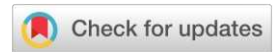


RESEARCH ARTICLE



Optimizing DNA Extraction Methods from Leaf and Wood Tissues to Support Dipterocarp Conservation and Sustainable Forest Management

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ABSTRACT

Dipterocarpaceae are economically important, contributing over 85% of Indonesia's timber exports. However, this crucial resource is increasingly threatened by illegal logging, habitat destruction, and the illegal timber trade, which jeopardize dipterocarp population. Furthermore, conservation efforts utilizing genetic and forensic techniques often encounter substantial challenges due to the complexities in DNA extraction protocol. To address this, the study aimed to enhance the efficiency of DNA extraction methodologies by comparing two methods: the modified cetyltrimethylammonium bromide (CTAB) and the Genomic DNA Mini Kit (Plant) from Geneaid Biotech Ltd. The research focused on leaf and wood samples from two species, specifically *Rubroshorea leprosula* (Miq.) P.S.Ashton & J.Heck and *Shorea laevis* Ridl. For each of these species, five leaf and five wood samples were extracted using both methods. The quality of the DNA extraction was evaluated using electrophoresis and quantified with a Qubit fluorometer. Higher DNA concentrations were obtained with the modified CTAB method compared to the GeneAid kit for both *R. leprosula* and *S. laevis*, particularly in leaf tissue. The GeneAid kit consistently exhibited low DNA yield efficiency compared to the modified CTAB method for both species. Additionally, PCR amplification of both leaf and wood samples confirmed that the extracted DNA was suitable for molecular analyses. These findings not only contributed to laboratory applications but also served as practical tools for species identification and genetic conservation for sustainable forest management and law enforcement.

Introduction

The tropical rainforests of Southeast Asia are rich in biodiversity, containing numerous plant species. Within these ecosystems, the Dipterocarpaceae family plays a crucial ecological and economic role, underscoring the need for effective conservation and sustainable use strategies [1,2]. Globally, there are 535 recognized species of Dipterocarpaceae across 17 genera distributed across four continents (Africa, Asia, Oceania, and South America) [3]. Indonesia and Malaysia exhibit the highest diversity of dipterocarp species [4], with these trees serving as essential components of lowland rainforests in regions including Sumatra, Kalimantan, Java, Sulawesi, Maluku, and Papua [5–10].

From an economic perspective, dipterocarps are vital, contributing over 85% of Indonesia's timber exports [11], despite several species being classified as endangered [12,13]. A total of 74% of the 491 Dipterocarpaceae species evaluated are listed as threatened on the IUCN Red List because of serious threats

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to this family from habitat destruction and illegal logging [14]. However, dipterocarp timber from natural forest concessions continues to dominate timber production, achieving a total production of 5.4 million m³ in 2017 [15], with meranti (*Shorea* spp.) contributing approximately 50% of the national production in 2020 [16]. The detrimental effects of environmental degradation and illegal logging extend beyond the decline of commercially and conservationally valuable species [17–19]. Such activities significantly compromise the effectiveness of conservation and forestry policies, which rely heavily on accurate population data and genetic diversity of dipterocarps.

The current study highlights the pressing need to enhance conservation strategies for tropical forest. Börner et al. [20] reported that forest conservation efforts are largely ineffective, with an impact on forest cover of less than 1% per year across nine studies, except in Mexico and Indonesia. To address these challenges effectively, sustainable forest management and conservation initiatives must utilize technological support that provides accurate data on the origin of timber and the genetic diversity of tree species [21]. However, the absence of a comprehensive wood DNA database presents considerable obstacles to implementing policies aimed at verifying the origin of wood using forensic methods [21]. This situation complicates law enforcement efforts concerning the illegal timber trade and undermines the integrity of environmental policy.

The adoption of a DNA-based approach provides strategic advantages for the conservation of Dipterocarpaceae. Molecular techniques, including DNA extraction from both leaf and wood tissues, enable the procurement of high-quality genetic material, which is essential for effective conservation initiatives. This approach facilitates tracing the origin of wood to mitigate the effects of illegal trade and enhances the monitoring of genetic diversity within natural populations [22,23]. However, the extraction of DNA from wood is inherently more challenging than from leaf tissue because of the higher concentrations of phenolic compounds, cellulose, and lignin, as well as the presence of secondary metabolites [24–27]. Additionally, wood tissue typically exhibits a lower DNA concentration, even in living trees [28]. DNA in dry wood and heartwood is more susceptible to degradation [28,29] because of its significantly lower water content compared to other plant tissues, such as leaves or young stems [30].

