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# Molecular Profiling of *Dillenia suffruticosa* in Bangka Islands Using *rbcL* Gene

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**Abstract**

*Dillenia suffruticosa or Simpur is known for their multiple uses in various medical and food packaging purposes. Considering their applications and valuable potential, implementing conservation strategies is required to ensure their survival and preserve their genetic diversity. In this study, we complemented the traditional methods with DNA barcoding for identifying plant species. The current study was designed to accurately identify D. suffruticosa collected from Bangka Islands in Indonesia, through the molecular analysis using rbcL gene as marker genetic. DNA was extracted from the leaves, and PCR was performed to amplify the chloroplast rbcL regions with P609f/P610r primers. Sanger sequencing was conducted, and the homology and divergence of the amplified biological sequences were analyzed using BLASTn. A Neighbor-Joining phylogenetic tree was developed, and genetic distance measurements were computed using the Maximum Composite Likelihood method. Based on our analysis, the specimen was successfully identified by DNA barcoding. An approximately 606 base-pair sequence of the rbcL gene from was amplified from D. suffruticosa, which showed 99% similarity to a species from Hawaii. Furthermore, phylogenetic analysis demonstrated that the rbcL barcode loci used was effective in assigning samples accurately at the genus level, but rather challenging in resolving taxa at the species level. This study was the first to identify the D. suffruticosa in Bangka Islands with the help of DNA barcoding method and suggest that rbcL acts as a reliable marker for identifying a member of medicinal plants at the genus level. Future research should include more genetic markers to enhance species differentiation among Dillenia and other members of Dilleniaceae, leading to a more effective identification method..*

## 1. INTRODUCTION

*Dillenia suffruticosa* is a member of the class Magnoliopsida and the family *Dilleniaceae.* This plant is widely distributed in the forests of the Bangka Islands and has good tolerance to various environmental conditions including ex-mining land [1]. However, this species grows best in moist and swampy environments as thickets and wasteland [2].

*D. suffruticosa* has few local names as Simpor, Simpoh, and Sempur. Local people in Bangka Belitung mostly called this plant as

Simpur or Simpur Air and utilized their leaves as wrappers for traditional foods. Some traditional dishes from Bangka Belitung wrapped in simpur leaves including grilled fish, tempeh, lontong, lakso, and pepes **[3]**. The extract of leaves, fruits, roots, and flowers have been investigated containing a biologically active natural product source including phenolics, alkaloids **[4]**, flavonoids, saponins, tannins **[5]**, polyphenols, and triterpenoids **[3]**. In addition, the recent studies have shown the potential of *D. suffruticosa* as medicinal plants for their antimicrobial **[5]**, antidiabetic **[6]**, antioxidant **[4]**, and anticancer activities **[7]**. Considering of their applications and valuable potencies, implementing conservative strategies to protect Simpur species is essential for ensuring their survival and maintaining their diversity.

Research on plant morphology is essential for advancing conservation efforts, understanding evolutionary processes, ecological studies, plant functions, and species relationships. Morphological characteristics are important for distinguishing plant species, understanding species distribution, and population dynamics **[8]**. However, accurate identification based solely on morphology can be challenging due to the absence of certain distinguishing features, requires reliable references, time-consuming, limited taxonomic expertise **[9]**. In this context, molecular profiling to identify the DNA barcode provides a reliable and efficient alternative for accurately identifying *D. suffruticosa*.

A standardized DNA marker less than 700 base pairs in length showed promise to discriminate species and phylogeny by comparing it to reference sequences in a DNA database. The ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene stands out as a potential standard core coding region for the barcoding of all land plants which recommended by The Consortium for the Barcoding of Life (CBOL) plant working group (PWG) **[10]**. In addition to its widespread presence and the simplicity of its amplification and alignment, *rbcL* is the most extensively characterized plastid coding region in GenBank **[11]**. This marker has been extensively studied within the plastid genome and is represented across all major groups. It is commonly used for identifying algae, peptidophytes, and flowering plants **[38]**. The research suggests that the *rbcL* gene also has greater ability to distinguish between species compared to other chloroplast markers such as the matK gene. The *rbcL* gene proves effective in accurately identifying selected medicinal plant species, with clear and consistent results in every sample tested. It meets most of the necessary criteria and can be used together with other markers for more reliable identification **[39]**.

