

Pharmacognostic Evaluation and Molecular Identification of *Cinnamomum myrianthum* Merr. Leaves

Rhian Jaymar D. Ramil^{1,2*}, Philip Joshua S. Aliga², Jimmbeth Zenila P. Fabia³, Mae Ann R. Batuyong-Lista⁴, Francisco M. Heralde III⁵

¹Department of Pharmacy, College of Health Sciences, Mariano Marcos State University, City of Batac, Ilocos Norte, PHILIPPINES.

²Functional Foods and Nutraceutical Research Center, Research Directorate, Mariano Marcos State University, City of Batac, Ilocos Norte, PHILIPPINES.

³Center for Cellular and Molecular Medical Research, Research Directorate, Mariano Marcos State University, City of Batac, Ilocos Norte, PHILIPPINES.

⁴Department of Biological Sciences, College of Arts and Sciences, Mariano Marcos State University, City of Batac, Ilocos Norte, PHILIPPINES.

⁵Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, PHILIPPINES.

Correspondence

R. Rhian Jaymar D

Department of Pharmacy, College of Health Sciences, Mariano Marcos State University, City of Batac, Ilocos Norte, PHILIPPINES; Functional Foods and Nutraceutical Research Center, Research Directorate, Mariano Marcos State University, City of Batac, Ilocos Norte, PHILIPPINES.

E-mail: rramil@mmsu.edu.ph

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ABSTRACT

Introduction: *Cinnamomum myrianthum* Merr. is a narrowly distributed endemic species in the Philippines with limited scientific information on its botanical identity and quality attributes. This study was undertaken to establish the pharmacognostic profile of *C. myrianthum* leaves as a scientific basis for species authentication, standardization, and phytochemical and biological investigation. **Methods:** Mature leaves were collected, taxonomically authenticated, and evaluated using standard pharmacognostic procedures. Organoleptic, macro- and micromorphological, and physicochemical characteristics were determined, including moisture content, loss on drying, ash values, extractive values, and pH. Molecular authentication was further performed through chloroplast *rbcL* DNA barcoding, followed by sequence similarity analysis and phylogenetic reconstruction. **Results:** *C. myrianthum* leaves exhibited characteristic sensory features, including an olive-green lamina, distinct aromatic odor, glabrous texture, and mildly bitter taste. Morphological examination revealed opposite to sub-opposite, petiolate leaves with triplinerved venation and a typical dorsiventral leaf anatomy. Physicochemical evaluation showed total ash (4.58%), acid-insoluble ash (1.94%), moisture content (7.29%), loss on drying (6.81%), and slightly acidic pH (5.37), all within acceptable limits for crude herbal materials. DNA barcoding generated a 606 bp *rbcL* consensus sequence with 44.55% GC content, showing >99.8% sequence similarity with authenticated *Cinnamomum* species and confirming its taxonomic placement within the genus. **Conclusion:** This study establishes the first pharmacognostic reference standards for *C. myrianthum* and provides essential baseline data for its botanical authentication, quality control, and future phytochemical and pharmacological studies, supporting its scientific utilization and conservation as an endemic Philippine medicinal plant.

Keywords: Cinnamon, DNA Barcoding, morphological, organoleptic, physicochemical

INTRODUCTION

Cinnamomum Schaeff. is a taxonomically diverse genus within Lauraceae, consisting of approximately 250 recognized species predominantly occurring in tropical and subtropical ecosystems across Southeast Asia, China, and Australia¹. The Philippines harbors considerable diversity within the genus *Cinnamomum*, with approximately 25 documented species, of which 18 are recognized as endemic to the country². Among these is *Cinnamomum myrianthum* Merr., which is an endemic species distributed in the lowland forests of northwestern Luzon, particularly in Ilocos Norte and Cagayan provinces.

The genus *Cinnamomum* has long been recognized for its pharmacological importance, with documented use in traditional health systems for the management of gastrointestinal, respiratory, inflammatory, metabolic, and cardiovascular disorders³⁻⁵. Several Philippine *Cinnamomum* species have likewise been reported in local ethnomedicinal practices for the treatment of skin diseases, pain, diabetes, and gastrointestinal conditions⁶. Phytochemical studies of well-investigated species such as *C. cassia*, *C. verum*, *C. burmannii*, and *C. loureiroi* have identified diverse bioactive metabolites associated with antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anticancer activities⁵.

