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DNA Barcoding for Species Identification and Antioxidant Evaluation of Kapul (*Baccaurea spp.)*: Implication for The Conservation of Native Plants of Kalimantan

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Abstract: This study aimed to characterize the molecular identity of three morphologically distinct Kapul types from West Kutai, East Kalimantan, using DNA barcoding based on the matK and rbcL genes, and to evaluate the antioxidant activity of their leaf extracts. Leaf samples were subjected to DNA extraction, PCR amplification, Sanger sequencing, and subsequent BLAST comparison against reference sequences available in the GenBank database. The BLAST analysis revealed high sequence similarity with several *Baccaurea* species, with sequence identity values generally exceeding 98%. However, the highest similarity matches were not consistently assigned to a single species across different barcode regions, indicating limitations of the matK and rbcL markers in resolving species-level identification among closely related taxa. Antioxidant evaluation of ethanolic leaf extracts demonstrated strong activity, with IC_{50} values of 6.94 ppm, 10.30 ppm, and 15.08 ppm for Samples 1, 2, and 3, respectively. These findings reveal high molecular similarity among the investigated Kapul types and provide preliminary evidence of their antioxidant potential, while highlighting the need for additional molecular markers to improve species-level identification and support future conservation studies.
Keywords: Antioxidant activity; *Baccaurea* spp.; DNA barcoding; matK; rbcL

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INTRODUCTION

East Kalimantan is recognized as one of the biodiversity-rich regions in Indonesia, characterized by extensive tropical rainforest ecosystems that support a wide range of plant resources. These biological resources have long been utilized by indigenous communities, including the Dayak people, who rely on locally available plants for traditional medicine and daily healthcare practices. Medicinal plants are commonly prepared as herbal remedies, either as primary treatments or as complementary therapies alongside modern medical interventions. However, the scientific documentation, authentication, and biological characterization of many ethnomedicinal plants remain limited, as their identification is frequently based on vernacular names and morphological characteristics. Such limitations may lead to uncertainty in species identification and hinder the development of reliable information regarding their phytochemical properties and biological potential. Therefore, systematic studies integrating taxonomic identification, phytochemical evaluation, and biological activity assessment are essential to support the documentation and sustainable utilization of traditional medicinal plants (Lestari et al., 2023).

Among the native plant resources of Kalimantan, species belonging to the genus *Baccaurea* (Phyllanthaceae) have attracted increasing attention due to their ecological,

ethnobotanical, and nutritional importance. Several *Baccaurea* species are traditionally consumed as fruits and used by local communities, including *Baccaurea macrocarpa* (Tampoi), *Baccaurea lanceolata* (Liposu), and other locally recognized Kapul varieties. Previous studies have demonstrated that *Baccaurea* species contain diverse secondary metabolites associated with biological activities. The bark of *B. macrocarpa* has been reported to contain alkaloids, flavonoids, phenolic compounds, steroids, and triterpenoids, although saponins were not detected (Erwin et al., 2019). Phenolic compounds and flavonoids are considered important contributors to antioxidant activity due to their ability to donate electrons or hydrogen atoms and neutralize free radicals. Phytochemical analyses of Liposu (*B. lanceolata*) and Tampoi (*B. macrocarpa*) fruits revealed the presence of phenolics, flavonoids, and tannins, with extracts exhibiting significant free radical-scavenging activity depending on the extraction solvent and plant part evaluated (Abu Bakar et al., 2014). Furthermore, Erwin et al. (2019) demonstrated that *B. macrocarpa* bark extract exhibited strong antioxidant activity using the DPPH assay, with an IC_{50} value of 11.15 ppm. These findings indicate that *Baccaurea* species represent potential sources of natural antioxidants; however, information regarding the biological properties of several locally occurring species remains limited.

Despite their ecological and economic importance, the species diversity and taxonomic identity of *Baccaurea* in Kalimantan remain insufficiently documented. Accurate species identification is particularly challenging within closely related plant groups because several taxa may share overlapping morphological characteristics, especially when reproductive structures are unavailable. Morphological identification based solely on vegetative traits may therefore result in uncertainty and misidentification among closely related species. This limitation emphasizes the importance of integrating molecular approaches with conventional taxonomy to improve species documentation and biodiversity assessment.

DNA barcoding has emerged as an effective molecular approach for species identification by utilizing short standardized DNA sequences as genetic markers. This technique provides sequence-based evidence that can complement morphological identification and improve taxonomic resolution, particularly for closely related or morphologically similar taxa (Bhavana et al., 2021; Wardani et al., 2022). DNA barcoding has been widely applied for species authentication, biodiversity assessment, and characterization of cryptic or unidentified species (Letsiou et al., 2024). In plants, chloroplast DNA regions are among the most frequently used barcode markers due to their conserved structure, relatively low recombination rate, and availability across diverse plant taxa (Sundari et al., 2019). Several chloroplast loci have been proposed for plant DNA barcoding, including *atpF-H, matK, ndhF, rpoC1, trnH-psbA, rbcL, rpl32-trnL*, and *rpoB* (Paksoy et al., 2022).

Among these markers, *matK* and *rbcL* are considered core barcode regions recommended for plant identification. The *rbcL* gene generally provides high amplification success and broad taxonomic coverage, whereas *matK* exhibits greater sequence variation and may provide higher discriminatory power among closely related taxa. The combination of these two loci has been widely applied to improve taxonomic resolution in different plant groups. For example, DNA barcoding analyses in Fabaceae demonstrated that the combination of *matK* and *rbcL* improved species identification performance compared with morphological classification alone (Wardani et al., 2022). Similarly, studies involving *Acacia* species have successfully utilized *matK*-based barcoding for molecular identification and taxonomic assessment (Suriani et al., 2021). Recent investigations of invasive terrestrial plants in India also reported

that combining *matK* and *rbcL* markers improved phylogenetic resolution compared with using individual loci, highlighting the complementary value of these markers in plant discrimination studies (Lonare et al., 2025). However, the effectiveness of DNA barcoding is influenced by marker variability, taxonomic complexity, and the completeness of available reference databases. Therefore, additional molecular markers and comprehensive reference libraries may be required for accurate species-level identification (Varsha Rani et al., 2026).