This study was the first to identify the *D. suffruticosa* on Bangka Islands, Indonesia, through molecular profiling using the *rbcL* gene. The DNA of Simpur leave samples was extracted and the obtained sequences were compared with those of other related species available in GenBank databases to assess the effectiveness of the *rbcL* gene in identifying members of the *Dillenia*.

## 2. MATERIALS AND METHODS

### Plant material collection

Specimen of the species were collected from Pelawan Forest in Bangka Islands, Indonesia. Photographs were taken to record the locations where samples were collected and to show the features of the site. Additionally, inflorescences, leaves, stem, and fruits were documented and collected for analysis of their morphological characteristics. The samples were morphologically examined based on the methods described by Hoogland **[40]** and Tan and Latiff **[2]**.

### DNA extraction

Young leaf samples of Simpur were collected, placed in Ziploc bags containing silica gel for preservation, and transported to Biodiversity Indonesia (BIONESIA) Laboratory in Denpasar, Bali for DNA analysis. Genomic

DNA was extracted from 10 g leaf materials using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

**PCR amplification and sequencing**

The DNA marker was amplified using the primers P609 F: (5'-GTA AAA TCA AGT CCA CCR CG-3') and P610 R: (5'-ATG TCA CCA CAA ACA GAG ACT AAA GC -3') **[12]**. The PCR reaction mixture (26 µL) consisted of 2 µL of template DNA, 1.25 µL of each forward and reverse primer (10 mM), 9 µL of nuclease-free water, and 12.5 µL of Ready mix. The PCR amplification for *rbcL* was carried out using Applied Biosystems™ 2720 Thermal Cycler as follows: initial denaturation for 3 minutes at 94°C, followed by 38 cycles of denaturation at 94°C for 30 seconds, 30 seconds of annealing at 53°C, extension at 72°C for 60 seconds, and final extension of 2 minutes at 72°C **[13]**. The PCR amplicon size was visualized on 1% Agarose gel stained with Nucleic Acid Gel Stain (GelRed®) and bidirectionally sequenced using The Sanger sequencing method at PT. Genetics Science Jakarta.

**DNA barcoding identification**

Sequence chromatogram was edited, assembled into consensus sequence, and converted into FASTA format. The contig sequence was employed in BLASTn to assess for closely matching sequences. The aligned sequences were analyzed for evolutionary relationships using MEGA XI **[14]**. A phylogenetic tree was constructed with *Psidium guajava* designated as the outgroup. The *rbcL* sequences were aligned using MUSCLE, and a phylogenetic tree was generated using the Neighbor-Joining method based on the Tamura-Nei model with 1,000 bootstrap replicates for support **[15]**. Genetic distance values were calculated using the Maximum Composite Likelihood method to enable comparison among the samples **[16]**.

**Two-dimensional DNA barcoding**

The sequenced data was used to generate unique QR code, linking directly to the respective *rbcL* sequences which enabling precise identification of the species. The DNA barcode and QR code were generated using an online Bio-Rad DNA barcode generator (https://biorad-ads.com/DNABarcodeWeb/) and QR code generator (https://www.qr-code-generator.com/) respectively **[17]**.

## 3. RESULTS and DISCUSSION

**Morphological characters**

*Dillenia suffruticosa* from Bangka Island had common features which mainly can be identified from the trunk, leaf, flower, and fruit morphology. The Simpur species in this study was shrub and had small to big sized of sturdy trunk, up to 6 m high (Figure 1A/F). The leaves had enormous size, could be more than 30 cm in length, alternate, and mostly elliptic. The leaf surface was slightly woolly on both side and had 15 pairs or more of secondary veins (Figure 1B/C). The inflorescence was terminal, with one or two branches, forming a raceme, and the flower exhibited bright yellow petal (Figure 1D). The fruit was star-shaped and roseate in color (Figure 1E).