Despite this broader recognition, *C. myrianthum* remains largely unexplored. No comprehensive

pharmacognostic data currently exist to support its identification, authentication, or quality evaluation, limiting both its scientific documentation and prospects for phytochemical and pharmacological investigation. Given the high degree of endemism among Philippine *Cinnamomum* species, establishing reliable diagnostic and quality control parameters for *C. myrianthum* is critical not only for species authentication but also for the conservation and sustainable use of indigenous plant resources.

As interest in medicinal plants as sources of novel bioactive compounds continues to grow, pharmacognostic characterization constitutes a necessary foundation for subsequent phytochemical, pharmacological, and bioprospecting work. Accordingly, this study established the pharmacognostic profile and molecular identity of *C. myrianthum* leaves through organoleptic, macromorphological, micromorphological, physicochemical, and DNA barcoding analyses. The resulting data provide reference standards for species authentication and quality assessment, and serve as a scientific basis for future research and conservation efforts involving this endemic Philippine plant.

MATERIALS AND METHODS

Collection and authentication of plant materials

Collection of *Cinnamomum myrianthum* was conducted in compliance with Philippine ethical and regulatory requirements, with prior informed

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consent obtained from the local government unit of Pasuquin, Ilocos Norte, and the Protected Area Management Board of the Metropolitan Ilocos Norte Watershed Forest Reserve, and a gratuitous permit secured from the Department of Environment and Natural Resources in accordance with Republic Act No. 9147 (Wildlife Resources Conservation and Protection Act).

Mature leaves were collected from a forested area in Brgy. Sapat, Pasuquin, Ilocos Norte, under the supervision of local taxonomist Michael A. Calaramo. Voucher specimens were prepared, authenticated, and deposited at the Herbarium of Northwest Luzon under voucher number HNUL 0020807.

Organoleptic evaluation

The organoleptic characteristics of fresh leaves of *C. myrianthum* were evaluated following standard pharmacognostic procedures⁷. Leaf morphometric parameters, including length and width ($n = 10$), were measured using a graduated ruler, and gross morphological features, including leaf shape, were documented. Leaf color was assessed under diffuse daylight using a colorimeter (3NH, Guangdong, China). Surface characteristics, including texture, consistency, brittleness, and fracture characteristics, were determined through tactile examination and manual fracture analysis. Odor was evaluated by gently crushing fresh leaf samples between the fingers and characterizing both odor intensity and sensory quality. Taste evaluation was performed cautiously using a minimal quantity of fresh leaf material solely for preliminary organoleptic characterization without ingestion. Prior to assessment, the plant material was taxonomically authenticated, and no documented reports of acute toxicity associated with minimal gustatory exposure to *Cinnamomum* leaves were identified in the literature. The evaluation was conducted exclusively for descriptive pharmacognostic purposes and did not involve formal human sensory testing beyond routine qualitative examination.

Morphological evaluation

Macromorphological assessment involved the systematic documentation of gross diagnostic characteristics, including leaf type, lamina morphology, margins, apices, bases, venation patterns, and phyllotaxy. For micromorphological analysis, fresh mature leaves were washed with distilled water, and free-hand transverse sections were prepared from the midrib region using a sharp razor blade. The sections were mounted directly in glycerin on clean glass slides, covered with a coverslip, and examined under a compound light microscope (ICOE Optics Expert, Ningbo, China). Representative photomicrographs were captured to document diagnostic anatomical features.

Physicochemical evaluation

To establish the pharmacognostic specifications of *C. myrianthum* leaves, physicochemical parameters were evaluated following standard pharmacognostic procedures⁷. Briefly, moisture content was determined using a moisture analyzer (Shimadzu, Kyoto, Japan) while loss on drying was measured by drying powdered leaf samples at 100–105°C to constant weight. Total ash was determined by incineration of the dried powdered leaf samples in a muffle furnace (Sci Finetech, Seoul, South Korea) at 500–600°C until carbon-free ash was obtained, followed by determination of acid-insoluble and water-soluble ash using dilute hydrochloric acid and distilled water, respectively. Water-soluble extractive value was determined by hot aqueous extraction, whereas ethanol-soluble extractive value was obtained by 24 h maceration in ethanol, followed by filtration, solvent evaporation, and drying to constant weight. The pH of the aqueous leaf extract was measured with a calibrated pH meter (Apera Instruments, Shanghai, China).

