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MOLECULAR CHARACTERISATION OF A NOVEL NIGERIAN PLANT AS SYZYGIUM SPP OF THE MYRTACEAE FAMILY USING DNA BARCODING

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ABSTRACT

This research focuses on the taxonomy and DNA barcoding of *Syzygium guineense* (*S. guineense*), commonly known as 'water pear' or 'water berry', a medicinal plant of great importance in Nigeria. Molecular characterisation is required to accurately identify medicinal plants from families known for complex taxonomy and a multitude of species. We used a dual approach combining taxonomical and molecular barcoding techniques. The plant specimen was initially identified by a taxonomist as *S. guineense*. Organoleptic and microscopic evaluations described the leaves as shiny green, ovate, 7–13 cm long, papery in texture, fragrant when crushed, with entire margins and obtuse apices. Microscopy revealed paracytic stomata, while chemomicroscopy confirmed the presence of cell wall materials and inclusions. Phytochemical screening indicated flavonoids, alkaloids, saponins, glycosides, and tannins, consistent with diagnostic features of *Syzygium* species. Molecular identification was performed using the Ribulose Bisphosphate Carboxylase (rbcl) gene marker. The rbcl gene is used as a DNA barcode for species identification and classification. Genomic DNA was extracted and amplified via end-point PCR, yielding a 542 bp fragment with 43.17% GC content, consistent with reported rbcL sequences in the Myrtaceae family. BLAST analysis revealed 100% query cover and 99.08% identity with multiple *Syzygium* species and other Myrtaceae members, confirming genus-level placement. However, the rbcL marker did not conclusively resolve the plant as *S. guineense* at the specie level. This finding highlights the limitations of relying solely on morphological and phytochemical traits for plant identification. Additional molecular markers are required to resolve closely related species with overlapping characteristics.

Keywords: DNA Barcoding, Pharmacognostic, Phytochemicals, *Syzygium guineense*, Taxonomy

INTRODUCTION

The precise and accurate identification of plant species, particularly those that are morphologically similar or genetically complex, presents a persistent challenge in botanical research and its applied fields (Mursyidin *et al.*, 2018). This challenge is exacerbated in scenarios where only processed or incomplete plant materials are available, rendering traditional morphological identification methods impractical or impossible (Buddhachat *et al.*, 2019).

The genus *Syzygium*, well-recognised for its diverse species, has been reported to possess remarkable phytochemical compositions (Hoang *et al.,* 2021). With approximately 1200 species, it is one of the most abundant genera within the Myrtaceae family, distributed across Africa, Nigeria, extending eastward to the Hawaiian Islands, India, Southern China, and southward to Australia and New Zealand (IOR, 2012; Wangkhem *et al.,* 2020; Hatt *et al*., 2023). A vast numbers of species within the myrtaceae family are still undescribed as plants belonging to this family are often hard to classify and described (Thornhill *et al.,* 2015). It is difficult to differentiate complex species within the Myrtaceae based on morphological characters alone (Ismail *et al.,* 2020). Conventional methods of plant identification, based on visual insights such as color, flower structure, leaf shape, and texture, are often time-consuming and require expert knowledge, which can be limiting given the vast number of plant species and their inherent similarities at various taxonomic levels (Zin *et al.,* 2020). Correct authentication of medicinal plants is important, as some of these species are declining due to illegal logging and deforestation. In addition, correct botanical identification is an important aspect of quality; a key aspect in herbal formulation. Hence, to overcome these limitations of imprecise identification based on morphological traits alone, integrating DNA barcoding will serve as an efficient and quick approach for the precise identification of tree species.

The *rbcL* gene, though often used as a general plant barcode, is especially useful in helping to distinguish species within difficult groups like *Syzygium* making it a reliable tool for species-level authentication (Dong et al., 2013). The rbcL gene remains a primary barcode marker for plants and it is frequently combined with other markers like matK or ITS in a multi-locus approach to enhance identification accuracy (Dokane, 2024; Saunders and McDevit, 2012). The relative stability of the RbcL gene sequence across diverse plant taxa also minimises intra-specific variability while maximising interspecific divergence, thereby facilitating accurate species delimitation (Nicholas *et al.,* 2012).