**Sequenced *rbcL* gene**

PCR amplification of the *rbcL* gene yielded clear and distinct bands of the expected size on the agarose gel, confirming primer specificity (Figure 2). The amplified product sizes were ~606 bp and the DNA sequence were shown in Table 1. In further, we used the sequence to develop the DNA QR code and barcode for *D. suffruticosa* from Bangka Islands.

**Sequence analysis with closely related species**

The sequence was used as a query in a BLAST search at NCBI to identify similar sequences within the same or different genera of the *Dilleniaceae* family. The results showed that *D. suffruticosa rbcL* sequences in this study

shared the identity up to 99% at the nucleotide level with other *Dillenia* species. Precisely, our query sequence shared maximum identity of 99,49% with *D. suffruticosa* collected from Hawai. However, highly genetic identity values were also observed between our sample with other species, *D. philippinensis* and *D. alata* up to 99% (Table 2).

Reconstruction of phylogenetic tree revealed clear differentiation between the *Dillenia* species with other genera of *Dilleniaceae*, including *Tetracera, Hibbertia, Davilla,* and *Doliocarpus*. However, all closely related species within these groups were clustered simultaneously and fell under the same clade. Moreover, some reference sequences that belong to distinct species, *D. philipinensis* and *D. alata*, *T. billardierei* and *T. nordtiana*, as well as *D. validus* and *D. subandinus* were well not separated and formed a sister group (Figure 3).

Pairwise analysis showed the differences ratios among the *rbcL* nucleotide sequences. Only a few genetic distance were observed between the species ranges from 0,004-0,029 (Table 3). The nucleotide composition of all 4 bases of *D. suffruticosa* from Bangka as well as reference sequences were calculated. The nucleotide base composition across the sequences were observed as follows T (29%), C (22%), A (27%), and G (22%). AT bases were higher (56%) compared to GC bases (44%) (Table 4). A higher AT content was also observed in *rbcL* sequence of species in the NCBI database

**DNA barcode**

The DNA barcodes typically contain only the Latin names and sequence data of species. In this study, we generated QR codes and two-dimensional DNA barcode images from *rbcL* sequences for precise identification of *D. suffruticosa* from Bangka (Figure 4).

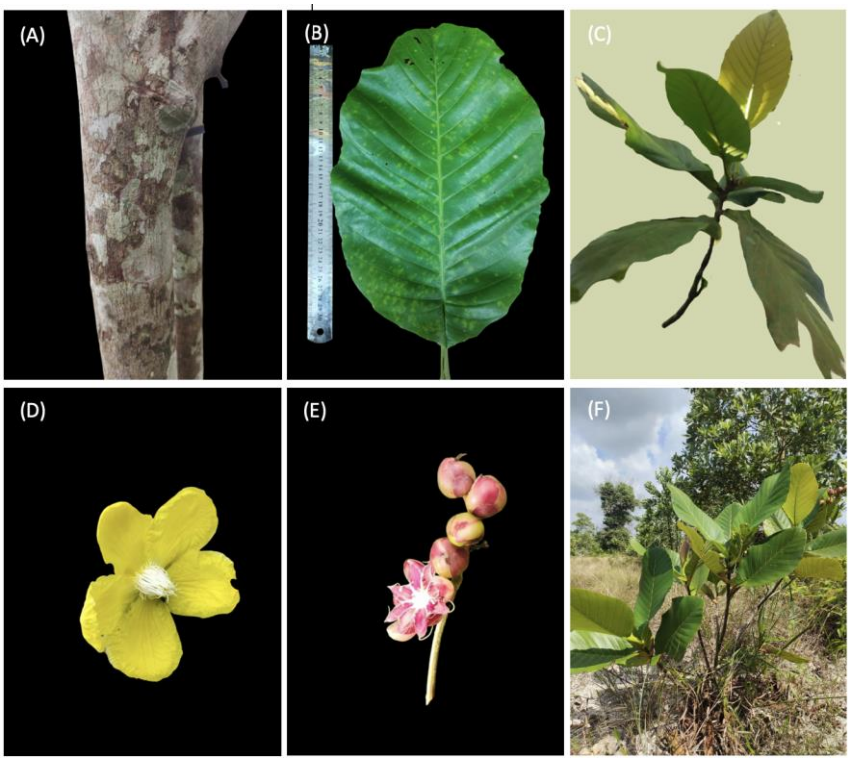


**Figure 1.** *Dillenia suffruticosa*. (A) trunk; (B) leaf; (C) alternate leaf arrangement; (D) flower with yellow petal; (E) stellate-red fruit; (F) young tree.