All physicochemical measurements were performed in triplicate ($n = 3$). Data were expressed as mean $\pm$ standard error of the mean (SEM).

Since the study primarily aimed to establish baseline pharmacognostic standards, only descriptive statistical analysis was employed.

Molecular identification through DNA barcoding

Genomic DNA of *C. myrianthum* was extracted, amplified, and sequenced by Apical Scientific Sdn. Bhd. following standard molecular protocols. Briefly, genomic DNA was isolated from silica gel-dried leaf samples using the NucleoSpin Plant II Mini Kit, and DNA quality and concentration were assessed spectrophotometrically. The chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcl*) region was amplified using *rbcl*-specific primers under optimized PCR conditions. Amplified products were purified and subjected to bidirectional sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit. The consensus *rbcl* sequence generated from *C. myrianthum* was aligned with homologous *Cinnamomum* sequences retrieved from GenBank using Clustal Omega. Phylogenetic reconstruction was performed using the EMBL-EBI Simple Phylogeny tool. A total of eight (8) reference sequences representing selected *Cinnamomum* species were included in the analysis. The resulting phylogram was used to visualize sequence relationships among taxa.

RESULTS AND DISCUSSION

Organoleptic Evaluation

The organoleptic characteristics of *C. myrianthum* leaves revealed a medium earthy olive-green adaxial surface and a softer, muted green abaxial surface with a slight yellowish undertone, pigmentation patterns consistent with those reported in other *Cinnamomum* species, including *C. verum* and *C. cebuense*⁸ (Table 1).

A distinct clove-like aromatic odor was observed, suggesting the presence of volatile phenylpropanoids, such as eugenol, which are commonly reported in *Cinnamomum* essential oils and recognized as important chemotaxonomic markers⁹. Morphologically, the leaves were asymmetric, ranging from ovate to lanceolate, reflecting the foliar variability commonly observed among Philippine *Cinnamomum* taxa¹⁰ (Figure 1).

The leaves were glabrous, with a smooth, firm texture comparable to those reported for *C. verum* and *C. zeylanicum*, and exhibited a slightly bitter taste, which may indicate the presence of tannins and aromatic secondary metabolites such as eugenol and cinnamaldehyde^{11–13}. The mean leaf dimensions were 118.8 mm in length and 28.9 mm in width, which are relatively smaller than those reported for other related species^{8,10,13}. These size differences may result from factors such as leaf age, elevation, or microclimate, as the dimensions of *Cinnamomum* leaves are known to vary with environmental conditions¹⁴.

Morphological evaluation

The macromorphological and micromorphological characteristics of *C. myrianthum* are presented in Table 2 and Figures 1-2. Leaves were opposite to sub-opposite, petiolate, and exhibited a distinct triplinerved venation pattern, characterized by a prominent midrib extending toward the apex and two lateral primary veins that did not reach the leaf apex, with reticulate secondary venation. The lamina possessed an entire margin, narrowly acuminate apex, and cuneately acute base. These morphological traits are consistent with diagnostic features reported in other *Cinnamomum* species, including *C. verum*, *C. mindanaense*, *C. cebuense*, and *C. tamala*^{8–11,13,15}, supporting the taxonomic placement of *C. myrianthum* within the genus and highlighting conserved foliar characters within the family Lauraceae (Table 2).

The transverse section of *C. myrianthum* leaves exhibited anatomical features characteristic of dicotyledonous members of the Lauraceae. The midrib region showed a centrally located vascular bundle composed of distinct xylem and phloem tissues, embedded within parenchymatous

Table 1. Organoleptic features of *C. myrianthum*

Parameters	Observations
Color	Upper surface: A medium, earthy olive green (Pantone 576 C); Lower surface: A soft, muted green with a slightly yellow undertone (Pantone 5773 C)
Odor	Distinct aromatic clove-like
Shape	Asymmetric and ovate to lanceolate
Texture	Hard and smooth (glabrous), fibrous
Taste	Mild bitterness
Size (mm)	Length 118.8 ± 1.74 ; Width 28.9 ± 0.74