For the genus *Baccaurea*, molecular information remains limited, and several species are poorly represented in public sequence databases. Previous studies have reported challenges in resolving species relationships within *Baccaurea* using single barcode regions, suggesting that closely related species may share high sequence similarity in commonly used chloroplast markers (Saswita et al., 2023a). Furthermore, several *Baccaurea* species remain data deficient, limiting the reliability of database-based molecular identification approaches (Saswita et al., 2023b). Consequently, integrating molecular characterization with morphological identification is necessary to improve the documentation of *Baccaurea* diversity, particularly for underexplored taxa from Kalimantan.

In addition to taxonomic characterization, evaluating the biological potential of *Baccaurea* species may provide complementary information regarding their utilization value. Antioxidant activity assessment, particularly through the DPPH radical-scavenging assay, is widely used to evaluate the free radical-neutralizing capacity of plant extracts (Molyneux, 2004; Prior et al., 2005). However, antioxidant information regarding several locally recognized Kapul types remains limited, particularly for leaf extracts. Therefore, an integrated approach combining DNA barcoding and antioxidant evaluation may provide valuable baseline information regarding the molecular identity and biological potential of indigenous *Baccaurea* resources.

The present study aimed to characterize three morphologically distinct Kapul candidates from East Kalimantan using DNA barcoding based on the chloroplast *matK* and *rbcL* genes and to evaluate the antioxidant activity of their leaf extracts. The findings are expected to contribute baseline molecular and biological information for further taxonomic, biodiversity, and conservation studies of *Baccaurea* species in Kalimantan.

METHOD

This study was conducted from July to November 2025 in two laboratories, namely the Pharmaceutical Chemistry Laboratory, Faculty of Pharmacy, Universitas Nahdlatul Ulama Kalimantan Timur and Genetic Research Center, YARSI Research Institute, YARSI University. The research was divided into two main parts: 1) species determination through morphological and DNA barcoding analysis; 2) and phytochemical screening continued with antioxidant assay.

Sample collection

Leaf samples used in this study were collected from three different locations in Kutai Barat Regency, Kalimantan Timur, Indonesia. A total of three samples were analysed, each known by local names, namely kapul (S1), kapul kecil (S2) and kapul kurap (S3). Kutai Barat Regency was selected according to previous research (Akhmadi and Sumarmiyati 2015), and the search for the plants was focused on the Long Iram area. The research team communicated with local informant and visited the plant locations, asked for written permission from the owner when possible, then collected leaves, and documented the plants.

Morphological Identification

Species determination was performed through the preparation of herbaria from three samples, which were then used for morphological analysis. The determination was carried out at the Laboratorium Ekologi dan Konservasi Biodiversitas Hutan Tropis Fakultas Kehutanan dan Lingkungan Tropis, Universitas Mulawarman particularly Samarinda: Herbarium Universitas Mulawarman (SHMU), based on Angiosperm Phylogeny Group IV. The result of species determination was informed through a formal letter. Since our team requested the herbaria to be returned, the herbaria do not have voucher number.

DNA Extraction

DNA extraction was performed following the manufacturer's protocol of the Wizard® Genomic DNA Purification Kit Promega (Catalogue No. A1120). DNA electrophoresis was subsequently conducted as a standard method to assess the quality of the extracted DNA. DNA quantity and DNA/Protein ratio were measured using Microplate Readers Tecan Infinite ® 200. All samples were extracted and measured in duplicate.

Primer Design

Primers for PCR were designed using the primer3 website (https://primer3.ut.ee/). The primers for DNA barcoding marker genes, namely *rbcL* gene and *matK* gene, were designed using *Baccaurea ramiflora* Lour chloroplast, complete genome (NCBI Reference Sequence NC_057309.1) and the primer for 18sRNA gene were designed using *Phyllanthus emblica* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: P140605211 (NCBI Reference Sequence: LC089029.1). Primers were synthesized using Macrogen service.

Amplification of *rbcL* and *matK* genes with PCR

Conventional PCR amplification was conducted by preparing a reaction mixture; contained the following reagents : 12.5 µL of GoTaq Green, 1 µL each of left and right primers, 1 µL of template DNA, and 0.3 µL of bovine serum albumin (BSA) and 9.2 µL Nuclease-free water was then added to adjust the final volume. For the PCR sequencing procedure, the reagents used were identical to those applied in the conventional PCR; however, the reaction volume was increased twofold. The reaction mixture was gently homogenized before being subjected to PCR amplification under the appropriate thermal cycling conditions. PCR amplification was carried out under the following conditions: initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 57°C for 30 sec, and 72 °C for 1 min; final extension at 72°C for 5 min; and a hold at 4 °C. DNA electrophoresis was performed to evaluate the quality of the PCR products. Touchdown PCR amplification of the *rbcL*/*mat*K gene regions was performed to improve amplification specificity. The reaction was carried out in a total volume of 50 µL containing 25 µL of GoTaq Green Master Mix (2×), 2 µL each of left and right primers, 2 µL of template DNA, and 0.3 µL of bovine serum albumin (BSA). Nuclease-free water was added to reach the final reaction volume. PCR amplification was conducted using an initial denaturation at 95 °C for 3 min, followed by 15 touchdown cycles consisting of denaturation at 95 °C for 30 s, annealing starting at 60–65 °C and decreasing by 1 °C per cycle until the target annealing temperature was reached, and extension at 72 °C for 60 s. This was followed by 25 additional cycles at a constant annealing temperature, with denaturation at 95 °C for 30 s, annealing at the final touchdown temperature for 30 s, and extension at 72 °C for 60 s. A final extension was performed at 72 °C for 7 min. All samples were amplified in duplicate for each primer.

