International Journal of Agriculture System
Vol. 10 Issue 2, December 2022
Nationally Accredited Journal Decree No. 48a/E/KPT/2017
P-ISSN: 2337-9782, E-ISSN: 2580-6815. **DOI: 10.20956/ijas.v10i2.3966**

Comparison of DNA Isolation Methods that Derived from Leaves of a Potential Anti-Cancer Rodent Tuber (*Typhonium flagelliforme*) Plant

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ABSTRACT

The content of polysaccharides, polyphenols, proteins, and RNA compounds is the main problem often found in Plants DNA isolation, which inhibit the process of DNA isolation. Comparing the methods of plant DNA isolation is necessary for obtaining the DNA with good quality, purity, high concentration and efficiency time and cost. This study aimed to determine the best DNA isolation method that derived from leaves of a potential anti-cancer Rodent Tuber (Typhonium flagelliforme) plant by comparing the conventional DNA isolation method (cetyl trimethyl ammonium bromide/CTAB) and 2 commercial kits (Promega Wizard™ Genomic DNA Purification Kits, and Geneaid Genomic Mini Kit). The results showed that the CTAB method yielded a higher amount of DNA (>100 ng/µL) at the cost of 0.49 USD per sample, in comparison with Promega method (69.19 to 157.68 ng/µL) at 3.28 USD per sample and Geneaid method (8.15 to 18.52 ng/µL) at 2.06 USD per sample. Based on the purity of isolated DNA (A260/280), CTAB method produced relatively similar DNA quality to Promega kit (1.8-2.0). On the other hand, Geneaid method resulted in a lower purity value at 1.15 to 1.60.

Keywords:
Comparison; DNA-Isolation; CTAB; Promega, Geneaid

1. Introduction

Rodent tuber (*Typhonium flagelliforme*) is a medicinal plant that has many health benefits. This plant has a potential to be used in cancer treatment (Sianipar et al., 2021), such as breast cancer, cervix cancer (Purwaningsih et al., 2014), lung cancer (Iskandar and Asril, 2018) and leukemia (Katrin et al., 2012; Singh et al., 2013). Syafruddin et al. (2018) stated that rodent tuber extract contains ribosome inactivation protein (RIP). This protein can damage DNA or RNA cancer cell, so it can damage the cancer cell without disturbing the surrounding tissue (Purwaningsih et al., 2014). Sianipar and Purwaningsih (2018) stated that leaves, stem, tubers and root of rodent tuber contain secondary metabolites as anti-cancer potential, such as flavonoid (Farida et al., 2012), alkaloid, sterol, saponin, cerebroside and glycoside, β-daucosterol and β-

sitosterol (Purwaningsih et al., 2017). β-sitosterol is a sterol group that is abundant in some plants, including rodent tuber. β-sitosterol can be used in cancer treatment because it is able to induce apoptosis of cancer cells (Novotny et al., 2017; Rajavel et al., 2018).

Molecular biology makes easy to study secondary metabolites that has anti-cancer effects in plants (Subedee et al., 2020). DNA isolation is an important step in genetic and molecular studied (Ferniah and Pujiyanto, 2013). The main problem is often found in plants DNA isolation is the content of polysaccharides, polyphenols, proteins, and RNA compounds, which inhibited the process of DNA isolation (Inglis et al., 2018; Heikrujam et al., 2020). There are various DNA isolation methods available. Hikmatyar et al. (2015) stated that DNA isolation with CTAB method showed thick DNA bands that appear little or without smear when visualized under UV light. Similar research has also been conducted by Laurent et al. (2015). Whereas according to Hanum et al. (2018), isolation of rice DNA of local variety of South Sumatera using DNA method of Genomic DNA Purification System Kit from Promega has higher quality and quantity compared with CTAB method. Moreover, Hengkengbala et al. (2018) stated that plant DNA isolation using Geneaid Genomic DNA Mini Kit Plant method give better results than the CTAB method and the innuPrep Plant DNA Kit (Germany).