Table 2. Morphological profile of *C. myrianthum*

Parameters	Observations
Phyllotaxy	Opposite to sub-opposite
Venation	Triplinerved, three primary nerves are prominent: the midrib extends to the base of acumen, lateral veins (parallel to the midrib) are not reaching the tip, reticulate secondary veins
Petiole	Present
Apex	Narrowly acuminate
Margin	Entire
Base	Cuneately acute
Three-veined distribution	Present



Figure 1. Representative leaves of *C. myrianthum*, lower surface (A), upper surface (B).

ground tissue. Such an organization is consistent with the typical vascular architecture reported in *Cinnamomum* species. The lamina displayed a dorsiventral organization, consisting of upper and lower epidermis covered by a thin cuticle. Beneath the upper epidermis, a compact layer of palisade parenchyma was observed, while the spongy parenchyma comprised loosely arranged cells with intercellular spaces. The clear differentiation between palisade and spongy mesophyll confirms the dorsiventral nature of the leaf (Figure 2).

These micromorphological features are consistent with previously reported leaf anatomy in *Cinnamomum* species^{13,16,17}, which similarly exhibit a single palisade layer beneath the upper epidermis and a well-developed spongy mesophyll. The presence of a centrally positioned vascular bundle and parenchymatous ground tissue further aligns with anatomical descriptions across Lauraceae, supporting the taxonomic

placement of *C. myrianthum* and highlighting conserved foliar structural traits within the genus.

Physicochemical assessment

The physicochemical parameters of *C. myrianthum* provide important quality indices for the authentication and standardization of the crude leaf material (Table 3). The total ash value (4.58%) was comparable to those reported for other *Cinnamomum* species^{15,18}, indicating a normal level of inherent inorganic constituents. The acid-insoluble ash value (1.94%) remained within acceptable pharmacopeial limits, indicating minimal siliceous contamination and appropriate post-harvest handling, whereas the water-soluble ash value (2.29%) reflected the presence of water-soluble inorganic mineral components commonly evaluated in herbal drug standardization. The ethanol-soluble extractive

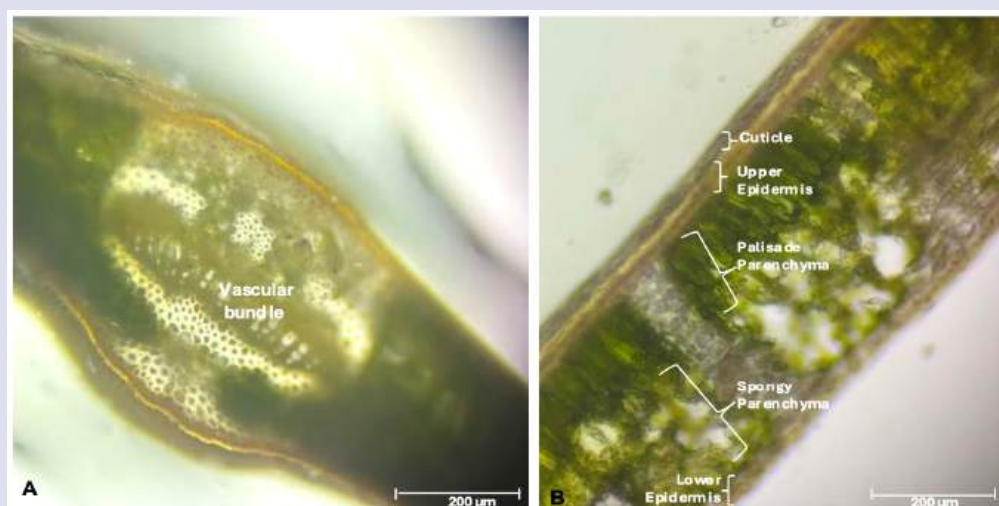


Figure 2. Transverse leaf section of *C. myrianthum* showing the (A) midrib and (B) lamina anatomy.