Ethnopharmacological surveys have shown that herbal remedies and their products are the most preferred type of alternative and complementary medicine (ACM) globally (Okunola *et al.,* 2020). Hence, a need for correct identification of these commonly used medicinal plants.

Syzygium guineense, a common medicinal plants used in traditional medicine in Africa, is a highly variable species, known locally in Nigeria as 'adere' or 'ori' in Yoruba, 'malmoo' in Hausa (IOR, 2012). it is a common small tree, 10 -20 m in height with edible fruits often found along riverbanks. It is commonly known as water berry in parts of Africa. The reported Phytochemical compounds found in parts of the plant are flavonoids, tannins, alkaloids, phenols, saponins, and cardiac glycosides (Abera and Adane, 2018). The reported ethno-medicinal uses of the leaves and bark of *S. guineense* include the management of gastrointestinal upsets, infertility in women, menstrual pain, malaria, cough, epilepsy, diarrhoea, wounds, stomach pain, asthma, and sore throats (Abebe *et al.,* 2021; Flore *et al.,* 2023). *S. guineense*

have also been reported to possess several pharmacological activities.

The importance of Pharmacognosy has been widely felt in recent times. Pharmacognostic studies of medicinal plants such *Syzygium guineense* will ensure plant identity, lay down standardisation parameters which will help prevent adulterations. Such studies will help in the authentication of the plants and ensure reproducible quality of herbal products, which will lead to the safety and efficacy of this important medicinal plant. The pharmacognostic standardisation parameters, which are generally done, include: organoleptic characters, macroscopic study, microscopic study, physicochemical, phytochemical and fluorescence analysis (Chanda, 2013; Fakchich *et al.,* 2022)

Syzygium guineense is a highly variable species, leading to debate concerning its taxonomy, including its subspecies. Due to the large diversity and wide distribution of the genus Syzygium and given the difficulties in correct botanical identifications within species of this genus, taxonomy characterization merging with DNA barcoding appears as a suitable alternative (Wati *et al*., 2022; Flore *et al.,* 2023) for the correct identification and authentication of *Syzygium guineense* (Myrtaceae).

MATERIALS AND METHODS

Plant Collection, Taxonomic Identification and Preparation

The fresh parts of the plant bearing the leaves and fruits were collected from Dakache, Zaria in October, 2024. It was taken to the Botany Department, Kaduna State University for identification and authentication by the plant taxonomist, Mallam U.S Gallah. After botanical identification, a sufficient quantity of the leaves was collected, dried, pulverized, weighed (600g) and stored in an airtight container for future use.

Organoleptic /Morphologic Characteristics

The organoleptic features (shape, surface texture, colour, and odour) and morphological features (margin, venation, and apex) of the fresh leaf of *S. guineense* were assessed using the organs of sense and standard procedures, respectively (Evans, 2016; WHO, 2011).

Microscopic Characters

The transverse section of the leaf sample of *S. guineense* was cut using a blade, cleared with a chloral hydrate solution, and viewed under the microscope to observe some of its qualitative microscopic features.

Chemo Microscopical Evaluation

Chemo-microscopical evaluation of *Syzygium guineense* leaf powder was carried out. About 1g of the powdered leaves of the plant was cleared in a test tube with a hypo solution. The cleared sample was mounted on a microscope slide, using a drop (about 0.5 ml) of dilute glycerol. Using various detecting reagents, the presence of some cell inclusions and cell wall materials was evaluated as described by *Brain and Turner (1975) and Evans (2009).*

Deoxyribonucleic Acid (DNA) Barcoding of *S. guineense* using the Ribulose Bisphosphate Carboxylase (rbcl) Gene Marker

DNA Extraction of S. guineense Leaf

The total genomic DNA was extracted from the fresh leaf of *S. guineense* using the EasyPrepTM Genomic DNA Miniprep kit from Bioland Scientific LLC, USA. Following the manufacturer's instructions, two hundred milligrams (200 mg) of the leaf was ground using a pestle. Two hundred microliter (200ul) lysis buffer was added to open the cells, and the lyophilised tissues was transferred into a 2ml micro-centrifuge tube and extraction was carried out using ethanol to precipitate the DNA. After multiple washings, the pure DNA sample was eluted in a buffer and stored in a freezer at -20 °C.

