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GENOMIC DNA ANALYSIS OF *CHROMOLAENA ODORATA*

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ABSTRACT

The *Chromolaena odorata*, commonly known as Siam Weed, is a member of the Asteraceae family. Locally referred to as the Kapal Terbang plant, it is known for its health benefits, with various parts used to treat wounds, burns, and skin infections due to its anti-inflammatory, antimicrobial, and antioxidant properties. Siam Weed has been thriving in the market. However, the identity of the plant sparks confusion albeit the identical physical characteristics as *Chromolaena Odorata*. This research aims to identify genomic DNA analysis of the *Chromolaena odorata* plant using the Promega commercial kit. The extracted DNA will be quantified against a Lambda DNA/HindIII Marker standard through electrophoresis in a 1% agarose gel. Results indicate successful DNA extraction, enabling further analysis through agarose gel viewing and imaging. This process will help in avoiding false identification by accurately sizing base pairs. This study relates to a hadith by Prophet Muhammad (PBUH) illustrating that physical traits can reappear after skipping generations due to ancestral traits. The concept of genetic inheritance applies to both human and plant genetics. Different species may exhibit similar phenotypes, but the underlying genetic makeup is key to their identification. This approach could annotate the genetic foundations of the plant's medicinal properties, potentially leading to new healthcare applications. In addition to its scientific contributions, this research underscores the significance of Islamic scientific knowledge in genetics. The evidence for *Chromolaena odorata* DNA identification aligns with the hadith's implications on genetic variation and inheritance, demonstrating the relevance of historical wisdom in modern science.

Keywords: *Chromolaena odorata*; DNA extraction; electrophoresis; genetic inheritance.

1. INTRODUCTION

Chromolaena odorata (L.) R.M. King & H. Rob is a perennial shrub from Central and South America that has spread to Southeast Asia (McFadyen & Skarratt, 1996; Suksamrarn et al., 2004; Kouamé et al., 2012; Tanhan et al., 2007). It features a woody base, soft, hairy stems, and can grow up to 5 meters tall in sunny areas, often climbing on other vegetation. The plant has glandular, opposite leaves that are triangular to elliptical and emit a strong odor when crushed. Its flowers are

tubular and pale pink to white, forming clusters at the branch tips. The seeds, dark and 4 to 5 millimeters long, have a parachute-like structure for wind dispersion and can cling to fur, clothing, or machinery. The root system is fibrous, extending about 300 millimeters deep (Parsons & Cuthbertson, 1992; Ostermeyer, 2000). In Malaysia, this shrub grows near houses, ditches, and rubber plantations and adapts to various soils including mineral, peat, bris, and rocky types. Traditionally, it is used for treating ailments. It is also noted as one of the worst invasive species globally (Akinmoladun et al., 2007) and has numerous traditional uses for conditions like malaria and wounds (Omokhua et al., 2016). This study will use DNA-based methods to identify *Chromolaena odorata* in Malaysia. Our stride for genetic and DNA-based identification is driven by the following hadith by Prophet Muhammad PBUH: There came a person to the Prophet is from Banu Fazara and said: My wife has given birth to a child who is black, whereupon Allah's Apostle * said: Have you any camels? He said: Yes. He again said: What is this [sic: read their] colour? He said: They are red. He said: Is there a dusky one among them? He said: Yes, there are dusky ones among them|.] He said: How has it come about? He said: It is perhaps the strain (irq) to which it has reverted, whereupon he (the Prophet) said: It is perhaps the strain (irq) to which he (the child) has reverted (Sahih Al-Bukhari, 6847).

Currently, on many online sales platforms unidentified leaves are being marketed as *Chromolaena odarata* calling for DNA based identification to verify the species sold online. Protocols solely on the isolation of plant DNA have varied over the years as more methods are published (e.g., Doyle, 1990; Scott & Playford, 1996; Haymes et al., 2004). In this study, methods of identification will be based on genomic DNA in order to identify the *Chromolaena odorata* in Malaysia. Genomic DNA methods will be employed using a Promega kit for extraction and electrophoresis on a 1% agarose gel with the Lambda DNA/HindIII Marker (Abdel-Latif & Osman, 2017).

2. MATERIALS AND METHODS

Samples of plant with the same features as *Chromolaena odorata* near the lake at Kolej PERMATA Insan was collected and ensured to be free from contaminants. Sample was preserved in 80% ethanol (Mangeot-Peter et al., 2016) in a falcon tube to ensure preservation of nucleic acids and disruption of plant cells (Linke et al., 2010).

2.1. Genomic DNA Extraction

40 mg of sample was ground using a pestle and mortar before being preserved in a 4°C fridge in a 1.5 ml microcentrifuge tube. 600 µl of Nuclei Lysis Solution was added to the microcentrifuge tube using a pipette before vortexing for 3 seconds. The sample was then incubated in a water bath at 65°C for 15 minutes. After adding 3 µl of RNase into the microcentrifuge tube, it was inverted 5 times to mix the sample. Once more, the sample was incubated at 37°C for 15 minutes and was subsequently allowed to cool at room temperature (25°C) for 5 minutes. 200 µl of Protein Precipitation Solution was added to the sample, ensued by vortexing sample at high speed for 20 seconds. Then, the sample underwent centrifugation at 14000 x g for 3 minutes. Afterwards, the supernatant in the microcentrifuge tube containing DNA was removed using a pipette with caution, ensuring that only the protein pellet was left behind. The supernatant was transferred to a new 1.5 ml microcentrifuge tube containing 600 µl of isopropanol. The supernatant was mixed by inversion slowly 8 times and centrifuged at 14000 x g at 25°C for 1 minute. Supernatant was decanted and excess supernatant was absorbed using tissue paper. 600 µl of 70% ethanol was added to the

microcentrifuge tube and inverted to was DNA. Following centrifugation at 14000 x g for 1 minute at 25°C, the microcentrifuge tube was air-dried for 15 minutes upside-down on tissue paper to aspirate the ethanol. DNA pellet left in the tube is stored at 4°C for further usage (Promega Corporation, USA). The procedure is as shown below.