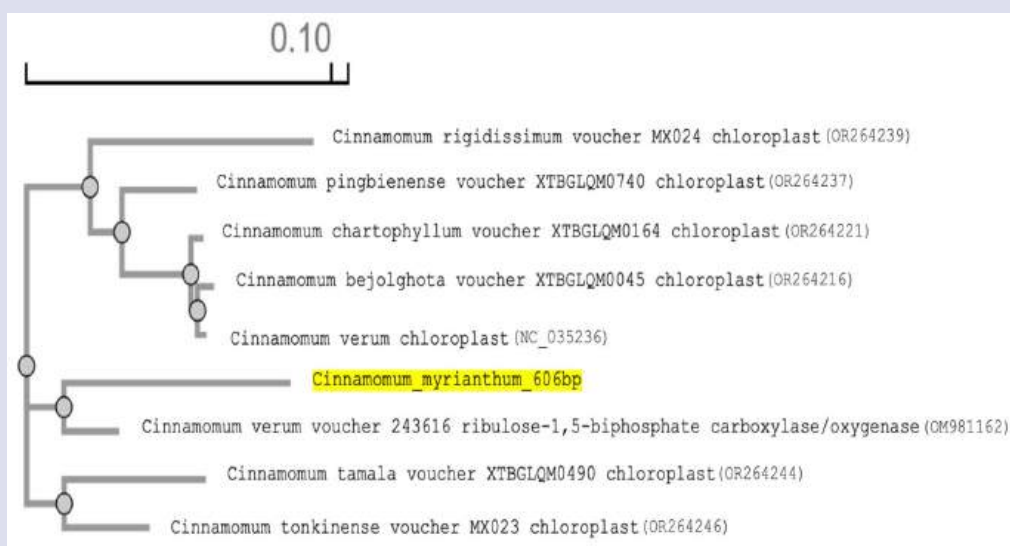


Figure 3. Phylogram showing the placement of *C. myrianthum* among selected *Cinnamomum* species generated using Clustal Omega Alignment and EMBL-EBI Phylogeny Tool

value (4.77%) indicates the presence of polar phytoconstituents, including phenolics, flavonoids, and glycosides, whereas the hexane-soluble extractive value (6.85%) suggests the presence of non-polar constituents such as essential oils and lipophilic compounds.⁵ The water-soluble extractive value (3.23%) reflects the presence of hydrophilic constituents, including sugars, tannins, organic acids, and other polar compounds commonly found in medicinal plants. This parameter serves as an indicator of the amount of water-soluble phytoconstituents present in the crude leaf material and its suitability for aqueous herbal preparations. The moisture content (7.29%) was within acceptable limits for crude herbal materials, indicating suitability for safe storage and reduced susceptibility to microbial deterioration, as moisture content below 12% is generally considered acceptable for minimizing microbial growth and degradation of herbal materials¹⁹. Moreover, the slightly acidic pH (5.37) may be attributed to the presence of organic acids and polyphenolic compounds, which are weakly acidic and commonly found in plant extracts²⁰. These findings support the identity, purity, and pharmaceutical quality of *C. myrianthum* as a potential medicinal plant resource.

Molecular identification using DNA barcoding

The molecular identity of *C. myrianthum* was determined through *rbcl*-based DNA barcoding. The assembled consensus sequence comprised 606 base pairs, with a GC content of 44.55%. BLASTn analysis of the *rbcl* sequence showed highly significant matches with members of the genus *Cinnamomum*, with the highest similarity observed with *C. cappara-coronde* and *C. verum*, exhibiting >99.8% sequence identity and near-complete query coverage (Table 4). These results provide strong molecular support for the placement of the specimen within the genus *Cinnamomum*. However, the chloroplast *rbcl* region is known to be highly conserved among members of Lauraceae and may offer limited discriminatory power at the species level. The observed high sequence similarity (>99.8%) with *C. cappara-coronde* and *C. verum* reflects the utility of *rbcl* for genus-level authentication rather than definitive species delimitation. Additional DNA barcode markers, such as *matK* and ITS, or complete chloroplast genome sequencing, would provide improved phylogenetic resolution and stronger species-level discrimination in future investigations.