Polymerase Chain Reaction (PCR) Amplification

The protocol described by Ismail *et al.* (2020) was followed. The RBCL primers (Rbcla F ATGTCACCACAAACAGAGACTAAAGC and Rbcla R GTAAAATCAAGTCCACCRCG were used. These primers were designed to amplify a portion of the rbcL gene, commonly used for plant DNA barcoding. The PCR amplification was carried out in a total reaction volume of 20 µL. The reaction mixture consisted of 10µL PCR premix, 6µL of molecular grade water, 1 µL of each primer (Forward and Reverse), and 2 µL and DNA template (Extracted genomic DNA). The PCR process underwent 35 cycles of denaturation, annealing and extension at 94℃ - 5 minutes, 94℃ - 30 secs, 54℃ - 30 secs, 72℃ - 1 min 72℃ - 5 min PCR conditions set at various times for the gene amplification.

Gel Electrophoresis

The amplification status of the PCR product was verified using gel electrophoresis. A 100 bp DNA ladder was used as a size reference, and the amplified DNA was placed onto a 1.2% agarose gel stained with drops of ethidium bromide (Ismail *et al.* 2020). This allowed for the detection of a unique band that represented molecules of varying sizes. The electrophoresis was operated at 110–120V for the duration of 30 minutes. The success of the amplification was confirmed by the presence of a clear band between 500 - 600 bp when viewed under the UV (Ismail *et al.* 2020; Tamura *et al.,* 2021). The PCR products was purified for Sanger sequencing

DNA Sequencing and Data Analysis

The amplified DNA product was sent for gene sequencing. After sequencing, the resultant sequences were edited manually and were analysed using Finch TV software (Ismail *et al.* 2020). This was compared to reference sequences in the NCBI BLAST database to determine the ten closest matches. BLAST searches were applied to the obtained sequences from the GenBank databases. The GenBank is an NIH genetic sequence database, an annotated collection of all publicly available DNA sequences. From that search, we pulled out DNA sequences of plants that looked similar to our query sequence for comparison. This was done by aligning the retrieved sequences side by side to view similarities and differences using the bioinformatics software, CLUSTAL X (version 1.81). This step is crucial to detect exactly where our plant's DNA marches or differs plants' DNA. DNA is made up of Adenine (A), Thymine (T), Guanine (G) and Cytosine (C) nucleotides. Nucleotide composition analysis was used to quantify the AT and GC contents present in the query sequence. This helps in identification as different plants may have different proportions of these nucleotides.

RESULTS AND DISCUSSION

Organoleptic /Morphologic Characteristics

The organoleptic/morphological characteristics of *S. guineense* are described in Table 1.

Table 1: Organoleptic Characteristics of *S. guineense* Leaf

Characters	Observations
Color	Shiny Green
Odour	Fragrant smell when crushed
Taste	Sour
Surface texture	Hard/papery
Size	7-13.0cm length, 2- 5.36cm width
Shape	Ovate –elliptic
margin	Entire
Apex	Obtuse to rounded

Qualitative Microscopic Characters

The microscopic observation of the upper epidermal layer of the *S. guineense's* leaf showed the presence of paracytic type of stomata, guard cells and epidermal cells (Plate 1)

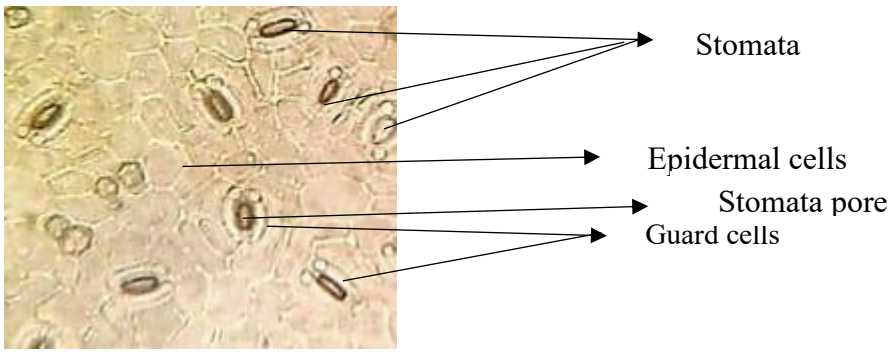

Plate 1: Photomicrograph of the upper epidermal layer of *Syzygium guineense* leaf at magnification (× 100)