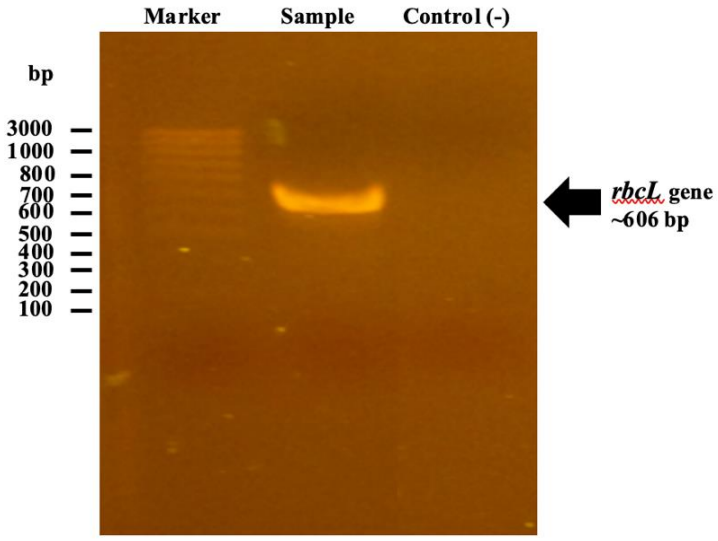


**Figure 2.** Amplified partial *rbcL* gene on agarose gel 1%. Marker and negative control were also shown in this study

**Table 1.** Partial *rbcL* sequence of Simpur Bangka

**DNA sequence**

TTGTAAAATTCCAGTCCACCGCGGAGACATTCATAAACTGCTCTACCATAGTTCTTAGCGGATAACCCCAATTT
AGGTTTAATAGTACATCCCAATAGAGGACGCCCATACTTGTTCAATTTATCTCTCTCAACTTGGATGCCATGAG
GCGGGCCTTGGAAAGTTTTCGTATAAGCAGGAGGGATTCGAAGATCCTCCAGACGTAGAGCGCGAAGGGCTTT
GAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAGGGG
TAAGCTACATAAGCAATAAATTGAGTTTCTTCTCCAGGAACGGGCTCGATGTGGTAGCATCGTCCTTTGTAACG
GTCAAGACTAGTAAGTCCATCGGTCCACACAGTTGTCCATGTACCAGTAGAAGATTCGGCAGCTACCGCGGCC
CCTGCTTCTTCAGGCGGAACTCCAGGTTGAGGAGTTACTCGAAATGCTGCCAAGATATCAGTATCTTTGGTTTC
ATAGTCAGGAGTATAATAAGTCAATTTGTAATCTTTAACACCAGCTTTGAATCCAACACTTGCTTTAGTCTCTGT
TTGGGGGTGACATAA

**Table 2.** Accession number and BLAST result of Simpur Bangka

| Field ID | Specimen | Base-Pair | Gene | Species closed to | Accession Number | Query Cover (%) | Identity (%) |
|---|---|---|---|---|---|---|---|
| ARN24 | *Dillenia suffruticosa* | 605 | *rbcL* | *Dillenia* sp. | FJ860354.1<br>FJ860352.1<br>FJ860348.1 | 98<br>98<br>97 | 99,49<br>99,32<br>99,32 |