Comparative studies of plant DNA isolation methods are important to obtain DNA with good quality and purity, as well as high concentrations. The DNA can be used for various downstream applications such as genomic sequencing study, DNA multiplication using polymerase chain reaction (PCR), quantitative PCR (qPCR), DNA markers identification and characterization, such as random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), single nucleotide polymorphism (SNP), short tandem repeat polymorphism (STRP), and a variable number of tandem repeat (VNTR), as well as southern blotting, which require DNA with good quality and quantity (Lucena-Aguilar et al., 2016). In addition, there are other factors that must be considered, i.e. time and cost. This study aimed to determine the best DNA isolation method for rodent tuber by comparing the conventional DNA isolation method (cetyl trimethyl ammonium bromide/CTAB) and 2 commercial kits (Promega Wizard™ Genomic DNA Purification Kit and Geneaid Genomic DNA Mini Kit Plant). The results of this study are expected to be used as a base for determining the best DNA isolation method of rodent tuber plants.

2. Materials and Methods

2.1 Experimental Location

The study was conducted at the Laboratory for Biotechnology, Building 630, Directorate of Laboratory Management, Research Facilities, and Science and Technology Park, Serpong, South Tangerang City, Indonesia. This study started on 3 June 2020 and ended on 31 August 2020.

2.2 Material

Plant material used for this study was rodent tuber plants that derived from the Collection Park, the Laboratory for Biotechnology. The fresh young leaves of rodent tuber from three accessions were used as plant materials (Figure 1). These accessions were (1) Ny. Meneer, Semarang, Indonesia; (2) Sungai Merdeka, Samboja District, Balikpapan, Indonesia; and (3) Matesih, Karanganyar Regency, Solo, Indonesia.

Figure 1. Rodent tuber plant for DNA Isolation; a) Matesih-Solo, b) Ny. Meneer Semarang, c) Sungai Merdeka-Balikpapan

2.3 DNA Isolation Methods

DNA isolation was carried out with three different methods, i.e. the conventional extraction method with CTAB (Aboul-Maaty and Oraby, 2019) and two commercial DNA isolation kits. The commercial kits were Promega Wizard™ Genomic DNA Purification Kits (Promega) and Geneaid Genomic Mini Kit (Geneaid). General description methods applied in the study were shown on Table 1. The parameters observed were the concentration and purity of DNA, time, and cost.

Table 1. Description of the methods applied in the study

Kit Name/method	General Description	Specifications
CTAB	Material preparation step was required, manual operation, extraction without membrane, with phenol and chloroform, purification without enzymes	Sample: 50 mg Elusion volume of DNA: 200 µL
Promega Wizard™ Genomic DNA Purification Kits	Manual operation, Solution-Based DNA Extraction Method	Sample: 40 mg Elusion volume of DNA: 100 µL
Geneaid Genomic DNA Mini Kits (Plant)	Manual operation, spin column: glass fiber membrane optimized for genomic DNA extraction	Sample: 50 mg Elusion volume of DNA: 100 µL

2.3.1 Sample Preparation

The leaves of rodent tuber were prepared before DNA isolation process. They were washed with running water and detergent, followed by drying. Subsequently the dried leaves were cut into small pieces, and then freeze in the liquid nitrogen. The leaves were finally ground into fine powder using a mortar and pestle.

2.3.2 DNA Isolation with CTAB Method

The leaves powder of 50 mg was added into a 2 mL microcentrifuge tube, followed by 1 mL of CTAB solution (20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl) and 100 µL of 0.2% β-mercaptoethanol. Then vortex vigorously at high speed for 10 seconds. The mixture was incubated at 65ºC for 45 minutes, while tapping the tube for every 15 minutes. After adding 500 µL chloroform-isoamyl alcohol (24:1), the sample was mixed by inverting the tube for 12 times. The next step was centrifugation for 1 minute at 13,000× rpm, and the supernatant was collected. After adding 1/10× volume of NaOAc pH 5.2 and 1× volume of isopropanol, then it was incubated for 30 minutes at –20ºC, before centrifugation for 10 minutes at 13,000× rpm, following by removing the supernatant. The 200 µL 70% iced-cold EtOH was added, and then the pellet was dried for 30 minutes on sterile tissues. Finally, 200 µL buffer TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) was poured into the solution and mixed it by gently tapping before storing at –20ºC.