Transverse Section of *S. guinnense* Leaf

The transverse section (T.S) of the *S. guineense* leaf (Plate 2) showed the presence of mesophyll tissues, vascular bundles, cuticles, lower and upper epidermis

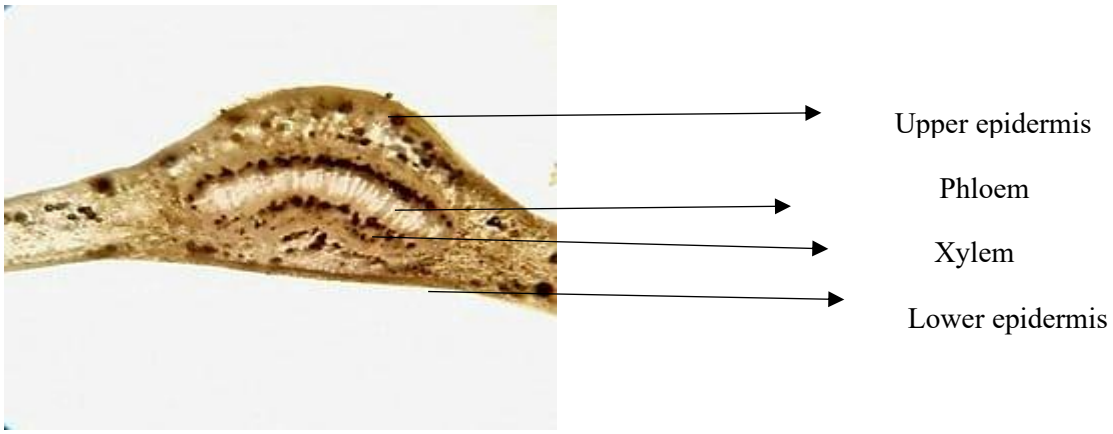

Plate 2: Photomicrograph of the Transverse Section of *Syzygium guineense* Leaf at Magnification (×100)

Chemo-Microscopy Features of *Syzygium guineense* Powered Leaves

The chemo-microscopic features of the powdered leaves of *S. guineense* (Table 2) showed the present of cellulose cell wall, lignified cell wall, starch, tannins, calcium oxalate crystals and the absent of calcium carbonate.

Table 2: Chemo-Microscopic Features of *S. guineense* Leaf Sample Powder

Test	Observation	Observation
Cellulose cell wall	Blue colour	Present
Lignified cell wall	Red colour	Present
Starch	Blue black colour	Present
Tannins	Greenish-black color	Present
Calcium carbonate	No effervescence	Absent
Calcium oxalate crystals	Transparent crystals disappeared	Present

Preliminary Phytochemical Profile

The preliminary phytochemical profile of *S. guineense* (Table 3) showed the presence of tannins, flavonoids, alkaloids, saponins, cardiac glycosides and carbohydrates, with the absence of anthraquinones.

Table 3: Phytochemical Profile of *S. guineense* Crude Extract

Phytochemicals	Test	Observation
Cardiac glycosides	Keller –Killani	Positive
Saponins	Frothing	Positive
Alkaloids	Drangedorf	Positive
	Wagner	Positive
Flavonoid	Sodium hydroxide	Positive
Tannins	Ferric chloride	Positive
Steriods/	Lieberman-Buchard	Positive
Carbohydrates	Molisch	Positive
Anthraquinones	Bontrager	Negative

PCR amplification and Gel electrophoresis

The pure DNA of *S. guineense* leaf was successfully extracted and amplified. This was confirmed by the gel electrophoresis analysis (Fig. 1), which revealed a single band of the amplified DNA of the sample (SG) between 500 and 600bp.