Table 3. Physicochemical qualities of *C. myrianthum*

Parameters	Values (% , expect pH, Mean ± SEM, n = 3)
Total Ash	4.58 ±0.19
Acid Insoluble Ash	1.94 ±0.29
Water Soluble Ash	2.29 ±0.18
Ethanol Soluble Extractives	4.77 ±0.20
Water Soluble Extractives	3.23 ±0.01
Hexane Soluble Extractives	6.85 ±0.06
Moisture Content	7.29 ±0.43
pH	5.37 ±0.06
Loss on Drying	6.81 ±0.03

Table 4. Top BLASTn Hits for the *C. myrianthum* *rbcl* Sequence Against the NCBI Nucleotide Database

Accession	Species	Identity (%)	Query Coverage (%)
MK243400.1	<i>Cinnamomum cappara-coronde</i>	99.83	99
OM981162.1	<i>Cinnamomum verum</i>	99.83	99
OM981159.1	<i>Cinnamomum verum</i>	99.83	99

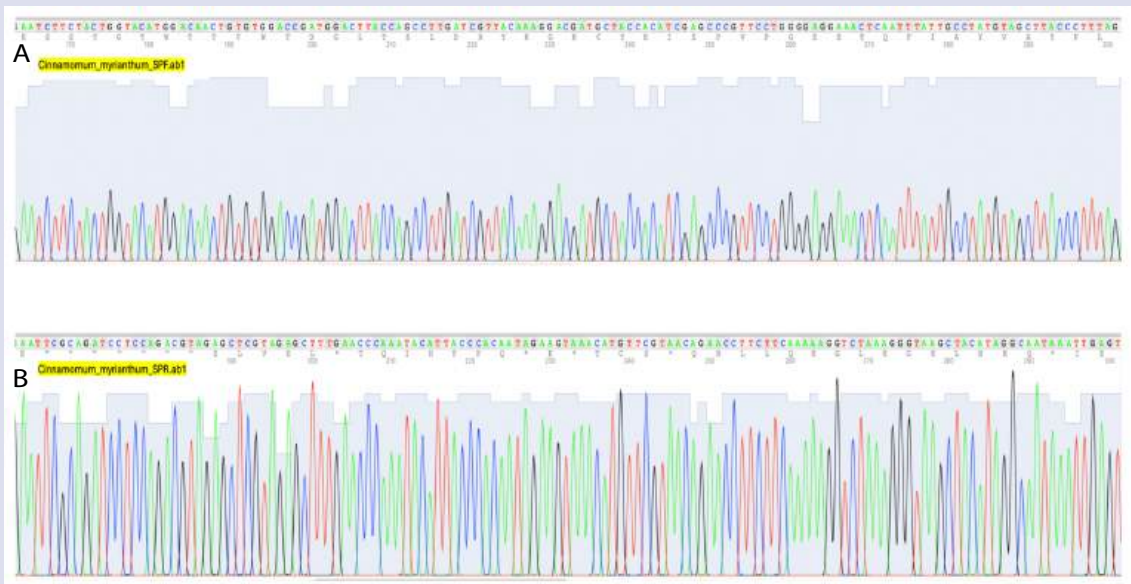


Figure 4. Chromatograms of *C. myrianthum*: (A) forward-strand sequence; (B) reverse-strand sequence.

Phylogenetic analysis of *rbcl* sequences (Figure 3) placed *C. myrianthum* within the *Cinnamomum* clade and indicated a distinct position relative to the other representative species included in the analysis. The tree primarily supports the placement of the specimen within *Cinnamomum* rather than resolving detailed evolutionary relationships among closely related species. The phylogram is regarded as a preliminary representation of sequence similarity.

Chromatogram analysis of both forward and reverse reads (Figure 4) demonstrated generally high-quality sequencing data, with the forward read showing well-resolved peaks and minimal background noise, while minor peak overlaps were observed in limited regions of the reverse read. These findings provide strong molecular support for the taxonomic authentication and phylogenetic distinctiveness of *C. myrianthum*.

CONCLUSION

The present study establishes a comprehensive pharmacognostic profile of *C. myrianthum* Merr. and provides essential reference standards for its botanical authentication and quality assessment. The integration of morphological, physicochemical, and DNA barcoding approaches

provides a reliable framework for identifying and standardizing this endemic species as crude medicinal plant material. These baseline data not only support future phytochemical and pharmacological investigations but also contribute to the scientific recognition, sustainable utilization, and conservation of *C. myrianthum* as a potentially valuable indigenous medicinal resource of the Philippines.

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