Capillary Electrophoresis Sequencing

Sequencing was conducted in accordance with the Macrogen sequencing service protocol, which included PCR product purification (Catalogue No. SEQNAU-0004) followed by Sanger sequencing of the PCR products using forward and reverse primers (Catalogue No. SEQNSI-0001). The sequencing results were analyzed using Bioedit software for left, right, and consensus sequences. The sequences were then compared with blast nucleotides in the NCBI GenBank database.

Preparation of Leaf Ethanolic Extract

Plants leaves were washed with distilled water and subsequently air-dried in a well-ventilated environment at ambient temperature. The dried material was then finely ground utilizing a grinder and subjected to extraction procedures with 96% technical-grade ethanol using a plant material-to-solvent ratio of 1:5 or 1:6 thus the plant material in submerged condition. The resulting mixture was macerated at room temperature and filtered through filter paper. The filtrate was concentrated using waterbath (Memmert) at 50 °C to obtain a concentrated extract. This extract was subsequently transferred to an airtight container equipped with silica gel.

Phytochemical Screening

Flavonoid

Four drops of concentrated HCL were added to the extract solution, followed by the introduction of 9mm pieces of magnesium ribbon. The formation of flavonoids was confirmed through observable colour changes, particularly the development of a pale pink hue or the fading of colour in specific solutions (Asman, et al, 2024). Quercetine was used as positive control.

Phenolic

One mL extract solution was mixed with 200µL of 5% $FeCl_3$ Solution. The appearance of a color change, such as blue, green, purple or red indicates the presence of phenolic like tannins (Maheshwaran, et al, 2024). Gallic acid was used as positive control.

Saponin

The extract will be reconstituted in 20 mL of distilled water. The graduated cylinder will be well-shaken for 15 minutes. The presence of saponin indicated by a 2 cm layer of foam (Maheshwaran, et al, 2024).

Alkaloid

Mayer's Test

Two drops of Mayer's reagent were added to a few mL of the filtrate. A brownish-yellow complex that in that form of precipitates is formed. The formation of precipitates indicates the presence of alkaloid in the sample (Maheshwaran, et al, 2024). Powdered coffee beans were used as positive control.

Wagner's Test

Two drops of Wagner's reagent were added to a few mL of the filtrate. The formation of reddish brown precipitate indicates the presence of alkaloid in the sample (Maheshwaran, et al, 2024). Powdered coffee beans were used as positive control.

Dragendorff's Test

Add 1 mL reagent to 2 mL of plant extract solution. A positive result is the formation of a brownish or yellowish precipitate, which is a complex compound of potassium alkaloid (Maheshwaran, et al, 2024). Powdered coffee beans were used as positive control.

Tannins

A small quantity of the leaf extract was added to a test tube and then an equal volume of gelatin solution was added to it and NaCl was added as well. The mixture was shaken gently and allowed to stand for a few minutes. Positive results are indicated by the formation of a precipitate or turbidity (Maheshwaran, et al, 2024). Tea was used as positive control.

Steroid and Triterpenoid

Steroid and terpenoid content were examined using Liebermann-Burchard reagent. A series of color changes will occur, typically starting from pink and progressing to blue, green and then to deep green, indicating the presence of sterols such as cholesterol (Maheshwaran, et al, 2024).

Antioxidant Assay

The antioxidant activity was assessed using the DPPH radical scavenging methods. Extract solutions were prepared at concentration of 3,6,9,12 and 15 ppm. Subsequently 2 mL of each extract was mixed with 1 mL of 100ppm DPPH solution in a test tube and the mixture was incubated for 30 minutes in the dark and protected from light before being measured by Spectrophotometer B-One 100. Measurements were carried out at a wavelength of 514 nm. Quercetin was used as positive control with variation of concentration 1, 2, 3, 4, and 5 ppm respectively.The antioxidant activity of the samples was expressed as percentage inhibition (PI), calculated using the formula : %scavenging activity = (Abs control – Abs sample)/Abs control x 100. The experiment was conducted in triplicate. The experiment was conducted in triplicate, and the results were reported as the mean ± standard deviation of the scavenging activity (Prior et al, 2005). The concentration values as X and %scavenging activities as Y for three samples and quercetin were plotted to obtain linear regression equation y=a+bx. The inhibition concentration 50 (IC50) for each sample was calculated according to the equation obtained.

RESULT AND DISCUSSION

Based on previous studies by Akhmadi and Sumarmiyati (2015), Kapul plants are commonly found in West Kutai Regency, particularly in Mencimai Village and Long Iram**.** This study aimed to locate three Kapul species as reported in earlier research, with the assistance of local community members. Three Kapul samples were successfully collected. The first Kapul sample, according to local community information, was known as Kapul Putih**.** It was found growing in a household yard and had been intentionally planted. Local residents identified the tree as Kapul putih based on prior fruiting. The second Kapul sample was collected from Muara Mujan Village, Tering area, where it was growing in a local garden. The owner of the tree identified it as Kapul kecil (locally known as *jentikan*), which had also been intentionally planted and had previously borne fruit. The third Kapul sample was obtained from a forest area near the mining access road in Ujoh Halang**.** According to local informants, this sample grew wild and had previously fruited, and it is locally known as kapul kurap**.**

The sampling locations, plant habitus, and leaf (and flower) morphology are presented sequentially in Figure 1. During the sampling process, local informants played a crucial role in identifying and locating plant species that are becoming increasingly rare. This highlights the importance of local ecological knowledge in supporting species conservation. Species determination of the three Kapul samples was conducted by the Faculty of Forestry, Universitas Mulawarman and using Angiosperm Phylogeny Group IV resulted as follows: sample 1 is identified as *Baccaurea macrocarpa* (Miq.) Mull.Arg with common name as Kapul, sample 2 is

identified as *Baccaurea microcarpa* (Airy Shaw) Haegens with common name as Kapul Kecil, and sample 3 is identified as *Baccaurea bracteata* Mull.Arg with common name as Kapul.