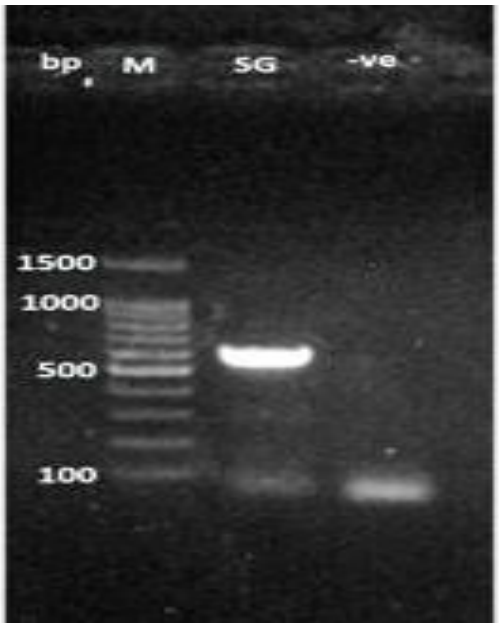

Figure 1: Electrophoresis Analysis of PCR Amplified Products using Rbcl-Specific Primers. M Representing DNA Ladder, SG Representing the PCR Amplification of the *S. guineense,* PCR Product

Sequencing and Blast analysis

A Chromatogram of the nucleotide sequence of *S. guineense* PCR product (Fig.2) was developed and analysed using Finch software. The sequence was subjected to blast analysis and compared with known sequences in the GenBank database. Table 4 shows the extracted result from the blast.

Figure 2: A Chromatogram of the Query Nucleotide Sequence from the PCR Product for Taxonomic Resolution

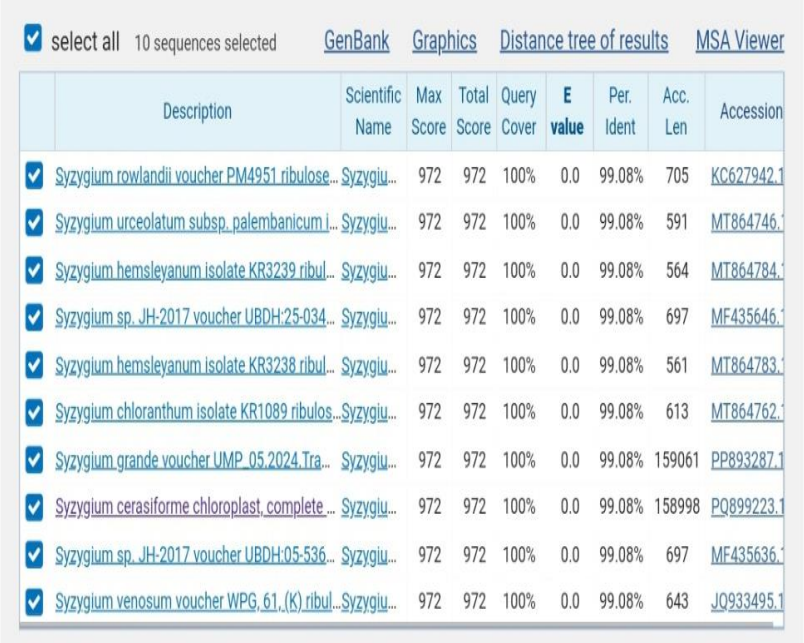

Figure 3: Nucleotide Blast Result Showing a High Query Cover (100%) with High Percentage Identity (≈ 99.08%) to Multiple Species of *Syzygium* in the Database without Identifying a Single Exact Species as *Syzygium Guineense* (Ambiguity at Species Level)