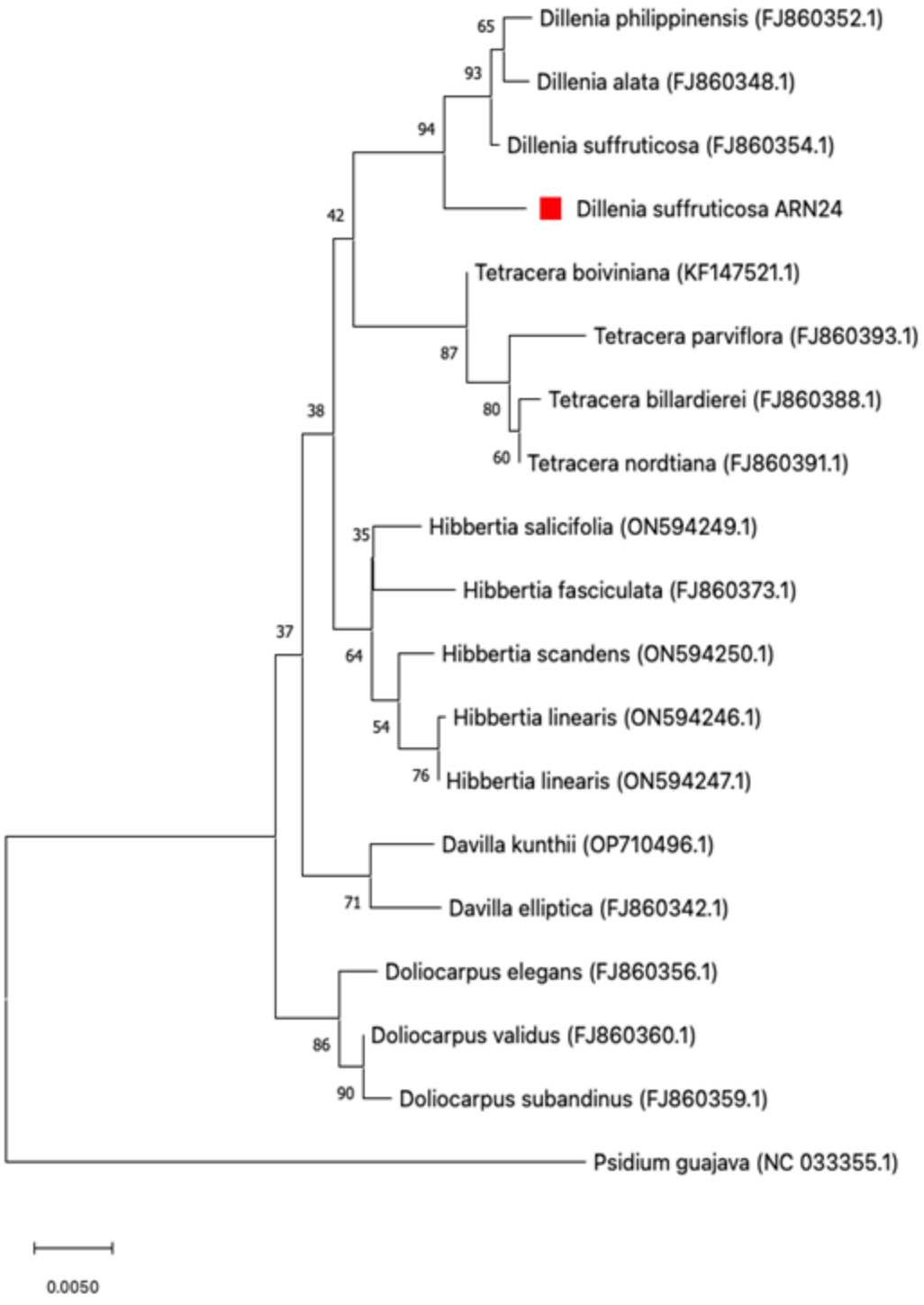


**Figure 3.** Neighbor joining tree representing relationship of Simpur Bangka with closely related species. *Psidium guajava* was used as an outgrup to root the phylogenetic tree. Value of 0,005 shows the substitution rate per nucleotide site