2.3.2 DNA Isolation with Promega Wizard™ Genomic DNA Purification Kit Method

The leaf powder of 40 mg was added into a 1.5 mL microcentrifuge tube. After adding 600 µL of nuclei lysis solution, vortex vigorously at high speed for 3 seconds. Sample was incubated at 65ºC for 15 minutes. After adding 3 µL of RNase solution to the cell lysate, the sample was mixed by inverting the tube for 4 times. Afterwards the sample was incubated at 37ºC for 15 minutes. Sample was allowed to cool in room temperature for 5 minutes before proceeding to the next step. After adding 200 µL of protein precipitation solution, then vortex vigorously at high speed for 20 seconds. The next step was centrifugation for 3 minutes at 14,000×g. The precipitated proteins formed a tight pellet. The supernatant containing the DNA (leaving the protein pellet behind) was removed and it was transfer to a fresh 1.5 mL microcentrifuge tube containing 600 µL of room temperature isopropanol (some supernatant may remain in the original tube containing the protein pellet). The residual liquid in the tube was left to avoid contaminating the DNA solution with the precipitated protein. After gently mixing the solution by inversion until the thread-like strands of DNA formed a visible mass. The next step was centrifugation for 1 minute at 14,000×g at room temperature and the supernatant was removed carefully. After adding 600 µL 70% ethanol and gently inverting the tube for several times to wash the DNA. The next step was centrifugation for 1 minute at 14,000×g at room temperature. The tube was inverted to clean absorbent paper and the pellet was air-dried for 15 minutes. After adding 100 µL of DNA rehydration solution, rehydrated the DNA by incubating at 65°C for an hour. Periodically mixing the solution by gently tapping the tube.

2.3.3 DNA Isolation with Genaid Genomic DNA Mini Kit Method

The leaf powder of 25 mg was added into a 1.5 mL microcentrifuge tube. After adding 400 µL of GP1 buffer or GPX1 buffer and 5 µL of RNase A into the sample tube, the sample was mixed by vortex for 10 seconds. The next step was sample incubation for 10 minutes at 60ºC. During incubation, the sample was mixed by inverting tube every 5 minutes. Subsequently, add 100 µL of GP2 buffer and the sample was mixed by vortex and followed by incubation on ice for 3 minutes. A filter column was placed in a 2 mL collection tube then transferred the mixture to the filter column. The next step was centrifugation for 1 minute at 1,000×g. The supernatant carefully transferred from the 2 mL collection tube to a fresh 1.5 mL microcentrifuge tube. After adding a 1.5 volume of GP3 buffer (make sure isopropanol was added), the sample was vortex immediately for 5 seconds. A GD column was placed in a 2 mL collection tube. After transferring 700 µL of the sample (and any remaining precipitate) to the GD column, the next step

was centrifugation for 2 minutes at 14,000×g. Then the remaining sample was added into the GD column, followed by centrifugation at 14,000×g for 2 minutes. Subsequently, a 400 µL of W1 buffer was added into the GD column. The next step was centrifugation for 30 seconds at 14,000×g. Followed by adding 600 µL of washing buffer (make sure ethanol was added) into the GD column. The sample was centrifuged for 30 seconds at 14,000×g and for 3 minutes at 14,000×g to dry the column matrix. The dried GD column was transferred into a fresh 1.5 mL microcentrifuge tube. Subsequently, a 100 µL of pre-heated elution buffer or TE was added into the center of the column matrix. The sample was incubated for 3-5 minutes in the room temperature to ensure the elution buffer or TE was completely absorbed. The final step was centrifugation for 30 seconds at 14,000×g to elute the purified DNA.

