CONFERENCE PROCEEDING

Genomic DNA Analysis of *Chromolaena Odorata*

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ABSTRACT

The *Chromolaena odorata* is often referred to as the Siam Weed and is a member of the *Asteraceae* family. Known locally as the Kapal Terbang plant, it is noted for its various health benefits given that several parts of this herb have been used to treat wounds, burns, and skin infections in addition to possessing qualities namely anti-inflammatory, antimicrobial, and antioxidant properties. Hence, the purpose of conducting this research is to contribute more information on the DNA extraction of *Chromolaena odorata*. Subsequent DNA extraction protocols will hinge upon the application of a commercial kit from Promega. The quantification of extracted DNA will be meticulously assessed against a Lambda DNA/ HindIII Marker standard, employing electrophoresis within a 1% agarose gel in the research findings. This systematic approach holds promise for shedding light on the genetic underpinnings of the plant's medicinal properties, potentially paving the way for novel applications in the field of healthcare and medicine. In addition, it endeavours to provide scientific evidence to support the identification of *Chromolaena Odorata*.

Keywords: *DNA extraction; DNA identification; Chromolaena odorata; Siam Weed; Pokok Kapal Terbang*

INTRODUCTION

Chromolaena odorata (L.) R.M. King & H. Rob is a perennial shrub (McFadyen & Skarratt, 1996; Suksamrarn et al., 2004) emanating from central and South America that has established its roots throughout Southeast Asia (Kouamé et al., 2012a; Tanhan et al., 2007). The shrub has a woody base with soft stems and undergo etiolation in shady areas, prolonging its life as a creeper by growing on other vegetation where it can grow up to 5 meters tall. The plant is hairy and glandular, while the leaves are opposite, triangular to elliptical with serrated edges. When crushed, the leaves produce a pungent, aromatic odour. Its panicles of 10 to 35 tubular flowers range in colour from white to pale pink and form at the ends of the branches. The *Chromolaena odorota*'s achenes seeds have little hair on them.

They are primarily dispersed by the wind, but they can also stick to fur, clothing, and machinery to travel great distances via the little hairs aforementioned. They are characterized by their dark coloration and measure between 4 to 5 millimetres in length. These seeds have a slender and elongated shape, and they feature a white hair-like structure that forms a parachute. As the seed undergoes drying, these hairs

transition from white to a brown hue. The plant's root system is fibrous in nature, typically extending to a depth of around 300 millimetres (Parsons & Cuthbertson, 1992; Ostermeyer, 2000).

In Malaysia, *Chromolaena odorata* can be found around houses, ditches, and rubber plantation areas. This plant easily grows in open areas and pastures that get a lot of sunlight (Lai et al., 2006). *Chromolaena odorata* has the ability to thrive in various types of soil, whether mineral soil, peat soil, bris soil or even problematic soils. Furthermore, rocky soil areas such as roadsides can also grow this plant as long as there is access to sunlight and rain. Traditionally, this plant is used to relieve flatulence, itchy skin, and diarrhoea in babies. It is also used to reduce acne, reduce swelling, treat worms, shingles, and scabies, relieve high fever, bathe mothers after childbirth, spacing pregnancy, as massage oil ingredients, treat haemorrhoids and control glucose levels in the blood (Mustapha et al., 2017).

Due to the momentous invasive nature of *Chromolaena odorata* in the humid tropics of Southeast Asia, Africa, and the Pacific Islands (Akinmoladun et al., 2007), the weed was crowned one of the world's worst invader species by the International Union for Conservation of Nature. The species, particularly the biotype found in Asia and West Africa, has many ethnopharmacological uses, including treatment of malaria, wounds, diarrhoea, skin infection, toothache, dysentery, stomach ache, sore throat, convulsions, piles, coughs, and colds (Omokhua et al., 2016a). Henceforth, DNA-based species identification of *Chromolaena odorata* can be useful as a tool for quality control and safety monitoring of herbal pharmaceuticals and nutraceuticals and will amplify the medical potential and commercial profitability of herbal products.

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. DNA extraction will be based on the commercial kit from Promega. DNA quantity will be determined against the Lambda DNA/ HindIII Marker standard by electrophoresis in a 1% agarose gel for best results (Abdel-Latif & Osman, 2017).