**Table 3. Pairwise distance using maximum composite likelihood in *rbcL* gene**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Dillenia suffruticosa* ARN24 | | | | | | | | | |
| 2 | *Dillenia suffruticosa* (FJ860354.1) | 0,008 | | | | | | | | |
| 3 | *Dillenia philippinensis* (FJ860352.1) | 0,010 | 0,003 | | | | | | | |
| 4 | *Dillenia alata* (FJ860348.1) | 0,012 | 0,003 | 0,003 | | | | | | |
| 5 | *Hibbertia scandens* (ON594250.1) | 0,020 | 0,013 | 0,015 | 0,017 | | | | | |
| 6 | *Hibbertia salicifolia* (ON594249.1) | 0,020 | 0,012 | 0,013 | 0,015 | 0,007 | | | | |
| 7 | *Davilla kunthii* (OP710496.1) | 0,019 | 0,019 | 0,021 | 0,023 | 0,015 | 0,015 | | | |
| 8 | *Doliocarpus Validus* (FJ860360.1) | 0,022 | 0,022 | 0,025 | 0,023 | 0,017 | 0,015 | 0,016 | | |
| 9 | *Doliocarpus elegans* (FJ860356.1) | 0,022 | 0,026 | 0,029 | 0,027 | 0,017 | 0,015 | 0,016 | 0,004 | |

**Table 4.** Base composition of *rbcL* gene in *Dillenia* sp

| **Sequence** | **T (%)** | **C (%)** | **A (%)** | **G (%)** |
|---|---|---|---|---|
| *Dillenia suffruticosa* ARN24 | 29 | 22 | 27 | 22 |
| *Dillenia suffruticosa* (FJ860354.1) | 29 | 19 | 27 | 25 |
| *Dillenia philippinensis* (FJ860352.1) | 29 | 19 | 27 | 25 |
| *Dillenia alata* (FJ860348.1) | 29 | 19 | 27 | 25 |
| Avg. | 29 | 20 | 27 | 24 |


**Figure 4.** DNA barcode (A) and QR code (B) generated using *rbcL* sequence of Simpur Bangka

## 4. Discussion

Indonesia is home to 5490 medicinal plant taxa, spanning 245 families and 1,809 genera. Of these, 3312 species are native, 1754 have been introduced, and 342 species have an uncertain origin **[18]**. *Dillenia suffruticosa* as member of *Dilleniaceae* is known for their multiple uses in various medical and food packaging purposes **[19];[20]**. For instance, extracts from the leaves, fruits, roots, and flowers have been studied and identified as a highly promising source of antioxidant and

anticancer properties as saponins, triterpenes, sterols, polyphenolic compounds, betulinic acid, koetjapic acid, lupeol, vitexin, tiliroside, and kaempferol ( **[21];[22]**. In particular, Simpur was widely distributed in all areas of Bangka Island and commonly used by the local ethnic. The leaves as wrappers for traditional foods [3], woods for wild animal deterrent, and leaves for any medicinal purposes. Several studies reported that the local community of Bangka Islands often used the boiled water from Simpur leaves to treat diabetes mellitus **[41]** and diarrhea **[42]**. In addition, the pounded of fresh leaves are mostly utilized for anti-bruise medication by Suku Lom Bangka **[43]**. In this study, we proposed the morphological characters and DNA barcode based on *rbcL* locus of *D. suffruticosa* or Simpor bini by locals in Brunei to initiate and enhance the conservation strategies of this species.

The gene *rbcL*, which encodes the large subunit of the enzyme ribulose bisphosphate carboxylase/oxygenase, is often used as a marker for comparing species at the family and genus levels. However, it is not effective for distinguishing between different species due to its limited ability to show differences. The gene is about 1430 base pairs long, so at least two pairs of primers are required to fully sequence the entire coding region **[38]**. In addition, a multiple loci approach has been adopted in plant DNA barcoding because the recommended single barcode regions including *rbcL*, matK, and psbA-trnH have failed to effectively distinguish between different species due to slow evolutionary rate of chloroplast DNA. However, other findings suggested that the *rbcL* gene has greater ability to distinguish between species compared to other chloroplast markers and proves effective in accurately identifying some medicinal plant species **[39]**.

