respectively from white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* fruit.



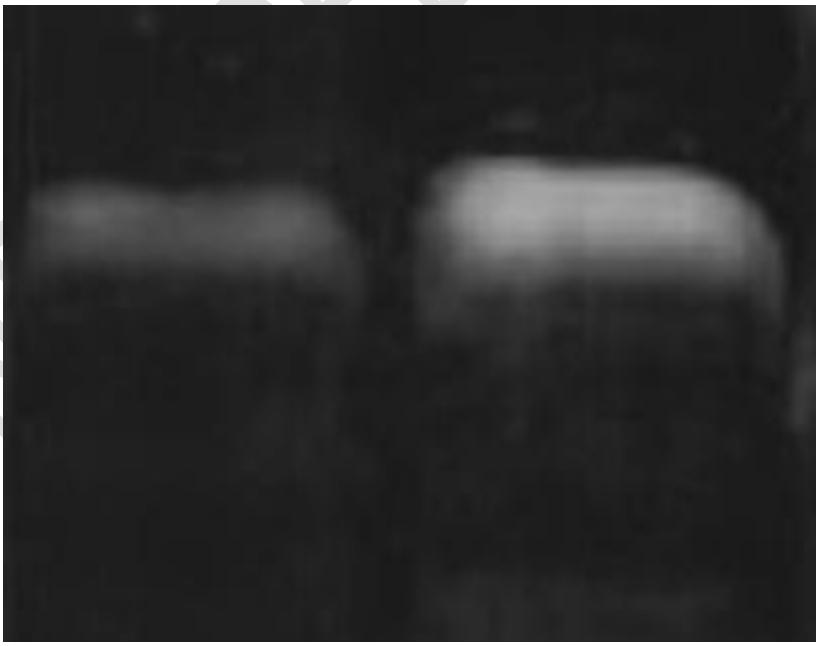
A- ***Borassus flabellifer;*** **B-** ***Cocos nucifera***

Fig: 1 DNA from white edible part of *Cocos nucifera* and *Borassus flabellifer* using simple method of extraction.



A- ***Borassus flabellifer;*** **B-** ***Cocos nucifera***

Fig: 2 Agarose gel image of isolated DNA from white edible part of *Cocos nucifera and Borassus flabellifer* using phenol-chloroform method of extraction.



A- ***Borassus flabellifer;*** **B-** ***Cocos nucifera***

Fig: 3 Agarose gel image of isolated DNA from white edible part of *cocosnucifera* and *Borassus flabellifer* using CTAB method of extraction.

4.DISCUSSION

According to the considerations provided by Harbrone [28], the complete isolated DNA using various ways of extraction was tested with Keller-killani and DPA tests. TABLE 1 shows the results: validates the existence of DNA. All the extraction procedures utilised to isolate the materials in the study yielded a positive result in this test [29].

From the results obtained from TABLE: 2 and TABLE: 3 show the absorbance of the isolated DNA at 260nm and 280nm. As per the statement of Ibrahim et al., 2010 the absorbance at 260nm indicated the presence of DNA [18]. The values of simple-0.988, Edward-0.949, PCI-1.05, CTAB-1.65 (in case of white edible part of *Cocos nucifera)* and the values of simple- 0.497, Edward-0.428, PCI-0.851, CTAB-1.28 (in case of flesh of *Borassus flabellifer* fruit*)* obtained at 260nm.These data showed that there was a significant amount of DNA in the sample isolated using simple, Edward, PCI, CTAB methods of extraction. Thus the absorbance at 280nm indicated the presence of protein. The values of simple- 0.678, Edward-1.036, PCI-0.58, CTAB-0.90 (in case of white edible part of *Cocos nucifera*) and the values of simple-0.398, Edward-0.605, PCI-0.490, and CTAB-0.67 (in case of flesh of *Borassus flabellifer* fruit*)* obtained at 280nm. These data showed that the presence of protein in the isolated DNA by using various methods [22].

The UV absorbance ratio ($A_{260}\backslash A_{280}$) is used for determining the protein contamination. For pure preparation of nucleic acid the A260\A280 ratio, which represent protein contamination should be between 1.8 to 2.0, according to Doyle *et al* [30]. The results obtained from the TABLE: 2 and TABLE: 3 show the values of (A260\A280) ratio for each of the isolated DNA using both the samples through various methods of extraction.

Among all the method of extraction that has been performed, the Edward method shows less purity because (A260\A280) ratio was found to be 0. 910, this indicates that the isolated DNA was contaminated with protein. On comparing with other methods, the Edward method was excluded for gel electrophoresis on consideration with poor purity due to very less A260\A280 ratio in case of isolated DNA [25].

The concentration level of isolated DNA from white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* fruit through various methods of extraction was recorded in the TABLE: 4 and TABLE: 5. From which it was observed that the concentration of DNA isolated using Edward method

was very less when compared to other methods (concentration for white edible part of *Cocos nucifera* - 4.7 µg\ml) and (concentration for flesh of *Brossasus flabellifer* fruit - 2.14µg\ml). Also concentration of DNA was found to be higher in case of DNA isolated using CTAB method (concentration for white edible part of *Cocos nucifera* - 8.25µg\ml) and (concentration for flesh of *Brossasus flabellifer* fruit - 6.45µg\ml [31]. As the CTAB method of extraction results in higher concentration of DNA, a trail of DNA isolation using CTAB method was made by varying the temperature (during the proceedings). Before that all the isolations were made at room temperature.

Thus, the temperature varying studies results in varying the absorbance ratio $A_{260\backslash 280}$ values and also the concentration level of DNA collectively. As the concentration of DNA was found to be high in the DNA isolation when it was conducted at low temperature (4°C). And the concentration for white edible part of *Cocos nucifera* was found to be 9.7µg\ml and concentration for flesh of *Brossasus flabellifer* fruit was found to be 10.6µg\ml. The concentration of isolated DNA at higher temperature was less on comparison with DNA isolated at room and low temperature using CTAB method it was concluded from the data obtained in TABLE: 4 and TABLE: 5.

Agarose gel results in **Fig: 1, Fig: 2, Fig: 3** shows that the purity level of DNA through effective migration. As Soni *et al*. [21] reveals that the difference in quantity and quality of isolated DNA.

Fig: 3 depict that the agarose gel of DNA isolated from white edible part of *Cocos nucifera* and flesh of *Borassus flabellifer* using CTAB method as it was indicated that the migration of isolated DNA was clear without any scattering when it was observed under the white and UV illuminator, which reveals that the CTAB method of DNA extraction was most effective and suitable method among all the other methods [23]. As the **Fig: 1 and Fig: 2** depict the agarose gel results of isolated DNA from simple and PCI method which were not effective and clear as the migration of DNA obtained in CTAB method [25].

5. CONCLUSION

The study emphasises the efficacy of "Ayurvedic medicine," an old Indian method that is still practised in various parts of the world. This ancient concept should be evaluated carefully in light of modern medical science, and if found to be appropriate, it can be applied in part. Finally, gel electrophoresis revealed that in terms of absorbance at 260&280nm, absorption ratio (A260/280), and isolated DNA content, the white edible portion of *Cocos nucifera* fruits outperformed the flesh of *Borassus flabellifer* fruits.

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