2.3.6 Visualization DNA Band After Isolation

DNA was visualized using gel electrophoresis. The gel of 50 mL was made by mixing 0.5 g agarose (Sigma) into 50 mL 0.5×TAE buffer. For homogenization, the gel was heated using a microwave oven for 3 minutes. In addition, 1 µL of Sybr Safe Dye (Promega) was added and homogenized with light shaking until homogeneous and cooled, then poured into the agarose gel mold. The hardened agarose gel was put into the electrophoresis chamber which already contained 0.5× TAE buffer, and the entire surface of the agarose gel was submerged with 0.5× TAE buffer. Furthermore, 5 µL of the sample was inserted into the well with the addition of 1 µL of loading dye 6× (Promega). After all the samples were put into the wells, the electrophoresis was run at 100 V for 30 minutes. Finally, the DNA bands were visualized that appear on the agarose under UV light using a Gel Doc UV Transluminator.

2.3.5 Quantification DNA After Isolation

DNA purity and concentration were measured using the Nanodrop ND 1000 Spectrophotometer (NanoDrop Technologies, USA) at a 260λ wavelength. DNA concentration was measured in ng/µL unit. Meanwhile, the level of DNA purity was measured by a wavelength ratio of 260/280, which is the ratio of DNA to protein.

3. Results and Discussion

3.1 Results

3.1.1. Timing and Cost

Figure 2 shown the comparison of time and cost for DNA isolation with 3 different methods. DNA isolation with Geneaid kit required the shortest time, which was 90 minutes, while DNA isolation using the CTAB method required the longest time, which was 297 minutes. This is because the isolation with the CTAB method needed the stages of making chemical solutions that were used for DNA isolation.

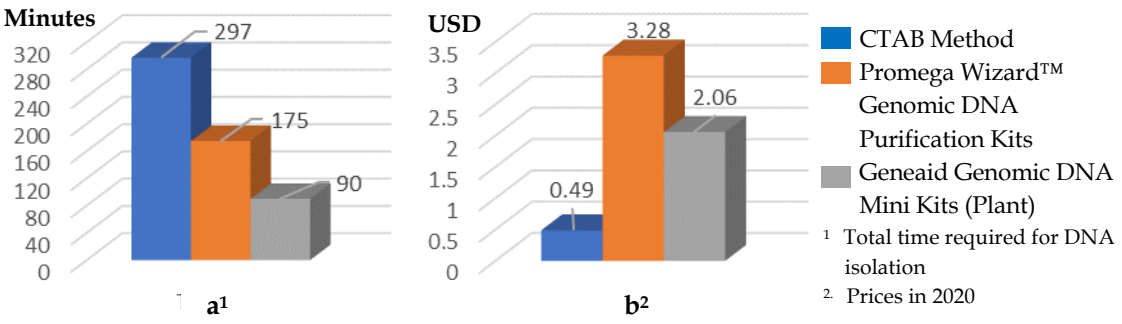

Figure 2. Comparison of DNA Isolation Method a) Time, b) Cost

3.1.2. 3.2 Visualization of DNA Band

Figure 3 presented the results of DNA visualization. All of the rodent tubers leaves samples isolated using the CTAB and Promega methods show the presence of DNA bands. The DNA bands isolated with the Promega kit had thicker bands and fewer contaminants compared to the DNA band isolated using the CTAB method. Promega kit showed clear bands and little or no smear on the visualization results. The clear band and the absence of smear on the visualization results indicated that the DNA had been isolated with good quality. Moreover visualization of isolated DNA with the Geneaid kit showed the presence of DNA bands from Matesih accession on all repetitions. In contrast, the DNA from Ny. Meneer and Sungai Merdeka had no band.