Some of the past studies have shown a similar overall appearance with our specimen of *D. suffruticosa* in terms of tree height, leaf size and structure, petal color, as well as fruit shape. Precisely, this medium-sized tree, growing up to 6 meters tall, features large elliptic to obovate leaves (15–35 cm) and vivid yellow flowers with five slender, scentless petals. It produces star-shaped fruit capsule opens completely well before sunrise, revealing its purple seeds surrounded by a bright red, fleshy aril **[23]**. In addition, it naturally grows in the moist, evergreen forests stretching from West Malaysia to Indonesia, Brunei Darussalam, and Philippines **[24]**. These features differentiate *D. suffruticosa* from other species, particularly with *D. indica* and *D. excelsa* which also has local name as Simpur **[25];26]**. In comparison, *D. indica* or elephant apple has a tree up to 30 meters in height. The leaves are elliptical, oblong, to lance-shaped and approximately 33 cm long and 8.5 cm wide. The flower type is solitary with terminal inflorescences. The petals are white with green stripes and are free from one another. The fruit is round (indehiscent), with the outer part covered by the green-colored sepals. The seeds are small, kidney-shaped (reniform), and wrapped in fine hairs **[27];[28]**. While *D. excelsa* or also known as Simpor laki in Brunei, is well represented by its tree habitus, reaching up to 40 meters in height. The leaves are elliptical to oblong, approximately 32 cm long and about 9 cm wide. The flowers are yellow with five separate petals. The fruit is white star-shaped (dehiscent), containing 18–20 carpels. Each carpel contains 1 to 3 brown seeds, each enclosed in a red aril **[19];[28]**.

Accurately identifying plant biodiversity is crucial for the preservation of rare endemic and endangered species, especially for the study of plant conservations since continues to be threatened by human acitivities **[29]**. The conventional taxonomy system has relied on morphological characteristics. However, this method becomes unreliable in cases of its reliance on morphological traits. Vegetative features, which can vary significantly under different environmental conditions, often lead to inconsistencies and confusion in species identification. Additionally, the presence of

cryptic plant species, those that appear identical morphologically but belong to separate evolutionary lineages, further complicates accurate classification. These challenges highlight the need to integrate molecular approaches, such as DNA barcoding, into taxonomic practices. By incorporating genetic information, we can achieve more precise and dependable plant identification, effectively addressing the limitations of morphology-based methods **[30]**. Among these, *rbcL* gene have been recommended as the primary plant barcoding markers by the Consortium for the Barcode of Life (CBOL) **[31]**. However, the choice of an ideal DNA barcode for plants remains a subject of debate, as the effectiveness of different loci can vary depending on the taxonomic group. The ability of these genes to distinguish species is not consistent across all plant species, making species identification challenging in some cases **[32]**. In this study, we successfully amplified ~606 bp rbcL sequence of *D. suffruticosa*. The size was in accordance with rbcL amplicon obtained from *Dilleniaceae* and other plant species which were approximately 600 bp with a full length of 1400 bp **[31];[12];[33]**. It also confirms that DNA sequences can be readily obtained using *rbcL* primers across flowering plant species in Sumatra **[34]**.

To assess the success rate of species identification, we compared the outcomes of morphological identification with those of molecular identification. The results demonstrated that DNA barcoding alone is sufficient to accurately assign DNA sequences to the correct species, as evidenced by the highest similarity with *D. suffruticosa* collected from Hawai which had become invasive species in these islands **[35]**. However, reconstruction of the phylogenetic tree showed the *rbcL* limitation to discriminate sequences on species level. The samples tested formed five distinct clades, grouped based on genus, and clustered different closely related species. The sequenced sample and NCBI reference for *D. suffruticosa* was align closely with *D. philipinensis* and *D. alata* indicating the high sequence similarity between the *rbcL* regions of respective species, which makes it difficult to distinguish between the sequences. Morphologically, it is clearly evident that *D. suffruticosa, D. philipinensis* and *D. alata* share a similar overall appearance, with the primary distinctions lying in their flower features; *D. suffruticosa*, the flowers are either nodding or oriented to the side, the petals are yellow and spread out, while the stamens, staminodes, and stylar branches are whitish in color; *D. philipinensis*, the flowers are generally upright or oriented to the side, the petals are white and fully expanded, while the gynoecium is dark red, the thick, firm stylar branches are also expanded and slightly curved backward; while *D. alata* shares the same floral architecture as *D. philipinensis* but differs in coloration, the petals and the outer set of stamens are yellow, whereas the carpels and the inner set of stamens are red **[36]**. The phylogenetic analysis underscored both the strengths and limitations of the *rbcL* gene in DNA barcoding and species differentiation within *Dilleniaceae*. It functions reasonably well in identifying flowering plant species, at least to the genus level **[34].**