Many studies have consistently shown that DNA degradation in wood samples can significantly hinder the amplification of molecular markers necessary for accurate species and origin identification [29,31]. In this study, DNA was extracted from leaf tissue, which served as a baseline control. Leaves typically yield higher-quality DNA due to their lower content of secondary metabolites, especially for young leaves [32]; therefore, if extraction is successful from leaves but not from wood, the limitation can be attributed to the wood tissue itself rather than to the extraction method. The limitations associated with current extraction methodologies underscore the critical need to develop and optimize more reliable DNA extraction techniques. Therefore, this study aimed to optimize two DNA extraction methods, the modified cetyltrimethylammonium bromide (CTAB) method and the GeneAid Genomic DNA Mini (Plant) kit, specifically for leaf and wood samples of *Rubroshorea leprosula* and *Shorea laevis*, two commercially important species within the Dipterocarpaceae family that are widely used in construction [33,34]. The improvement and optimization of DNA extraction techniques are not merely procedural considerations but fundamental components that affect the reliability of subsequent genetic data [35]. The successful optimization of these methods for both species is expected to open up wider applications in genetic research within the Dipterocarpaceae family, thereby supporting conservation and sustainable management efforts. This study is expected to bridge the gap between molecular science and the practical application of sustainable environmental management policies.

Materials and Methods

Sample collection

This study utilized leaf and wood samples from two important species, *R. leprosula* and *S. laevis*. Samples of *R. leprosula* were collected from Tanjung Riau Village in the Sekupang District of Batam City, Riau Islands Province, Indonesia. Samples of *S. laevis* were obtained from the Bukit Bangkirai Natural Tourism Area in the Samboja District of Kutai Kartanegara Regency, East Kalimantan Province, Indonesia. DNA extraction and molecular analysis were conducted at the Forest Genetics and Molecular Forestry Laboratory, Department of Silviculture, Faculty of Forestry and Environment, IPB University. The collected fresh leaf and wood samples were dried using silica gel and stored in the same laboratory at room temperature. DNA isolation and PCR amplification were also performed at this facility. In this study, 10 samples, comprising five leaf and five wood samples from each species, were collected. DNA extraction was carried out using a modified CTAB

method [36] as well as the Genomic DNA Mini Kit (Plant) from GeneAid, which also underwent specific modifications.

Materials

For the modified CTAB method, the lysis or CTAB extraction buffer used in the protocol for each reaction consisted of 100 µl of 1 M Tris–HCl, 280 µl of 5 M NaCl, 40 µl of 0.5 M EDTA, 200 µl of 10% CTAB, 5 µl of 0.2% mercaptoethanol, 100 µl of 1% polyvinylpyrrolidone (PVP), and 280 µl of distilled water. Additionally, Proteinase K (20 mg/mL) and RNase A (20 mg/mL), chloroform, cold isopropanol, 70% ethanol, and TE buffer are required for the extraction process. These components should be prepared for each sample or reaction that is being extracted. For the modified Genomic DNA mini kit (plant) method, it was necessary to use GP1 buffer or GPX1 buffer and RNase A, as well as GP2 and GP3 buffers, all of which were included in the kit. Additionally, the kit includes wash buffer, W1 buffer, and elution buffer which are also required.