METHODOLOGY

Samples Collection for Kapal Terbang Plant

Species identification using molecular DNA markers requires only a small sample of DNA. Therefore, *Chromolaena odorata* leaves are collected and ensured to be free from contaminants or pollutants and are not subjected to an excessive amount of environmental stress. Samples of plant with the same features as *Chromolaena Odorata* was found near the lake at Kolej PERMATA Insan. The sample needed to be preserved in 80% ethanol (Mangeot-Peter et al., 2016) in a falcon tube, as ethanol ensures the preservation of nucleic acids and disruption of plant cells (Linke et al., 2010).

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 then 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 cautiously removed using a pipette, 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	—

Gel Electrophoresis

When DNA is obtained, electrophoresis is conducted to separate DNA by size for purification and visualization.

Agarose gel for electrophoresis is prepared first. 0.3 g of agarose is measured. Agarose powder is mixed with 30 ml x TAE buffer in a microwavable flask. The mixture was microwaved for more than 40 seconds to 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 the agarose gel is ready, the comb is removed. A molecular weight ladder is added to the first lane of the gel. The DNA samples were mixed with a 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.

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 differently-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.

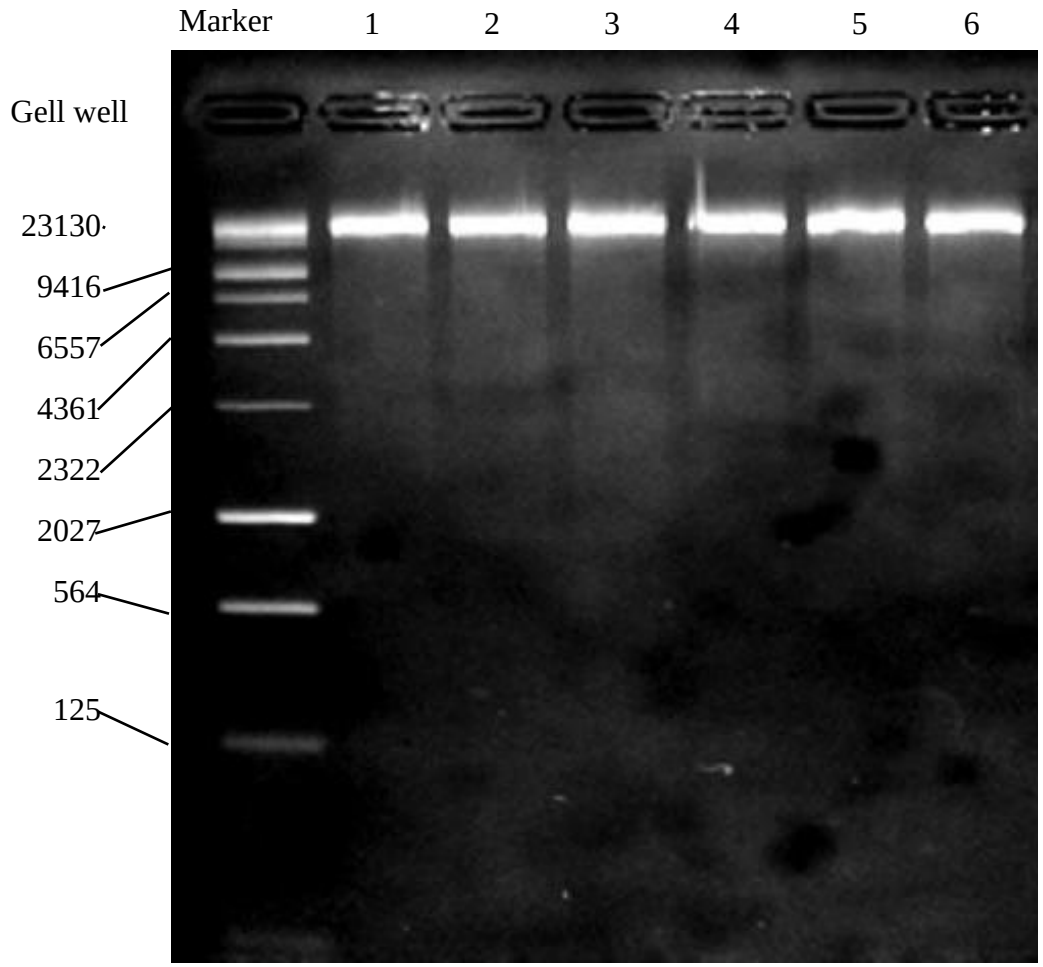

Figure 1. Gel Imaging of Sample

CONCLUSION

The result of this study shows that all samples of *Chromolaena odorata* or Kapal Terbang leaf collected and tested contain DNA material as identified through gel imaging. Further analysis of the DNA material of the 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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