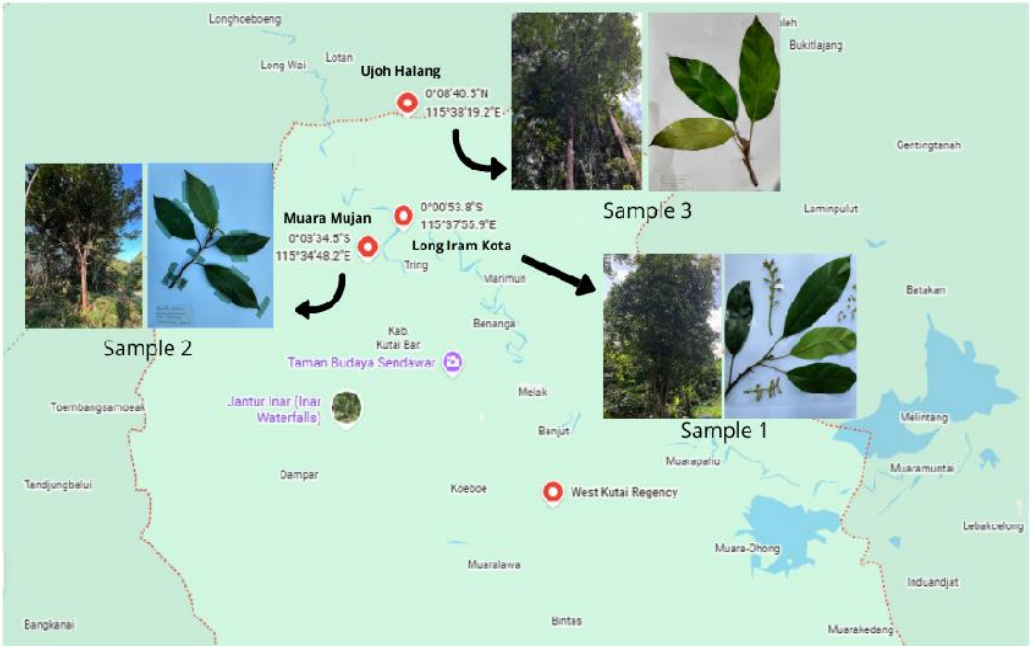

Figure 1. Map of samples collection site in Kutai Barat Regency Kalimantan Timur

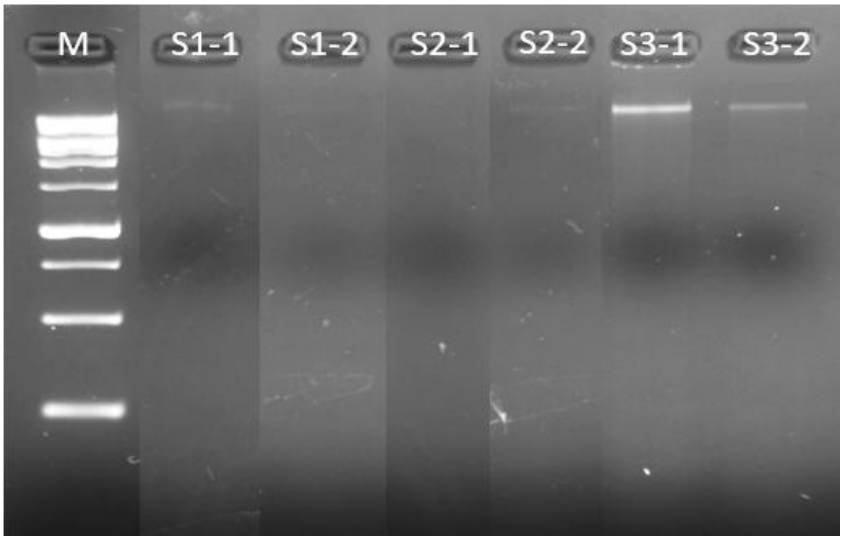

Figure 2. Genomic DNA extraction from each sample analyzed using agarose gel electrophoresis showed bands typical for genomic size. M: Marker DNA

Table 1. Microplate readers Tecan Infinite ® 200 read out for absorbances of DNA at 260 nm, protein at 280 nm, DNA concentration and DNA to protein ratio.

Sample ID-Replication	260	280	Conc ng/µ	Ratio
S1-1	0.0105	0.0073	10.05	1.44
S1-2	0.0061	0.0046	6.1	1.33
S2-1	0.0386	0.0222	38.6	1.74
S2-2	0.0141	0.0079	14.1	1.78
S3-1	0.12195	0.08565	121.95	1.42
S3-2	0.02855	0.02155	28.55	1.32

This study successfully amplified the *matK* and *rbcL* regions from DNA isolates of Samples 1, 2, and 3 using two pairs of matK primers (matK131 and matK289) and three pairs of rbcL primers (rbcL 13,rbcL32, and rbcL289), targeting overlapping regions. Samples 1 was successfully amplified with all primer pairs, whereas Sample 2 produced amplification products with rbcL13 and rbcL56 primers (Figure 3). No amplification was obtained from Sample 3, possibly due to insufficient DNA quality or quantity. The amplified products were subsequently subjected to Sanger sequencing. Sequence data were processed using bioEdit, including trimming of low-quality chromatogram regions. The trimmed forward and reverse sequences were then assembled to generate consensus suquences for subsequent molecular analysis.

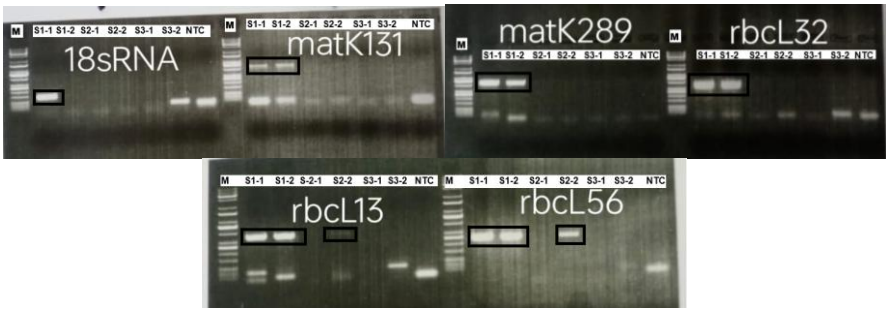

Figure 3. Touchdown PCR product shown Sample 1 was amplified using all primers and Sample 2 was amplified using primer *rbc*L13 and *rbc*L56 (left), sequencing for PCR preparation showed only Sample 1 was amplified (data not shown) thus Sample 2 was sequenced using previous touchdown PCR product, sample 3 was not amplified using all primers