DNA extraction

Leaf and wood tissues from *R. leprosula* and *S. laevis* were weighed, then placed in a 2 mL microtube along with three 3-mm stainless steel beads. These samples: *R. leprosula* leaf, *R. leprosula* wood, *S. laevis* leaf, and *S. laevis* wood were crushed using a Qiagen TissueLyser II at 30 Hz for 5 minutes, with the process repeated twice or until a fine powder was achieved. In the CTAB method, leaf samples from both species were cut into smaller pieces, weighed to 0.2 g, and placed in 2 mL microtubes. Wood cambium from *R. leprosula* and *S. laevis* was weighed to 0.7 g and 0.6 g, respectively, and placed in 2 mL microtubes. Each powdered tissue sample received 1,000 µL of 60 °C CTAB solution. *R. leprosula* leaf samples received 20 µL of 0.2% β-mercaptoethanol. *R. leprosula* wood samples received 20 µL of 0.2% β-mercaptoethanol and 20 µL proteinase K (20 mg/mL). *S. laevis* leaves received 10 µL each of 0.2% β-mercaptoethanol and proteinase K; wood samples received 20 µL of each reagent. Each sample type was homogenised using a vortex mixer, then incubated at 60 °C for 1 hour with stirring every 10 minutes. After incubation, 600 µL of chloroform–isoamyl alcohol (24:1) was added, followed by tube inversion for 1 minute. Samples were centrifuged at 10,000 rpm for 10 minutes. The clear supernatant was transferred to a new 2 mL tube, and 10 µL of RNase A (20 mg/mL) was added. The mixture was incubated at 37 °C for 1 hour, specific to each sample type. Another 600 µL of chloroform–isoamyl alcohol was then added and the tubes inverted for 1 minute. The sample was centrifuged again at 10,000 rpm for 10 minutes for each tissue type. DNA precipitation was carried out for each sample using cold isopropanol (1:1 with supernatant), with overnight incubation at –20 °C. After incubation, the tube was centrifuged at 11,000 rpm for 15 minutes and the supernatant discarded. The DNA pellet was washed with 500 µL each of cold 96% then 70% ethanol, each time inverting and centrifuging at 12,000 rpm for 10 minutes, for each sample type. The pellet was dried for about 15 minutes at room temperature and dissolved in 20 µL TE buffer for *R. leprosula* and 30 µL for *S. laevis*.

For the Geneaid method, the manufacturer's protocol was modified and specified for each tissue. For *R. leprosula* and *S. laevis* leaves, 0.2 g of tissue were combined with 400 µL of GP1 buffer and 5 µL of RNase A. For *R. leprosula* wood, 0.7 g of tissue with 400 µL GP1 buffer was used, for *S. laevis* wood, 0.4 g with 400 µL GPX1 buffer and 5 µL RNase A, then vortexed. Each tissue mixture was incubated at 60 °C for 30 minutes and inverted every 5 minutes. During this incubation, the elution buffer was pre-heated: 50 µL for *R. leprosula* samples, 80 µL for *S. laevis* samples, ready for elution. After incubation, each sample received 100 µL GP2 buffer, was vortexed, and put on ice for 3 minutes. All were then centrifuged for 5 minutes at 14,000 × g to separate the supernatant. This supernatant was transferred to a filter column in a 2 mL tube and centrifuged at 1,000 × g for 1 minute. Flow-through was discarded as per sample, and the column removed. The remaining supernatant was moved to a clean 1.5 mL tube, 1.5 volumes of GP3 buffer added, and vortexed 5 seconds. For each sample type, 700 µL of this mixture was applied to the GD column and centrifuged at 14,000 × g for 2 minutes, repeated for remaining lysate. The GD column was rinsed with 400 µL W1 buffer and 600 µL wash buffer, each followed by centrifuging at 14,000 × g for 1 minute. The dried GD column was placed in a clean 1.5 mL tube. DNA was eluted by adding 50 µL of pre-heated elution buffer for *R. leprosula* leaves/wood or 80 µL for *S. laevis* leaves/wood onto the column, left at room temperature for 10 minutes (*R. leprosula*) or 5 minutes (*S. laevis*), to ensure absorption. The samples were then centrifuged at 14,000 × g for 1 minute to recover high-quality DNA for analysis.

Qualitative and quantitative assessment of extracted DNA

The extracted DNA was then evaluated for quality and quantity through qualitative assessment on agarose gel and quantitative measurement using a Qubit Fluorometer. Qualitative evaluation was performed on a 1% agarose gel dissolved in TAE buffer and stained with GelRed (Biotium). The extracted DNA samples were then

electrophoresed at 100 V for 30 min, followed by the addition of a 1 kb DNA ladder to the wells of the gel. The successful isolation and quality of DNA were evaluated based on the presence and clarity of DNA bands during electrophoresis. DNA quality was assessed based on the band pattern observed on the gel, with high-quality DNA exhibiting a distinct, high-molecular-weight main band near the well, accompanied by minimal downward smearing and no additional bands indicative of RNA contamination.