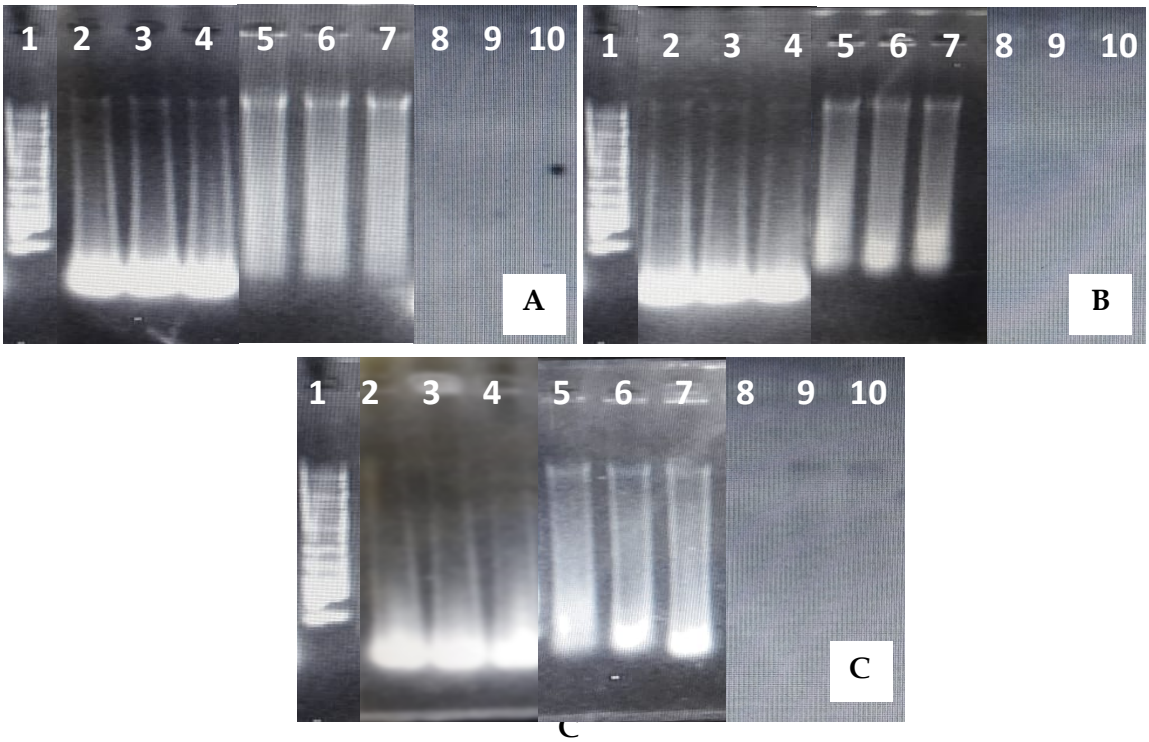

Figure 3. Visualization of DNA Isolation. 1 = 1 Kb DNA Ladder (Promega); 2, 3, 4 = CTAB Method; 5, 6, 7 = Promega Method; 8, 9, 10 = Geneaid Method a. Nyonya Meneer-Semarang Accesion b. Sungai merdeka-Balikpapan Accession c. Matesih-Solo Accession

3.1.3. Concentration and Purity of DNA

The results of measurements using Nanodrop ND 1000 Spectrophotometer (NanoDrop Technologies, USA) of DNA concentration and purity was shown on Table 2. The result represented that DNA isolation with CTAB methods had the highest concentration, that was >100 ng/µL at all samples with the purity on range 1.8 – 2.0. It showed that the DNA had good quality. The DNA concentration value with CTAB methods was higher than those using commercial kits. This concentration can still be increased by decreasing the volume of the TE buffer from 200 µL to 100 µL. The concentration of DNA isolated with the Promega kit showed a value of 79.65 – 157.68 with an average purity in the range of 1.8 – 2.0. Rodent tuber DNA of Sungai Merdeka and Matesih accessions had concentration value >100 ng/µL. The results of DNA isolation using the Geneaid kit show the smallest DNA concentration value, which was lower than 20 ng/µL with purity outside the range of 1.8-2.0.

Table 2. DNA concentration and purity

Accession	Repetition	CTAB		Promega		Geneaid	
		Conc. (ng.µL^{-1})	260/280	Conc. (ng.µL^{-1})	260/280	Conc. (ng.µL^{-1})	260/280
Ny. Meneer	1	187.47	1.91	95.82	1.89	8.60	1.17
	2	176.90	1.88	88.55	1.92	8.15	1.20
	3	180.52	1.93	69.19	1.95	8.45	1.44
Sungai Merdeka	1	182.82	2.01	120.57	1.80	14.52	1.57
	2	170.23	2.04	157.68	1.78	18.52	1.60
	3	204.43	1.97	102.66	1.89	16.07	1.15
Matesih	1	152.90	1.88	79.65	1.90	9.68	1.32
	2	177.59	1.84	147.33	1.73	8.23	1.34
	3	160.74	1.83	147.80	1.72	16.45	1.15