Table 2. PCR primers used in this study, expected product sizes, and PCR results

Primer ID	Position	Sequence (5' to 3')	PCR product (bp)	S1	S2	S3
rbcL13F	Left	ACAGAGACTAAAGC AAGTGTTGG	702	Amplified	Amplified	Not amplified
rbcL13R	Right	ATGCCCTTTGATTTC ACCCG				
rbcL32F	Left	TTGGATTCAAGGCT GGTGTT	716	Amplified	Not amplified	Not amplified
rbcL32R	Right	TTCTTCACATGTACC TGCAGT				
rbcL56F	Left	GCAGCATTCCGAGT AACTCC	485	Amplified	Amplified	Not amplified
rbcL56R	Right	AAATCAAGTCCACC GCGAAG				
matK289F	Left	TCAGAGGGAGTTAC AGTCATTG	942	Amplified	Not amplified	Not amplified
matK289R	Right	GCATATACGCACAA ATCGGTT				
matK131F	Left	TGTTGGAAAATGTG GATTATGCC	919	Amplified	Not amplified	Not amplified

Primer ID	Position	Sequence (5' to 3')	PCR product (bp)	S1	S2	S3
matK131R	Right	AGCATTTGACTCCG TACCAA				
18sRNAF	Left	ATGCGCCAAGGAAA ACGAAT	463	Amplified	Not amplified	Not amplified
18sRNAR	Right	GGTCGCGTCGGATA CATAGA				

Further analysis was conducted using BLAST nucleotide searches for the forward, reverse, and consensus sequences obtained from Sample 1 and 2. The BLAST result showed high sequence similarity with reference sequences belonging to the genus *Baccaurea.* Across the analyzed matK and rbcL regions, Sample 1 and 2 consistently showed close molecular similarity to *Baccaurea* taxa, supporting their placement within the genus. However, the closest BLAST matches were not consistently assigned to a single *Baccaurea* species across different barcode regions. Therefore, the BLAST result provides molecular evidence supporting genus-level affinity but are insufficient for definitive species-level identification (Rahmawati & Shabrina, 2024).

Morphological analysis identified Sample 1 as *Baccaurea macrocarpa*. The BLAST results showed that the matK sequences had the highest similarity to *B. ramiflora* and *B. lanceolata.* The matK131 consensus sequence (718bp) showed 100% query coverage and 99.86% sequence identity with both species, while the matK289 consensus sequence (823bp) showed 100% query coverage and 99.88% sequence identity with *B. ramiflora* and *B. lanceolata* (Table 4**).** Although *B. macrocarpa* was not among the top 10 matches, it was detected among the broader BLAST results. For the matK131 consensus sequence, *B. macrocarpa* occurred at rank 64 with 73% query coverage and 99.85% identity. For matK289, *B. macrocarpa* showed 98.57% identity with 86% query coverage in the forward sequence, 98.73% identity with 83% query coverage in the reverse sequence, and 99.15% identity with 86% query coverage in the consensus sequence.

The *rbc*L56 amplification of Sample 1 yielded a trimmed forward sequence of 418 bp, a reverse sequence of 408bp, and a consensus sequence of 368bp. The top BLAST matches showed 100% query coverage and 99.52%, 99.51%, and 99.73% identity for the forward, reverse, and consensus sequences respectively. *B. deflexa* represented the highest ranking match, while *B. macrocarpa* was also present among the top matches. Compared with the matK results, the rbcL sequences provided greater query coverage for the *B. macrocarpa* reference sequences. Nevertheless, several other *Baccaurea* species also showed similarly high sequence similarity. Previous DNA barcoding research on *mat*K in *Baccaurea* reported 484 conserved base pairs and four informative base pairs (Saswita et al., 2023a).

Sample 2 was morphologically identified as *Baccaurea microcarpa* ; however, this species is not yet represented in the NCBI database and is considered a data-deficient species within the genus *Baccaurea* (Saswita et al., 2023b). Molecular amplification was successful with two rbcL primers, although the sequence obtained using rbcL13 provided the best-quality result. The trimmed forward sequence was 562 bp in length. BLAST analysis showed that the top eight accessions were assigned to *B. deflexa,* each with 100% query coverage and 99.47% sequence identity.

Unlike Sample 1, the morphologically identified species *B. microcarpa* was not recovered as the closest BLAST match. This species is not currently represented by an appropriate reference sequence in the NCBI database, limiting direct molecular comparison. Therefore, the occurrence of *B. deflexa* as the highest BLAST match should not be interpreted as definitive evidence that Sample 2 belongs to *B. deflexa.* The molecular result instead indicates a high sequence similarity to an available *Baccaurea* reference species.

The molecular analysis demonstrated that the available barcode sequences from Samples 1 and 2 had high sequence similarity with members of the genus *Baccaurea*. This finding supports the placement of the investigated samples within the genus *Baccaurea* and indicates that *matK* and *rbcL* can provide complementary molecular information for *Baccaurea* characterization. . However, the results also demonstrate that high BLAST similarity does not necessarily correspond to definitive species identification. Different barcode regions produced different closest matches, including *B. ramiflora*, *B. lanceolata*, *B. deflexa*, and *B. macrocarpa*. Such variation indicates substantial sequence similarity among closely related *Baccaurea* taxa in the analyzed regions.

For Sample 1, the morphological identification as *B. macrocarpa* was supported by the presence of *B. macrocarpa* among the high-similarity BLAST results, particularly in the *rbcL* sequences, which showed relatively high query coverage. However, the highest-ranking matches were not consistently assigned to *B. macrocarpa*. The *matK* sequences showed even higher similarity to *B. ramiflora* and *B. lanceolata*, whereas the *rbcL* sequences showed high similarity to *B. deflexa* as well as *B. macrocarpa*. Thus, the molecular findings provide supporting evidence for the morphological identification but are insufficient to independently confirm the species identity.