Table 4: BLAST Results of rbcL Sequence from Syzygium Sample

Description	Scientific Name	Query Cover (%)	Identity (%)	Accession No.
Syzygium rowlandii voucher PMA951	*Syzygium rowlandii*	100	99.08	KC629749.1
Syzygium urecolatum subsp. palembangicum	*Syzygium urecolatum*	100	99.08	MT867446.1
Syzygium hemsleyanum isolate KR8238	*Syzygium hemsleyanum*	100	99.08	MT867451.1
Syzygium sp. HJ-2017 voucher UDRH25-934	*Syzygium* sp.	100	99.08	MT867440.1
Syzygium hemsleyanum isolate KR8238	*Syzygium hemsleyanum*	100	99.08	MT867432.1
Syzygium chloranthum isolate KR1089	*Syzygium chloranthum*	100	99.08	MT867437.1
Syzygium grande voucher LMP 05-2012	*Syzygium grande*	100	99.08	MT867428.1
Syzygium cerasiforme chloroplast complete	*Syzygium cerasiforme*	100	99.08	P09923.1
Syzygium sp. HJ-2017 voucher UDRH05-936	*Syzygium* sp.	100	99.08	MT867430.1
Syzygium wenseom voucher WPG G1_61	*Syzygium wenseom*	100	99.08	U92345.1

Characteristics of the Sequence

The nucleotide sequence was 542 base pairs in length. The base composition analysis revealed the following base counts: Adenine (A) = 147, Thymine (T) = 161, Cytosine (C) = 115, Guanine (G) = 119. The GC content of the sequence was 43.17%, and the AT content was 56.82% as shown in Table 5.

Table 5: Nucleotide Composition of rbcL Sequence from Syzygium Sample

Parameter	Value
Sequence length (bp)	542
Adenine (A) count	147
Thymine (T) count	161
Cytosine (C) count	115
Guanine (G) count	119
AT content (%)	56.82
GC content (%)	43.17

Phylogenetic relationship between the query sequence and other sequences in GenBank

The cladogram (phylogenetic tree) in Fig. 4 shows that the query sequence appears to cluster closely with other *Syzygium species* sequences

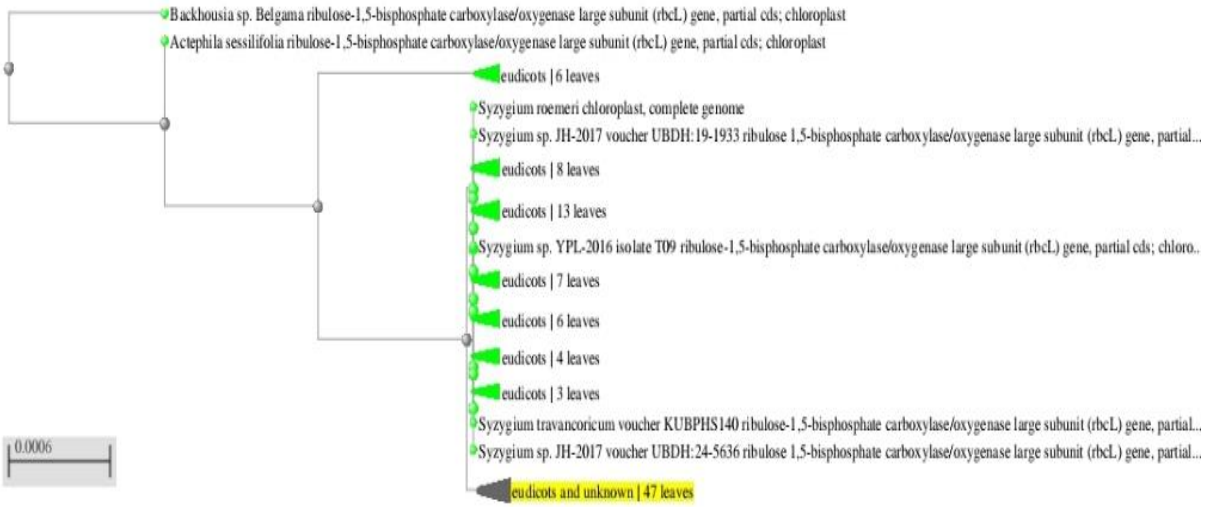

Figure 4: Cladogram showing the relationship between the Query nucleotide sequence and other sequence in the GenBank database. Clustering within the Syzygium genus with High similarity but no species-level resolution with Non-Syzygium taxa clearly separated from the Syzygium clade