Table 1. Genomic DNA Extraction Protocol

Step	Buffer and Reagents	Volume	Vortex	Incubation Temperature (°C)	Incubation Time	Centrifugation Setting
1	*Chromolaena odorata* sample	40 mg	—	—	—	—
2	Nuclei Lysis solution	600 µl	1-3 s	65	15 min	—
3	RNase	3µl	—	37	15 min	—
4	Protein Precipitation Solution	200 µl	20 s	24	20 sec	16,000 × g for 3 min
5	Isopropanol	600 µl	—	24	20 sec	16,000 × g for 1 min
6	Ethanol 70%	600 µl	—	24	20 sec	16,000 × g for 1 min
7	DNA Rehydration Solution	100 µl	—	65	1 hour	—

2.2. Gel Electrophoresis

When DNA is obtained, electrophoresis is then conducted to separate DNA by size for purification and visualization. Agarose gel for electrophoresis was prepared first. 0.3 g of agarose is measured and mixed with 30 ml x TAE buffer in a microwavable flask. The mixture was microwaved for more than 40 seconds until completely dissolve. The mixture was swirled mid heating. The agarose gel was cooled down for about 50°C. 3 µl of GelRed® is added for staining, the solution was swirled. The agarose gel is poured into a gel tray with the well comb in place. Bubbles can disrupt the gel; therefore, any bubbles are removed. The gel is then let to solidify for 15-20 minutes.

After agarose gel is ready, the comb is removed. Molecular weight ladder is added into the first lane of the gel. The DNA samples were mixed with loading buffer. It has a high percentage of glycerol, which enhances the density of your DNA sample, enabling it to settle at the bottom of the gel well instead of diffusing in the buffer; and it gives a visible dye that aids in gel loading thus allowing to gauge how far the DNA has migrated. Samples are then carefully added into the additional wells of the gel. The gel is then run at 80 V for 1 hour. Power is turned off and electrodes

are disconnected. The gel is carefully removed from the gel box. Ultraviolet (UV) rays were used to visualize the DNA fragments using a Bio-Rad gel imager (Baharum & Nurdalila, 2011).

3. RESULTS AND DISCUSSION

The study involved obtaining DNA profiles from *Chromolaena odorata* using the Wizard® Genomic DNA kit by Promega for the extraction process. The samples used were collected from two different Kapal Terbang plants identified by their characteristics, sourced from the nearby lake of Kolej PERMATA Insan. Both plants, labelled A and B, underwent DNA extraction to confirm the presence of genomic DNA from *Chromolaena odorata* in the samples. The research findings were verified and validated based on triplicates of genomic DNA from each sample. By implementing triplicates of genomic DNA of two samples in this study, we ensured the quality control of the extraction process.

The genomic DNA needed to align with the highest molecular weight marker on the ladder (Baharum & Nurdalila, 2011). Gel electrophoresis results (Figure 1) confirmed the presence of genomic DNA in the collected sample of *Chromolaena odorata*, using a Lambda DNA/ HindIII Marker for precise sizing due to the wide size range of the marker. This marker is well-suited for extracting genomic DNA involving different sized DNA fragments. The genomic DNA extraction of *Chromolaena odorata* is of utmost importance particularly during the onset of a study. It is the first step in affirming the species of the plant before proceeding with the development of a product using the sample. Going forward, the *Chromolaena odorata* can be analysed using Polymerase Chain Reaction (PCR) for supplementary information on its genetic material in future studies.

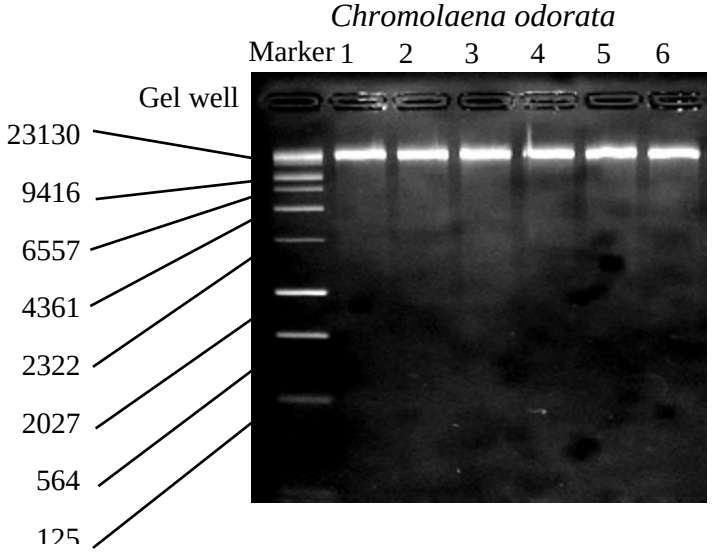

Figure 1. Gel Imaging of Sample with 1% TAE Agarose Gel

4. CONCLUSION

Result of this study shows that all samples of *Chromolaena odorata* or Kapal Terbang leaf collected and tested contains DNA material as identified through gel imaging. Further analysis on the DNA material of sample can be conducted, specifically through PCR testing to enable the sequencing of DNA, identification of beneficial properties, genomic identification of the species and to allow

further research and construction of the phylogenetic tree of the *Chromolaena odorata* species in Malaysia.

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