The discrepancy between morphological and molecular results may reflect the complementary nature of the two approaches. Morphological identification depends on diagnostic characters that may vary with developmental stage, environmental conditions, and the availability of reproductive structures. Closely related *Baccaurea* species may also exhibit overlapping morphological characteristics, making identification based only on vegetative characters difficult. Conversely, DNA barcoding is influenced by the discriminatory power of the selected loci. The high similarity observed among several *Baccaurea* species suggests that the *matK* and *rbcL* regions used in this study may not contain sufficient variation to clearly separate all closely related taxa at the species level. Previous research has also reported conserved regions within *Baccaurea matK* sequences, with limited numbers of informative nucleotide positions (Saswita et al., 2023a).

Such conservation may contribute to the difficulty in obtaining clear species-level discrimination using a single barcode region. Therefore, the use of additional markers and phylogenetic approaches may provide greater resolution than reliance on individual BLAST matches. The discriminatory power of DNA barcoding markers varies, with reported identification success rates of 21.1% for *rbcL*, 89.5% for ITS2, 87.5% for *trnH-psbA*, and 89.5% for both *rbcL* + ITS2 and *rbcL* + ITS2 + *trnH-psbA* combinations (Lee et al., 2025). These findings suggest that the use of multiple barcode markers can significantly improve species-level identification accuracy.

The results from Sample 2 further illustrate the limitations of BLAST-based species assignment. Although morphological analysis identified the sample as *B. microcarpa*, the highest available BLAST matches were assigned to *B. deflexa*, with 100% query coverage and 99.47% sequence identity. This apparent discrepancy should be interpreted cautiously because *B. microcarpa* is not represented by an

appropriate reference sequence in the NCBI database. Consequently, BLAST cannot identify a query sequence as *B. microcarpa* when a suitable reference sequence for that species is unavailable. Baccaurea spp. inventory presented 32 species are *not evaluated* including *B. macrocarpa* (Miq) Müll. Arg. and *B. bracteata* Müll. Arg., 2 *data deficient* including *B. microcarpa* (Airy Shaw) Haegens (Saswita et al., 2023b).

Table 3. Comparison of morphological analysis and DNA barcoding

Sample ID	Morphological analysis based on APG IV	DNA Barcoding using *rbcL* and *matK* marker genes
S1	*Baccaurea macrocarpa* (Miq) Müll. Arg.	*Baccaurea ramiflora*
S2	*Baccaurea microcarpa* (Airy Shaw) Haegens	*Baccaurea deflexa*
S3	*Baccaurea bracteata* Müll. Arg.	not amplified

This finding highlights an important limitation of database-dependent molecular identification. BLAST results are influenced not only by sequence similarity but also by the completeness, taxonomic accuracy, and representation of reference sequences in the database. Poorly represented or data-deficient taxa may be assigned to the closest available reference species, even when that species is not the true identity of the sample. Therefore, the highest-scoring BLAST accession should be interpreted as an indication of sequence similarity rather than definitive species identification.

Table 4. Morphological and BLAST-based molecular identification of Sample 1 using *matK* and *rbcL* barcodes

Sample ID	Morphology analysis using APG IV	DNA Barcoding according to BLAST result				
		Primer	Species Name	Query Cover	Percent Identify	Accesion Number
S1	*B. macrocarpa*	matK131 Forward	*B. ramiflora*	100%	99,31%	PP497828.1
			B. ramiflora	100%	99,20%	MW717296.1
			B. ramiflora	100%	99,20%	NC_057309.1
			B. lanceolata	100%	99,20%	AY552419.1
			B. javanica	100%	99,08%	AY579878.1
S1	*B. macrocarpa*	matK131 Reverse	*B. ramiflora*	100%	98,19%	PP497828.1
			B. lanceolata	100%	98,19%	AY552419.1
			B. ramiflora	100%	98,05%	MW717296.1
			B. ramiflora	100%	98,05%	NC_057309.1
			Maesobotrya vermeulenii	100%	98,05%	AY830273.1
S1	*B. macrocarpa*	matK131 Consensus	*B.ramiflora*	100%	99,86%	PP497828.1
			B. lanceolata	100%	99,86%	AY552419.1
			B. ramiflora	100%	99,72%	MW717296.1
			B. ramiflora	100%	99,72%	NC_057309.1
			Maesobotrya vermeulenii	100%	99,72%	AY830273.1
			B. macrocarpa	73%	98,85%	KJ708847.1
S1	*B. macrocarpa*	matK289 Forward	*B. ramiflora*	98%	99,32%	PP497828.1
			B. lanceolata	98%	99,32%	AY552419.1
			B. ramiflora	98%	99,21%	MW717296.1
			B. ramiflora	98%	99,21%	NC_057309.1
			B. ramiflora	98%	99,09%	PQ469841.1
			B. macrocarpa	86%	98,57%	KJ708847.1
S1	*B. macrocarpa*	matK289 Reverse	*B. ramiflora*	100%	99,53%	PP497828.1
			B. lanceolata	100%	99,53%	AY552419.1
			B. ramiflora	100%	99,41%	MW717296.1
			B. ramiflora	100%	99,41%	NC_057309.1
			B. javanica	100%	99,29%	AY579878.1
			B. macrocarpa	83%	98,73%	KJ708847.1

Sample ID	Morphology analysis using APG IV	DNA Barcoding according to BLAST result				
		Primer	Species Name	Query Cover	Percent Identify	Accesion Number
S1	*B. macrocarpa*	matK289 Consensus	*B. ramiflora*	100%	99,88%	PP497828.1
			B. lanceolata	100%	99,88%	AY552419.1
			B. ramiflora	100%	99,76%	MW717296.1
			B. ramiflora	100%	99,76%	NC_057309.1
			B. javanica	100%	99,64%	AY579878.1
			B. macrocarpa	86%	99,15%	KJ708847.1

Table 5. Morphology and molecular identification of Sample 1 through BLAST analysis using rbcL13, rbcL32 and rbcL56 barcodes, along with their respective accession numbers.