Quantitative assessment of DNA quality was performed using a Qubit Fluorometer v1.0 and the Qubit™ dsDNA BR Assay Kit (Invitrogen, Thermo Fisher Scientific). The required qubit tubes for the standards and samples were prepared. Two standards were used for calibration, which was performed each time prior to the sample quantification. The caps of the Qubit assay tubes were labeled, with the standard tubes labeled S1 and S2. The Qubit working solution was prepared by diluting the reagent 1:200 in the buffer (1 µL BR reagent + 199 µL BR buffer). A total of 190 µL of working solution was added to each standard tube, followed by the addition of 10 µL of the corresponding standard. The mixture was then vortexed thoroughly to ensure homogeneity of the solution. For each sample tube, 199 µL of the working solution was added, followed by 1 µL of the DNA sample, and the mixture was vortexed. The final volume is 200 µL in each tube. All tubes were incubated at room temperature for 2 minutes, after which the standards and samples were read using a Qubit Fluorometer v1.0.

PCR amplification

DNA isolated using both CTAB and commercial extraction methods was further assessed through PCR amplification employing the chloroplast *trnL-trnF* marker. Despite its relatively short length, the *trnL-trnF* region has proven to be a powerful molecular marker for the Dipterocarpaceae family [37–41]. PCR was performed in a 12.5 µL volume containing 6.25 µL 1× PCR master mix (MyTaq HS Red Mix 2×), 0.25 µL of forward and reverse primers (2 µM), 4.75 µL nuclease-free water (NFW), and 1 µL DNA template extracted using a GeneAid kit. DNA extracted using the CTAB method was first dissolved in a 1:10 ratio (1 µL of DNA and 9 µL of NFW), and 1 µL of the resulting solution was used as the template in the PCR reaction. Universal pair primer of forward primer 'c' (CGAAATCGGTAGACGCTACG) and reverse primer 'f' (ATTTGAACTGGTGACACGAG) were used [42]. The PCR profile consisted of an initial denaturation step (95 °C, 2 min), followed by 30 cycles of denaturation (95 °C, 45 s), annealing (55 °C, 45 s), and extension (72 °C, 1 min 30 s), with a final extension step (72 °C, 10 min). The amplified DNA was checked for quality using electrophoresis on a 1% agarose gel dissolved in TAE buffer, stained with GelRed Biotium. PCR products were loaded into gel wells, then the wells were filled with a 100-bp DNA ladder. The samples were electrophoresed at 100 V for 30 minutes. The resulting agarose gel was then photographed using a gel documentation system.

Results

Qualitative assessment of DNA quality from leaf and wood extractions of *R. leprosula* and *S. laevis*

In this study, the quality of DNA extracted using the modified CTAB method and the commercial GeneAid kit was analyzed by agarose gel electrophoresis. Figure 1a shows the results of leaf extraction using the same method. The DNA bands appear brighter in *R. leprosula* samples, whereas only a thin smear is visible in *S. laevis*. Conversely, Figure 1b shows the results of DNA extraction from wood tissue using the modified CTAB method. Smear-patterned DNA bands were observed in all *S. laevis* samples, while only two distinct DNA bands are visible in *R. leprosula* among the five analyzed samples.

Figure 2a shows leaf extraction using GeneAid, indicating the presence of smear bands in both species, although their intensity was lower than that of the CTAB method. However, different results were observed in Figure 2b, DNA extraction from wood tissue using the GeneAid kit did not produce DNA bands or smears in either of the tested species. The results of wood tissue extraction visualization showed much fainter DNA bands with thin smears in the modified CTAB method, and DNA bands that were not visible at all in the extraction results using the commercial GeneAid kit.

The absence of these bands can be interpreted as a low DNA concentration or possible limitations in agarose gel visualization, which may not be sufficient to detect very small amounts of DNA. Compared to the DNA bands from leaf extractions using CTAB or the GeneAid kit, the DNA bands appear thicker and brighter, although smears are primarily present in the modified CTAB method. This difference highlights that the type of tissue used in the extraction process significantly impacts the quality and efficiency of DNA extraction, due to variations in the anatomical structure and chemical composition of the tissue.

Wood tissues contain high levels of lignin, tannins, and other secondary metabolites that can inhibit the extraction process and reduce the quality of the resulting DNA. Gel electrophoresis-based evaluation remains important as an initial step, but it must be complemented by verification tests using PCR amplification to ensure the overall effectiveness of the method in the context of developing DNA extraction protocols from woody tissues and testing whether the extracted DNA is functional for molecular and genetic studies.

