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Full Length Research Article

MOLECULAR PHYLOGENY AND PHYTOCHEMICAL SCREENING OF LOCALLY USED ANTIMALARIAL PLANTS FOUND IN KADUNA STATE UNIVERSITY BASED ON *RBCL* GENE SEQUENCES

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ABSTRACT
This study investigated the molecular phylogeny and phytochemical composition of fourteen locally used antimalarial plants found within Kaduna State University, Kaduna, using *rbcL* gene sequences. The aim was to elucidate their genetic relationships and identify bioactive secondary metabolites that may underlie their therapeutic potential. Fresh leaves of each plant were collected, air-dried, and subjected to aqueous extraction for qualitative phytochemical screening, while genomic DNA was extracted and amplified using the *rbcL* primer through polymerase chain reaction (PCR). The amplified products were visualized on agarose gel electrophoresis, sequenced, and analyzed using BLAST and MEGA 11 software to construct phylogenetic relationships. Results showed that all plants contained at least four classes of secondary metabolites, with *Azadirachta indica*, *Artemisia annua*, *Cassia occidentalis, Carica papaya*, *Magnifera indica,* and *Senna siamese* exhibiting the richest phytochemical profiles, containing flavonoids, tannins, saponins, glycosides, and alkaloids. The PCR amplification produced distinct bands of approximately 700 bp, confirming successful amplification of the *rbcL* gene. Phylogenetic analysis grouped the species into four major clades consistent with their taxonomic families, Fabaceae, Meliaceae, Anacardiaceae, and Caricaceae, demonstrating close evolutionary relationships among related taxa. The study concludes that integrating molecular phylogeny with phytochemical screening provides valuable insights into the genetic diversity and therapeutic potential of medicinal plants, which can be candidates for further antimalarial drug discovery.

Keywords: *rbcL* gene, Phylogeny, Phytochemical screening, Antimalarial plants.

INTRODUCTION

Malaria remains one of the most life-threatening parasitic diseases worldwide, particularly in sub-Saharan Africa, where it poses significant public health and economic challenges (WHO, 2023). The disease, primarily caused by *Plasmodium falciparum*, continues to exert immense pressure on tropical and subtropical regions despite advances in control strategies. The emergence of multidrug-resistant strains of *Plasmodium* has further emphasized the urgent need for novel and effective antimalarial agents derived from natural sources (Azam *et al*., 2025). Medicinal plants have historically served as valuable reservoirs for bioactive compounds, and their use in traditional medicine for the treatment of malaria is deeply rooted in African ethno-botanical practices (Dawurung *et al*., 2021).

In Nigeria, several indigenous plants are traditionally employed in the management of malaria symptoms, including *Azadirachta indica*, *Carica papaya*, *Artemisia spp.*, *Cymbopogon citratus*, and *Cassia occidentalis* (Evbuomwan *et al*., 2023; Jada *et al.,* 2024). These plants are reported to contain secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids, which contribute to their therapeutic efficacy (Kumar *et al*, 2025). However, despite their ethno-medicinal relevance, scientific validation of their phytochemical constituents and genetic diversity remains limited. Understanding the phytochemical composition alongside molecular characterization provides information on the biochemical basis of their pharmacological activity and evolutionary relatedness (Sadhu *et al*., 2025).

Molecular phylogenetics, particularly through the use of chloroplast genes such as *ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit* (*rbcL*), has become a reliable tool for elucidating evolutionary relationships among plant taxa (Patwardhan *et al*., 2014). The *rbcL* gene is highly conserved and widely used as a molecular marker for species identification, DNA barcoding, and phylogenetic studies due to its universality and moderate rate of evolution (Hollingsworth *et al.,* 2009). Integrating molecular data with phytochemical screening provides a holistic understanding of plant biodiversity, bioactivity potential, and taxonomic classification (Sadhu *et al*., 2025).

This study, therefore, investigates the molecular phylogeny and phytochemical profiles of locally used antimalarial plants found within Kaduna State University. The study aims to assess the genetic relationships of the selected plant species using *rbcL* gene sequences and evaluate their qualitative phytochemical constituents.

MATERIALS AND METHODS

Collection of Samples

Fresh leaf samples of free-growing and cultivated plants were collected in the Botanical Garden and within the Main Campus of Kaduna State University (KASU). The leaves were air-dried at room temperature for three weeks. An electric grinding machine was thoroughly cleaned and dried beforehand to prevent contamination, in which the dried leaves were ground into fine powder.

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Extraction
For the aqueous extraction, a weighing balance was first zeroed, and a filter paper was placed on it. A quantity of 200 g of the sample was accurately weighed and transferred into a reagent bottle. Then, 2 L of distilled water was added to soak the sample and facilitate extraction for 48 hours. After allowing it to stand for a few minutes, the mixture was properly filtered using filter paper (Ogunniran *et al*., 2024; Htay *et al.*, 2023). The resulting filtrate was then subjected to qualitative tests with various reagents to determine the presence and quantity of flavonoids, tannins, saponins, alkaloids, glycosides, anthraquinones, and steroids.