Sample ID	Morphology analysis using APG IV	DNA Barcoding according to BLAST result				
		Primer	Species Name	Query Cover	Percent Identify	Accesion Number
S1	*B. macrocarpa*	rbcL13 Forward	*B. deflexa*	100%	99,84%	PV974157.1
			B.deflexa	100%	99,84%	PV974155.1
			B. deflexa	100%	99,84%	PV974154.1
			Baccaurea sp	100%	99,84%	MF435509.1
			B. deflexa	100%	99,84%	PV974158.1
			B.macrocarpa	100%	99,22%	PV974142.1
S1	*B. macrocarpa*	rbcL13 Reverse	NA	NA	NA	NA
		Consensus	NA	NA	NA	NA
S1	*B. macrocarpa*	rbcL32 Forward	*B. ramiflora*	100%	99,22%	PQ469841.1
			B. javanica	100%	99,06%	AY663570.1
			B. ramiflora	100%	99,06%	MW717296.1
			B. ramiflora	100%	99,06%	NC_057309.1
			B. ramiflora	100%	99,06%	PP497828.1
			B.macrocarpa	98%	99,36%	PV974142.1
S1	*B. macrocarpa*	rbcL32 Reverse rbcL32	NA	NA	NA	NA
S1	*B. macrocarpa*	Consensus	NA	NA	NA	NA
S1	*B.macrocarpa*	rbcL56 Forward	*B. deflexa*	100%	99,52%	PV974157.1
			B. mollis	100%	99,52%	PP962424.1
			B. deflexa	100%	99,52%	PV974155.1
			B. deflexa	100%	99,52%	PV974154.1
			Baccaurea sp.	100%	99,52%	MF435509.1
			B. macrocarpa	100%	99,52%	PP962421.1
S1	*B. macrocarpa*	rbcL56 Reverse	*B. deflexa*	100%	99,51%	PV974157.1
			B. mollis	100%	99,51%	PP962424.1
			B. mollis	100%	99,51%	MG838502.1
			B. deflexa	100%	99,51%	PV974155.1
			B. deflexa	100%	99,51%	PV974154.1
			B. macrocarpa	100%	99,51%	PP962421.1
S1	*B. macrocarpa*	rbcL56 Consensus	*B. deflexa*	100%	99,73%	PV974157.1
			B. mollis	100%	99,73%	PP962424.1
			B. mollis	100%	99,73%	MG838502.1
			B. deflexa	100%	99,73%	PV974155.1
			B. deflexa	100%	99,73%	PV974154.1
			B. macrocarpa	100%	99,73%	PP962421.1

Table 6. Morphology and molecular identification of sample 2 through BLAST analysis using rbcL13 barcodes, along with their respective accession numbers.

Sample ID	Morphology analysis using APG IV	DNA Barcoding according to BLAST result				
		Primer	Species Name	Query Cover	Percent Identify	Accesion Number
S2	*B. microcarpa*	rbcL13 Forward	*B. deflexa*	100%	99,47%	PV974157.1
			B.deflexa	100%	99,47%	PV974155.1
			B. deflexa	100%	99,47%	PV974154.1
			Baccaurea sp	100%	99,47%	MF435509.1
			B. deflexa	100%	99,47%	PV974158.1
S2	*B. microcarpa*	rbcL13 Reverse	NA	NA	NA	NA
S2	*B. microcarpa*	rbcL13 Consensus	NA	NA	NA	NA

The antioxidant evaluation in this study provides preliminary information on the biological potential of *Baccaurea* leaves that may complement their taxonomic characterization. All three samples exhibited strong DPPH radical-scavenging activity, with IC_{50} values of 6.94ppm, 10.30ppm, and 15.08 ppm for Samples 1, 2, and 3, respectively. These findings indicate that *Baccaurea* leaves possess considerable antioxidant potential and may provide an additional biological value for further investigation. However, the present results should not be interpreted as direct evidence that antioxidant potential will increase community conservation or lead to product development, as such outcomes require further socioeconomic, ecological, and applied studies.

Phytochemical screening showed that the ethanolic leaf extracts of Samples 1, 2, and 3 contained several secondary metabolite groups, including flavonoids, phenolics, tannins, steroids, and terpenoids (Table 7). Phenolic and flavonoid compounds are widely associated with antioxidant activity because they can participate in electron or hydrogen donation and stabilize free radicals (Prior et al., 2005; Erwin et al., 2019). Other antioxidant mechanisms may include radical scavenging, metal-ion chelation, and inhibition of oxidative reactions. The presence of these metabolite groups may therefore contribute to the antioxidant activity observed in the present study. Nevertheless, because the phytochemical analysis was qualitative, the contribution of individual compounds to the observed IC_{50} values cannot be determined directly. Further quantitative phytochemical analysis and compound profiling are required to establish the relationship between the chemical composition and antioxidant activity of Kapul leaves.

Table 7. Phytochemical screening of ethanol 96% extract of leaves of sample 1, sample 2, and sample 3.

	K+	K-	S1	S2	S3
Alkaloid Mayer	+	-	-	-	-
Alkaloid Wagner	+	-	-	-	-
Alkaloid Dragendorff's	+	-	-	-	-
Flavonoid	+	-	+	+	+
Fenolik	+	-	+	+	+
Saponin	+	-	-	+	-
Tanin	+	-	+	+	+
Steroid Terpenoid	+	-	+	+	+

The ethanol extracts of leaves from Sample 1, 2, and 3 exhibited very strong antioxidant activity based on their IC_{50} values. Using commonly applied DPPH

classification, antioxidant activity is categorized as very strong when IC_{50} is <50 µg/mL, strong at 50–100 µg/mL, moderate at 100–150 µg/mL, weak at 150–200 µg/mL, and very weak at >200 µg/mL (Molyneux, 2004; Fidrianny et al., 2015). Accordingly, all three leaf samples in the present study were classified as very strong antioxidants, with Sample 1 showing the highest antioxidant potential because it had the lowest IC_{50} values. The percentage of inhibition increased with increasing extract concentration (Table 8), indicating a concentration-dependent DPPH radical-scavenging responses. The DPPH assay is widely applied for evaluating free-radical scavenging activity because of its simplicity, rapidity, and practical applicability (Molyneux, 2004;Prior et al., 2005). The percent value of antioxidant activity shows the ability of an antioxidant compound in the sample to trap DPPH free radical into stable molecules with marked change in DPPH color from purple to yellow (Abeysinghe et al, 2021).