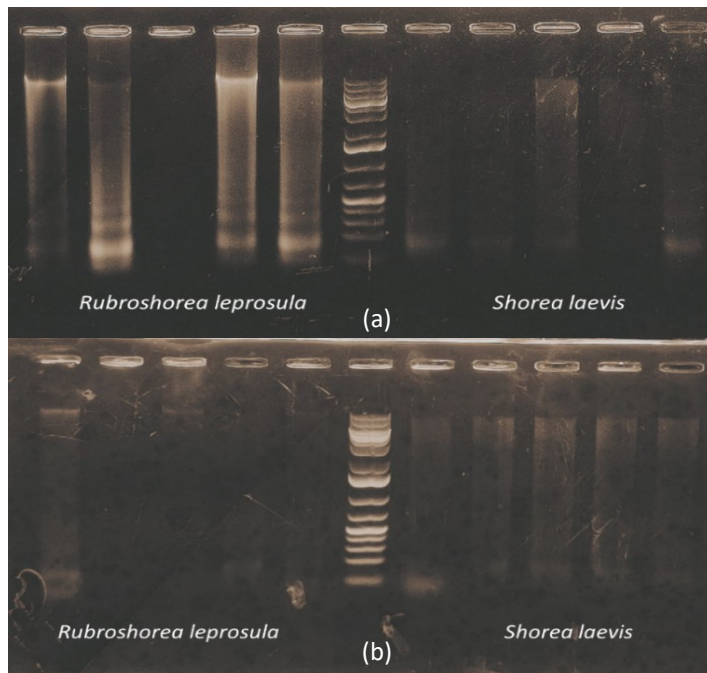


Figure 1. DNA extracted with the modified CTAB method and GeneAid kit was analyzed by agarose gel electrophoresis. (a) Leaf DNA yields show brighter bands in *R. leprosula*, while only faint smears appear in *S. laevis* and (b) Wood DNA extracted using the modified CTAB method shows smeared patterns in all *S. laevis* samples, while only two distinct DNA bands are visible in *R. leprosula*.

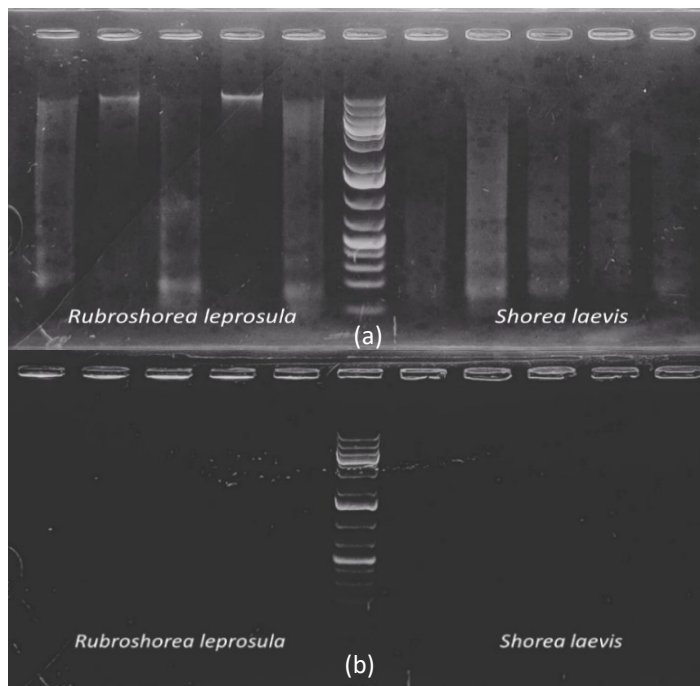


Figure 2. Visualization of (a) leaf DNA and (b) wood DNA extracted using the GeneAid Kit with the modification method. The commercial kits with the modification method reveal that both species exhibit smear bands of lower intensity in leaves, whereas wood extraction does not produce bands or smears in either species.