Qualitative Phytochemical Screenings
The phytochemical composition of the extract was analyzed at the Chemistry Laboratory, Kaduna State University, Kaduna, following the procedures described by Ogunniran *et al*. (2024), Htay *et al*. (2023), and Lisdiani *et al*. (2022). The extract was screened for the presence of flavonoids, tannins, saponins, alkaloids, glycosides, anthraquinones, and steroids.

DNA Extraction
Leaves (100 mg) from each plant were ground into fine powder and mixed with plant lysis buffer (PL buffer), proteinase K, and RNase A to ensure complete lysis and resuspension, followed by the addition of normal saline for better homogenization. The mixture was incubated at 60 °C for 10 minutes, then treated with PC buffer and kept on ice to precipitate detergents, proteins, and polysaccharides. After centrifugation, the supernatant was clarified, combined with binding/wash buffer 1 (WA1 buffer), and loaded onto a binding column, where nucleic acids were trapped while impurities were removed. The column was washed twice with wash buffer 2 (W2 buffer), centrifuged to eliminate residual ethanol, and then transferred to a clean tube for elution with elution buffer (EA buffer), yielding purified nucleic acids (Gnayem *et al.,* 2024).

Polymerase Chain Reaction
Primer (RBCLA) (6 µl) and water (8 µl) were added to the PCR tube. The extracted DNA (2 µl) was then added to the PCR tube. It was shaken and spun in a micro-centrifuge**.** The PCR machine was set, the reaction was carried out under the following thermocycling conditions: initial denaturation at 94^0C for 5 minutes, final denaturation at 94^0C for 30 seconds, anealing at 54^0C for 30 seconds, initial extension at 72^0C for 1 minutes, 35 cycles and final extension at 72^0C for 5 minutes (Saputri and Arsyadi, 2024).

Gel Electrophoresis and Visualization
Agarose powder (1.3 g) was measured and transferred into a conical flask. The agarose was dissolved by microwaving the mixture for 2 minutes. After removal from the microwave, the solution was allowed to cool for a few minutes. A casting tray was prepared for gel casting. Ethidium bromide (12 µl) was added to the cooled agarose solution. The PCR solution was loaded into the wells of the gel in the casting tray. A DNA ladder was loaded into one of the wells to serve as a size marker for determining the DNA fragment sizes. The electrophoresis system was run at 120 volts for 30 minutes. The gel was visualized under UV light to confirm the presence of the specific PCR product (Gnayem *et al.,* 2024).

Sequence Alignment
The FASTA format, together with an Outgroup sequence also in FASTA were prepared and uploaded into MEGA 11. Multiple sequence alignment of the DNA was carried out using the MUSCLE Algorithm, and the output was exported in MEGA format to a named folder. The alignment result was used to construct a phylogenetic tree (Obembe *et al.,* 2025).

Phylogenetic Relationship
MEGA version 11.0 was used to construct the phylogenetic relationships of plant DNA sequences and the sequence of the outgroup. The molecular evolutionary relationship was constructed using the Neighbor-Joining to determine the evolutionary relationships among plants (Jones *et al.,* 1992).

RESULTS

Table 1: Phytochemical Screening of the Leaf Aqueous Extract of the Plant Species

Phytochemical Constituents	AI	AO	AS	CC	CO	CP	DR	JC	KS	MI	PL	SS	TC	TI
Flavonoids	+	+	+	+	+	+	+	+	-	+	-	+	+	-
Tannins	++	+	+	+	+	+	+	+	+	+	-	++	+	-
Saponins	+++	-	-	-	++	+	+	+	+	+	+	+	-	++
Alkaloid	+	+	+	+	-	+	+	-	-	+	+	++	+	++
Glycoside	++	+	+	+	+	+	+	+	+	+	+	+	-	++
Anthraquinones	-	+	-	-	+	-	-	-	-	+	+	-	+	++
Steroids	+++	-	+	+	+	+	-	+	+	-	+	+	-	+

Keys: highly positive(+++), moderately positive (++), mildly positive (+), negative(-) *Azadirachta indica* (AI), *Anaccadium occidentalis* (AO), *Artemisia annua* (AS), *Cymbopogon citrus* (CC), *Cassia occidentalis* (CO), *Carica papaya* (CP), *Delonix regia* (DR), *Jatropha curcas* (JC), *Khaya senegalensis* (KS), *Magnifera indica* (MI), *Polyalthia longifolia* (PL), *Senna Siamese* (SS), *Terminalia catappa* (TC), *Termarindus indica* (TI)