The inhibition percentage obtained for *Baccaurea bracteata* in the present study deviates from the results reported by Lestari (2023), who documented an inhibition value of 68.31% for methanolic extract. Methanol is known to extract a broader range of polar antioxidant compounds compared with ethanol, which may result in higher radical-scavenging activity. Additionally, variations on plant age, environmental condition and harvesting time may further contribute to the observed differences in antioxidant capacity (Lestari et al, 2023). However, no previous studies have reported the IC_{50} value of *Baccaurea bracteata* leaves, suggesting that antioxidant capacity of this species has not yet been documented in the scientific literature. Consequently, the results of the present investigation serve as an initial reference for the IC_{50} profile of this plant and contribute to the broader understanding of its phytochemical and antioxidant characteristics. In this study, *Baccaurea macrocarpa* demonstrated an IC_{50} value of 6.94ppm, whereas Ariani (2024) reports an IC_{50} value of 15.10ppm for the ethanol extract of *Baccaurea macrocarpa* leaves (Ariani et al, 2024). This difference may be attributed to variation on the geographical origin and environmental growing conditions of the plant materials, which can significantly influence phytochemical composition and, consequently, antioxidant activity. It is important to note that lower IC_{50} values indicate stronger antioxidant potential, thereby suggesting that the sample analyzed in the present study exhibits a higher radical-scavenging capacity.

The antioxidant activity observed in the present study may be related to the secondary metabolites detected in the Samples extracts. Phenolic compounds and flavonoids are particularly relevant because their hydroxyl groups can donate hydrogen atoms or electrons to neutralize free radicals and stabilize the resulting radical species. Tannins, which are polyphenolic compounds, may also contribute to radical-scavenging activity through hydrogen donation and metal-ion chelation (Zhang, et al., 2016). Terpenoids may contribute through radical-quenching and interaction with reactive oxygen species, although their antioxidant effects depend strongly on their chemical structure. Thus, the simultaneous detection of phenolics, flavonoids, tannins, and terpenoids in Sample 1, 2, dan 3 provides a plausible chemical basis for the strong DPPH-scavenging activity observed in the present study (Pior et al., 2005; Apak et al., 2016; Shahidi & Ambigaipalan, 2015).

However, because the phytochemical screening was qualitative, the presence of these metabolite groups alone cannot establish which compounds are primarily responsible for the difference in antioxidant activity among the three samples. Quantitative determinations of total phenolic and flavonoid contents, together with chromatographic identification of individual compounds, is therefore required to confirm these associations.

Table 8. Antioxidant activity of sample 1, sample 2, and sample 3

Samples		Concentration (ppm)	Scavenging activity (%)±SD	IC_{50} (ppm)
Morphology Identify	**Molecular Sample Code**			
Baccaurea macrocarpa	S1	3	20,39±0,021	6,94
		6	43,30±0,007	
		9	67,83±0,005	
		12	84,63±0,111	
		15	88,40±0,004	
Baccaurea microcarpa	S2	3	13,21±0,011	10,30
		6	28,58±0,008	
		9	45,28±0,002	
		12	60,42±0,006	
		15	70,69±0,005	
Baccaurea bracteata	S3	3	7,23±0,004	15,08
		6	18,13±0,006	
		9	27,98±0,009	
		12	40,87±0,006	
		15	49,88±0,004	
Quercetine		1	11,92±0,007	3,58
		2	26,14±0,006	
		3	39,85±0,009	
		4	55,27±0,008	
		5	72,62±0,006	

For *Baccaurea microcarpa*, no specific studies related to this species have been identified in the existing literature. The absence of prior research highlights the limited scientific information available regarding its phytochemical composition and biological activity. Therefore, the findings of the present study contribute valuable preliminary data and serve as an important reference for future investigations involving this species.

CONCLUSION

This study demonstrated that the chloroplast *matK* and *rbcL* markers provide valuable molecular information for the characterization of Kapul (*Baccaurea* spp.) from East Kalimantan. The obtained sequences showed high similarity with members of the genus *Baccaurea*, supporting their application for genus-level identification. However, the inconsistent species-level assignments among BLAST results indicate that *matK* and *rbcL* alone may not provide sufficient resolution for distinguishing closely related *Baccaurea* taxa. Therefore, molecular identification should be integrated with morphological characterization and supported by additional molecular markers and more comprehensive reference databases to improve species-level identification accuracy.

The antioxidant evaluation further revealed that ethanolic leaf extracts from the three Kapul samples exhibited strong DPPH radical-scavenging activity, with differences in antioxidant capacity among samples. These findings provide preliminary molecular and biological information on indigenous *Baccaurea* resources from Kalimantan. Although this study does not directly evaluate conservation status or population dynamics, the generated data contribute baseline information for future

taxonomic studies, biodiversity assessments, and conservation efforts aimed at documenting and sustainably utilizing native *Baccaurea* species.

RECOMMENDATION

During this study, several challenges were encountered, including inconsistencies between morphological and molecular identification results, which may be influenced by phenotypic variation or limitations on reference databases. Additionally, some samples failed to amplify, possibly due to low DNA quality, degradation, or the presence of PCR inhibitors. Therefore, future studies are recommended to incorporate additional molecular markers such as ITS or *trn*H-*psb*A to improve species-level resolution, as well as to expand sampling size and geographic coverage across Kalimantan to better represent the genetic diversity of *Baccaurea* species and improve DNA extraction and amplification protocols.

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