Quantitative assessment of DNA concentration from leaf and wood extractions of *R. leprosula* and *S. laevis*

Table 1 presents the quantitative assessment of the DNA concentration using a Qubit fluorometer. Quantitative evaluation revealed variations among samples, tissues, and extraction methods. For *R. leprosula* samples, extraction from leaf tissue showed values ranging from 12.8 to 253.0 µg/mL, with two samples (samples 4 and 5) displaying extremely high values. In contrast, the extraction from wood tissue showed lower concentrations, ranging from 3.23 to 30.8 µg/mL. The use of the commercial kit produced variable DNA concentrations in leaves, whereas in wood tissue, most samples showed values that were too low, with one sample having a concentration below 3 µg/mL.

Table 1. DNA concentration (µg/mL) was measured using a Qubit Fluorometer for leaf and wood samples of *R. leprosula* and *S. laevis*, which were extracted using the modified CTAB method and the Geneaid kit. The CTAB method yielded higher concentrations in leaf samples, while the Geneaid kit produced consistently low yields in wood tissues of both species.

Species	Tissue type	Extraction method	DNA concentration (µg/mL)				
			Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
<i>Rubroshorea leprosula</i>	Leaf	CTAB	54.24	54.78	12.8	105.0	253.0
<i>Rubroshorea leprosula</i>	Wood	CTAB	30.8	3.23	9.58	9.79	9.16
<i>Rubroshorea leprosula</i>	Leaf	GeneAid kit	94.8	33.4	too low	21.2	107.0
<i>Rubroshorea leprosula</i>	Wood	GeneAid kit	too low	2.69	too low	too low	too low
<i>Shorea laevis</i>	Leaf	CTAB	26.6	15.6	33.5	20.6	26.1
<i>Shorea laevis</i>	Wood	CTAB	19.5	22.0	23.2	35.3	20.7
<i>Shorea laevis</i>	Leaf	GeneAid kit	20.5	45.5	17.1	20.3	20.7
<i>Shorea laevis</i>	Wood	GeneAid kit	2.86	2.47	too low	2.23	too low

For *S. laevis*, CTAB extraction from leaf tissue yielded DNA concentrations ranging from 15.6 to 33.5 µg/mL, whereas wood tissue produced 19.5 to 35.3 µg/mL, indicating that both tissues can generate measurable DNA despite considerable variation among samples. Extraction using the commercial GeneAid kit from leaf tissue resulted in concentrations between 17.1 and 45.5 µg/mL, whereas wood tissue showed very low concentrations, with two samples recorded as too low and three others below 3 µg/mL, showing a similar pattern to that observed in *R. leprosula*. As shown in Table 1, the extraction results from leaf tissue samples showed higher and more consistent DNA concentrations in both species than those from wood tissue samples. For extraction using the commercial GeneAid kit, the DNA concentration in leaf tissue was better than that in wood tissue. Meanwhile, the CTAB method was able to extract DNA from wood tissue, although at lower and more variable concentrations.

Verification of DNA quality from leaf and wood extractions of *R. leprosula* and *S. laevis* using PCR amplification

The extracted DNA, obtained using both CTAB and commercial kits, was further tested by PCR amplification using the cpDNA trnL-trnF marker. The PCR results showed that all samples were successfully amplified from all types of tissue, both leaves and wood, as indicated by the appearance of target DNA bands (Figure 3a and Figure 3b). The success of this amplification indicates that although the visualization of DNA quality on agarose gel was not optimal, especially in wood extraction using the commercial GeneAid kit, the DNA in the samples was still available in sufficient quantity and quality for PCR analysis.

This finding highlights the difference between DNA extraction visualization on an agarose gel and DNA verification using PCR amplification. The absence of DNA bands on the agarose gel does not directly indicate the complete absence of DNA, but rather an extremely low DNA concentration, which may have prevented DNA from being detectable during agarose gel visualization. Therefore, the successful PCR amplification in all tissue samples using both the conventional CTAB method and the commercial GeneAid kit indicates that the extracted DNA can still be utilized for further molecular applications.