3.2 Discussion

DNA isolation consists of 3 steps, which are: lysis of cells wall and membrane, purifying DNA from other compounds, and DNA precipitation (Travers and Muskhelishvili, 2015; Dairawan and Shetty, 2020; Rizko et al., 2020). The parameters used to determine the success of DNA isolation were DNA integrity, DNA concentration and purity. The integrity of DNA can be seen from the appearance of bands on the visualization results with agarose gel.

The problem often found in the DNA isolation of rodent tuber was secondary metabolites which often reduced the DNA purity. Polysaccharide compounds were one of the compounds that can interfered with the purity of DNA. DNA isolation using the CTAB method can reduce polysaccharides and secondary metabolites. It can be seen from the DNA purity data of rodent tuber accessions (Table 2). Most of the purity

results had a good value, ranging from 1.8-2.0 with DNA concentration value > 100 ng/µL.

Polyphenols and proteins were other compounds from the rodent tuber plant that can inhibit DNA purity. In the CTAB method, polyphenols can be treated with mercaptoethanol which was present in the extraction buffer composition. Protein contamination can be treated with isoamylalcohol chloroform. Chloroformisoamylalcohol was able to remove protein compounds without denaturing DNA because DNA was a hydrophilic compound that was insoluble in organic solvents. The deproteination ability of chloroform was based on the ability of chloroform to denature polypeptide chains that partially enter or were mobilized at the water-chloroform interface. Isoamylalcohol had a function as an emulsifier, which increased the surface tension area of the water-chloroform, therefore the deproteination process can be maximized.

Visualization of DNA isolated using the CTAB method showed smear on the agarose gel, at the bottom part (Figure 3). It was RNA. Some downstream applications such as sanger sequencing and next generation sequencing (NGS) require the DNA sample with RNA-free. In sanger sequencing, the presence of RNA in DNA samples can increase the chances of double peaks appearing. While in the NGS require that the DNA sample is RNA-free, the majority of the DNA must be equal or greater than 40-50 kbp, minimum DNA purity OD 260/280 should be 1.8-2.0, OD 260/230 should be >2.0. DNA quality by this method can be improved by using RNAse (Heikrujam et al., 2020). An additional RNAse increases cost 0.02 USD per sample.

The Promega Kit was a Solution-Based DNA Extraction Method (Table 2) which was similar to the CTAB method. The kit capable for isolating rodent tuber DNA. It was shown by the appearance of DNA bands on the agarose gel. The DNA bands that appear are single and firm. Single and firm bands indicate intact and undegraded DNA (Figure 3). This method was capable producing good quality DNA in the range 1.8-2.0 (Table 2) for the entire sample. However, the concentration of DNA with this method had a lower value than the CTAB method, which ranges from 79.65 – 157.68 ng/µL. Even so, the DNA was good enough to be used as DNA material in downstream applications, but DNA isolation using this kit required high cost.

The result of DNA visualization with the Geneaid kit showed low concentration and purity values <1.8. A purity value <1.8 indicated DNA was contaminated with polyphenolic compounds and other proteins. DNA isolation with the Geneaid kit was a silica-based membrane technology in the form of a spin column. This method has not been effective enough to remove polyphenolic compounds and proteins contained in rodent tuber. Sari et al (2014) stated the standard procedure with spin column recommended by the Geneaid kit is not good enough to isolate DNA. This is indicated by the value of DNA concentration <50 ng/µL. Proteinase-K addition to this kit increases the DNA concentration obtained by 2-3 times and increases the value of plant DNA purity.

4. Conclusion

The best DNA isolation was obtained using the CTAB method resulted in high concentration >100 ng/µL and the purity was in the range of 1.8-2.0 at the cost of 0.49 USD per sample. DNA isolation using this method takes longer than other methods because it needed the time of preparation of chemical solution. Nevertheless, once they were prepared, the chemical solutions could be used for DNA isolation several times.

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