The results of the clade analysis based on the *rbcL* chloroplast gene also showed bootstrap support value of 94%, 87%, 64%, 71%, 86% in clade group of *Dillenia, Tetracera, Hibbertia, Davilla,* and *Doliocarpus* respectively. It indicates that the species in a same clade are closely related genetically, and their lineage may trace back to a shared ancestor. The bootstrap scores are categorized as strong (>85%), moderate (70–85%), weak (50–70%), or poor (50% or less) **[44]**. A higher bootstrap value suggests greater confidence in the clade's placement within the phylogenetic tree. In this study, bootstrap values of >85% was obtained for *Dillenia, Tetracera,* and *Doliocarpus*, which is considered high. This indicates that the *rbcL* marker is a reliable molecular marker suitable for use in the phylogenetic analysis of these groups **[45]**.

However, moderate to weak bootstrap values were shown in clade *Davilla* and *Hibbertia* due to the limited number of characters supporting each node. This issue is common in recently diverged groups **[46]**. Additionally, the *rbcL* sequence proved to be relatively effective, with all species pairs showed any genetic distance values. The genetic difference among clades within the same genus were smaller than those between clades of different genera. These values indicate that a greater genetic distance reflects a more distant evolutionary relationship between taxa **[33]**. Genetic distance refers to the level of difference in a gene, which is determined by comparing the variations between different species or groups of population. The results showed a genetic distance of 0.008 between our sample and *D. suffruticosa* from Hawaii, indicating that there are 8 differing base pairs out of a total of 1000 base pairs. In this study, genetic distance in family *Dilleniaceae* showed rather low values **[37]**. The highest recorded difference is 0.029 of DNA between *Dillenia philippinensis* and *Doliocarpus elegans*. Genetic distances are categorized as low (0.010-0.099), medium (0.1-0.99), and high (1.00-2.00) **[47]**. The nucleotide composition percentages of *Dillenia suffruticosa* were very close to those of *Dillenia philippinensis* and *Dillenia alata*. The data also showed that Simpur Bangka has a different base composition compared to other *Dillenia* species, which had T (29%), C (19%), A (27%), and G (25%). However, *Dillenia* sp. showed a similar pattern with high levels of adenine and thymine, so the *rbcL* gene of this genus is classified as part of the A-T rich group. The A-T hydrogen bonds involve two hydrogen bonds, which are weaker than the three hydrogen bonds in G-C pairs. As a result, there is a higher likelihood of spontaneous mutations occurring in this group **[48].**

QR code and two-dimensional DNA barcode image were generated based on rbcL gene of *D. suffruticosa* which composed of AT rich sequence. Scanning the two-dimensional QR code located next to the DNA barcodes with a mobile device quickly retrieves the corresponding sequence and will make the species identification more unchallenging.

Closely related plant species have been successfully identified using a genetic marker, including *rbcL*. Previous studies have also suggested that additional regions such as matK and ITS can further enhance species differentiation **[31]**. Utilizing multiple marker combinations is particularly effective in cases where high sequence similarity exists among species or subspecies. While *rbcL* alone can reliably differentiate closely related species in some plants, combining it with other markers improves accuracy for species where *rbcL* alone is insufficient

## 5. Conclusion

We successfully amplified approximately 606 bp of *rbcL* sequence from *Dillenia suffruticosa*, which has 99% similarity with species from Hawai. The current research indicates that *rbcL* serves as a reliable marker for identifying a member of medicinal plants `up to the genus-level. To improve species differentiation among *Dillenia* or other members of *Dilleniaceae*, future research should incorporate additional genetic markers to develop a more robust and effective identification method.

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