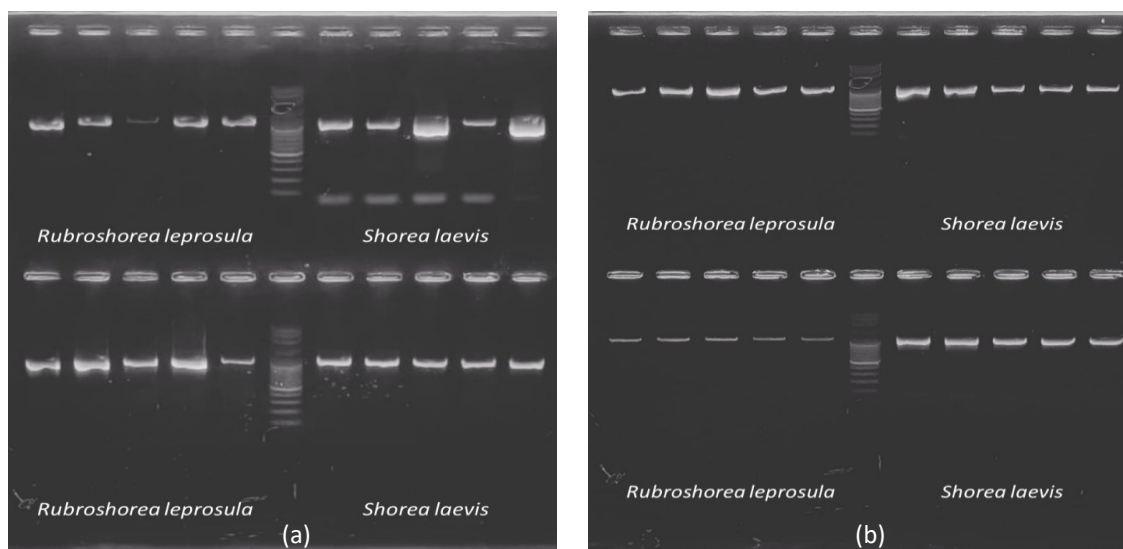


Figure 3. Amplification of trnL-trnF from (a) leaf DNA: CTAB method (top) and GeneAid kit (bottom) and (b) wood DNA: CTAB method (top) and GeneAid kit (bottom). All samples yielded clear PCR products with a single DNA band, indicating successful amplification of both extraction methods.

Discussion

The results of the DNA extraction analysis showed variations depending on the type of plant tissue used for extraction. Leaves consistently yield good-quality DNA during electrophoresis on agarose gel. The presence of living cells, especially in younger leaves, enhances the DNA extraction process compared to woody tissue [43,44]. Additionally, the higher water content in leaves facilitates the breakdown and separation of plant cells, thus optimizing the extraction procedure [45,46]. DNA extraction from wood tissue frequently demonstrates suboptimal quality, as observed through agarose gel electrophoresis. This reduction in quality is primarily due to high concentrations of secondary metabolites, such as lignin, tannins, and polyphenols [47,48], which bind to nucleic acids and impede the DNA extraction process, subsequently affecting both the quantity and purity of the isolated nucleic acids [49]. To address these challenges and enhance the efficiency of the extraction protocol, it is advisable to incorporate higher concentrations of mercaptoethanol and proteinase K [32]. Mercaptoethanol helps eliminate polyphenols [50], whereas proteinase K degrades proteins that may co-precipitate with DNA [35], thereby contributing to a more efficient and refined extraction process from wood tissue.

A comparative assessment of extraction methods revealed that the modified CTAB extraction method consistently yielded superior results for DNA extraction from wood tissue compared to commercial kits using silica membranes. Although the gel visualization results of the extracted samples may differ, all samples successfully underwent PCR amplification, underscoring the importance of evaluating extraction success based on functional outcomes rather than solely relying on visual assessments. Furthermore, Qubit fluorometer quantification highlighted substantial variation in DNA concentrations across tissues and methods, with CTAB providing more measurable yields from wood tissue, whereas the commercial kit yielded relatively higher concentrations in leaf tissue, particularly in *R. leprosula*, where two samples showed exceptionally high values compared to the others. Based on the assessment of DNA quality and quantity, both extraction methods (CTAB and the commercial kit) demonstrated distinct advantages. The CTAB method proved effective in extracting DNA from wood tissue of *R. leprosula* and *S. laevis*, where the commercial kit generally produced DNA with very low concentrations. CTAB ensured that measurable DNA could be obtained from wood samples, highlighting its reliability for challenging tissues. In contrast, the GeneAid commercial kit yielded higher DNA concentrations in leaf tissue, making it more advantageous for research requiring speed and reproducibility. Therefore, the CTAB method is recommended for wood tissue and large-scale studies with limited funding, while the commercial kit is suitable for rapid and efficient extraction from leaf tissue, despite being relatively more expensive.