Discussion

Based on key morphological features, the plant sample was identified taxonomically as *Syzygium guineense*. An organoleptic evaluation shows the leaves sample as simple, measuring 7–13.0 cm in length and 2–5.36 cm in width, shiny green, ovate-elliptic in shape, with an entire margin, rounded apex, and a hard, papery texture (Table 1). These characteristics are consistent with descriptions of *S. guineense* reported in existing literature (IOR, 2012, Badou *et al.,* 2020; Batiha *et al.,* 2020). Microscopic analysis revealed a paracytic stomatal type located on the lower epidermis. This is contrary to the report of Azila *et al.,* (2022), who indicated that, two closely related species of Syzygium presented with anisocytic stomata type. However, studies have shown that stomatal type shows interspecies variation and limited taxonomic significance within the *Syzygium* genus (IOR, 2012, Chatri *et al.,* 2020). Chemo-microscopic and phytochemical analyses are not traditionally included in classical taxonomy, However, they are now considered important in contemporary classification systems (Batiha *et al.,* 20200. In this study, the cleared powdered sample exhibited the presence of calcium oxalate crystals, tannins, and lignified cell walls, with no calcium carbonate detected (Table 2). Preliminary phytochemical screening of the crude extract (Table 3) confirmed the presence of tannins, flavonoids, saponins, cardiac glycosides, and steroids, while anthraquinones were absent. This result of the preliminary phytochemical does not agree with the report by Desalegn *et al.,* (2021) who reported that Flavonoid is absent while anthraquinones is present in the leaf extract of *Syzygium guineense*. Although traditional taxonomy, which relies on morphological and anatomical traits, is a fundamental strategy for plant identification, it frequently fails to reliably separate closely related species within complex genera (Amin and Park, 2025), such as Syzygium. This genus is distinguished by extensive species variety, overlapping morphological characteristics, and intraspecific variation, all of which present considerable hurdles to conclusive identification. Chemotaxonomy can identify cryptic species that may appear visually identical but differ in chemical composition, and it can operate as a "complementing tool" to enable robust plant identification (Amin and Park, 2025). However, it lacks the specificity needed for taxonomic precision because many chemicals are shared by multiple species. Therefore, relying simply on morphology and phytochemistry may result in misidentification. To address these constraints, molecular technologies such as DNA barcoding offers a more robust and reliable tools especially for specie level authentication (Wang *et al.,* 2024).

In this study, molecular identification of the plant sample identified morphologically as *Syzygium guineense* was conducted using the rbcL gene marker. From the result, following the successive PCR amplification of the extracted pure DNA the query nucleotide sequence was 542 base pairs in length. The base composition analysis revealed the following base counts: A = 147, T = 161, C = 115, G = 119. The GC content of the sequence was 43.17%, and the AT content was 56.82%, consistent with typical RbcL gene sequences from related species. This consistency underscores the suitability of RbcL for phylogenetic and species level identification studies within the genus *Syzygium*. The suitability of rbcl for plant identification has also been reported by Nicolas *et al.*, (2012) and Kang *et a*l. (2017)

The blast sequence result analysis indicated high similarities with ten (10) closely related species of *Syzygium* with query cover of 100% and pair identity of 99.08% but did not specifically reveal the specie name *guineense* for all ten (10) species identified**.** Also, the phylogenetic analysis based on the *rbcL* gene sequence revealed that the query sequence clustered closely with species from the genus *Syzygium* and shared high similarity with other members of the genus, supporting its placement within the genus, *Syzygium*. But again, the RBCL gene barcoding of the plant sample did not resolve to a specific species of *Syzygium guineense*.

This finding supports genus-level identification of the plant sample but also highlights the limitation of using a single barcode region like *rbcL* for precise species-level resolution of closely related and complex plant species such as *Syzygium* and related taxa (CBOL Plant Working Group, 2009; Hollingsworth *et al.,* 2011).

CONCLUSION

This study highlighted the value of having an integrated methodology when attempting to identify a closely related plant species accurately. The application of DNA barcoding using the rbcL gene confirmed the specimen's placement in the *Syzygium* genus but was unable to resolve it as *S. guineense*. This illustrates the profound difficulty species-

level identification poses in highly genetically and morphologically similar groups. The results suggest that a multidisciplinary approach combining the rbcl gene marker with other plants' barcode loci, such as matK, is needed for reliable identification of traditional medicinal plants with complex taxonomy, as this is crucial to ensuring the efficacy and quality standards in herbal medicine.

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