Robust DNA extraction methods are crucial for assessing the genetic diversity and population structure of dipterocarp families in tropical rainforests, which are increasingly at risk of extinction [51], as well as for identifying suitable seed sources for genetic restoration programs [52]. The effective application of these

methodologies will also encourage the establishment of comprehensive species verification systems within the timber supply chain [53,54]. Establishing a credible mechanism to ensure the legality and origin of timber is vital for global certification schemes such as the Forest Stewardship Council (FSC) and the Programme for the Endorsement of Forest Certification (PEFC) [55], as well as for national systems such as the Timber Legality Verification System (*Sistem Verifikasi Legalitas Kayu/SVLK*) [56]. A reliable DNA extraction protocol is essential for accurately verifying species identity and geographical origin [33,57–59]. Furthermore, successful DNA extraction supports the implementation of international policies, such as CITES, which aim to protect species of significant commercial value [60], while contributing to long-term conservation strategies and forest restoration efforts informed by genetic studies [60].

The successful extraction of DNA from wood represents a significant advancement in the field, serving as a crucial link between laboratory findings and practical applications in resource management, certification processes, and the ongoing fight against illegal timber trade [54]. Effective DNA extraction methods facilitate the genetic identification of both living trees and traded timber, providing essential references for tracing timber origins, verifying legality, and supporting law-enforcement initiatives [22,61]. Forensic DNA derived from timber allows for the comparison and correlation of samples sourced from global and national markets, particularly because many field samples are obtained in log form [62,63]. The ability to perform molecular analysis on timber samples also provides the genetic profiles of the populations being harvested for sale, which is vital for assessing the impact of logging on genetic diversity [64–66] and overall ecological health.

Despite these advancements, the existing limitations of wood DNA databases in forensic applications pose a major challenge, as the majority of genetic reference databases are predominantly based on leaves or fresh tissue [21,31,67], thus constraining the effectiveness of forensic DNA analysis of timber. The development of a reliable and efficient extraction protocol for wood would expand the wood DNA database, which is a foundational requirement for implementing DNA barcoding techniques and conducting comprehensive genetic population studies essential for wood identification in the international market [31,53]. This initiative aligns with key international efforts, including the World Forest ID project and the Global Timber Tracking Network, both of which are dedicated to establishing extensive DNA and isotope reference databases for wood species identification. Consequently, these DNA extraction protocols contribute to molecular and genetic studies and play a crucial role in timber certification processes, promoting sustainable forest resource management, particularly in Indonesia, and supporting the broader objectives of forest conservation and biodiversity protection.

Conclusions

This study provides compelling evidence that DNA extraction methods, particularly the modified CTAB extraction method, effectively address the challenges posed by secondary metabolite compounds compared to commercial kits. Successful PCR amplification indicates that the quality of the extracted DNA is suitable for molecular analysis, even without visual confirmation, particularly in wood tissue. The implications of these findings extend significantly beyond the laboratory. By enabling an accurate assessment of genetic diversity within dipterocarps, this study contributes significantly to targeted restoration and conservation efforts. Moreover, these methodologies provide essential support for verifying species identity, which is critical for ensuring legal compliance and regulatory enforcement in the timber trade, thereby aiding in the determination of the origin of wood. Additionally, the establishment of a comprehensive wood DNA database is facilitated by the efficient extraction protocols described in this study. Thus, a reliable wood extraction protocol is vital for advancing molecular and genetic research, aligning with broader conservation objectives, informing the development of effective environmental policies, and enhancing the management of forest resources at the national level.

Author Contributions

NA: Data Curation, Methodology, Visualization, Formal analysis, Writing – Original draft, Writing - Review & Editing; **HHR:** Conceptualization, Supervision, Investigation, Validation, Writing – Original draft, Writing - Review & Editing; **FGD:** Supervision, Investigation, Validation, Writing - Review & Editing; **WCA:** Investigation, Validation, Writing - Review & Editing; **IZS:** Supervision, Investigation, Validation, Writing - Review & Editing; **IK:** Methodology, Visualization, Writing - Review & Editing; **DS:** Methodology, Visualization, Writing - Review & Editing, and **AS:** Conceptualization, Supervision, Investigation, Validation, Writing – Original draft, Writing - Review & Editing.

AI Writing Statement

During the preparation of this work the authors used Grammarly in order to improve the English language clarity and grammar. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Conflicts of interest

There are no conflicts to declare.

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