Table 1, presents the qualitative phytochemical constituents of the studied plant samples. The analysis revealed varying distributions of secondary metabolites across the samples. Flavonoids were widely detected in most samples, although they were absent in KS, PL, and TI. Tannins were present in nearly all samples, with strong reactions (++/+++) observed in AI and SS, while PL and TI showed no detectable tannins. Saponins exhibited marked variability, with very high concentrations (+++) in AI and notable presence in CO and TI, but were absent in several samples including AO, AS, and CC. Alkaloids were moderately present in most samples, with higher intensity observed in SS and TI, whereas CO, JC, and KS tested negative. Glycosides were consistently detected across the

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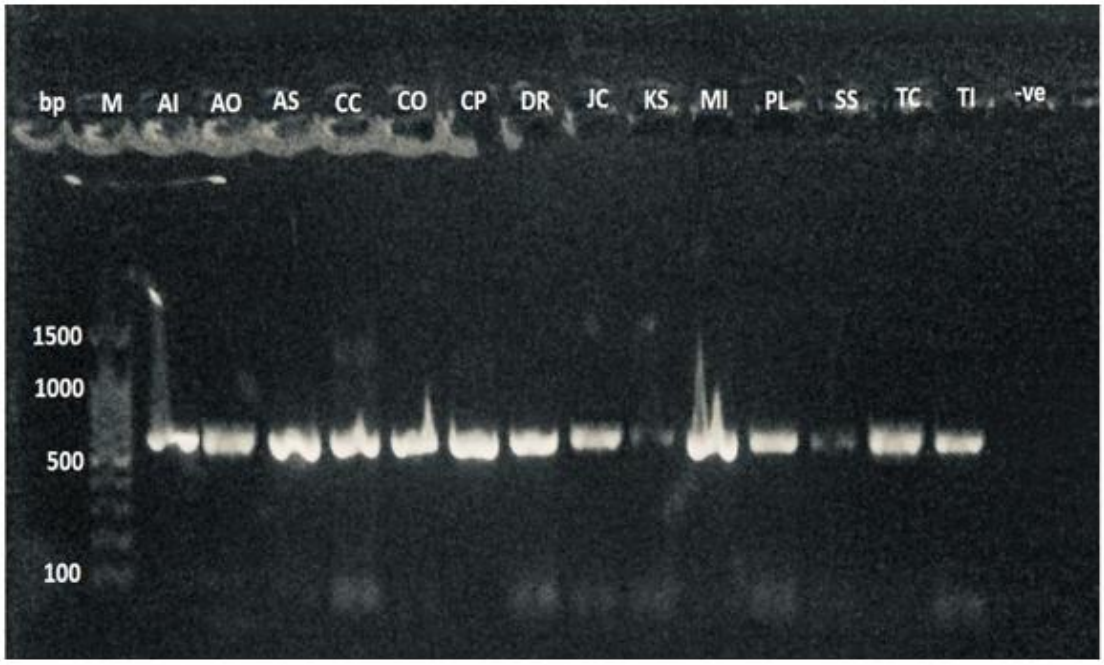
majority of samples, except TC, indicating their widespread occurrence. Anthraquinones showed limited distribution, being present in only a few samples such as AO, CO, MI, PL, TC, and TI. Steroids were prominently present in AI, while moderate to weak presence was observed in several other samples, and they were absent in AO, DR, MI, and TC.



Keys: BP= Base pair, M= Marker or ladder, AI= *Azadirachta indica*, AO= *Anaccadium Occidentalis*, AS= *Artemisia spp*, CC= *Cymbopogon citrus*, CO=*Cassia occidentalis*, CP=*carica papaya*, DR= *Delonix regia*, JC= *Jatropha curcas*, KS= *Khaya senegalensis*, MI= *Magnifera indica*, PL= *Polyalthia longifolia*, SS= *Senna Siamese*, TC= *Terminalia catappa*, TI= *Termarindus indica*, -VE= Negative control

Figure 1: Gel Electrophoregram of the *rbcL* gene in different plant species analysed

The gel electrophoregram of the *rbcL* gene revealed distinct bands corresponding to the amplified regions of DNA from plant species. The sizes of the bands were estimated to be approximately 700bp when compared to the DNA ladder. Clear, sharp bands indicate successful DNA extraction and PCR amplification. Smearing or faint bands suggest suboptimal amplification (Figure 1).

BLAST Result

The BLAST analysis showed a high sequence similarity between all the plant species and related atimalarial plants when performing nucleotide BLAST at NCBI, with percent identity ranging from 99.8% to 100%, with E-values indicating significant matches. The high similarity confirms that the amplified sequences belong to the targeted regions. Closely related species were identified, supporting the hypothesis of shared ancestry or functional similarity.

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Figure 2: Sequence Alignment

The multiple sequence alignment revealed conserved regions across the species. Variations were observed, indicating possible evolutionary divergence. Conserved regions suggest functional or structural importance in antimalarial activity. Variations provide insight into species-specific adaptations or evolutionary changes (Figure 2).

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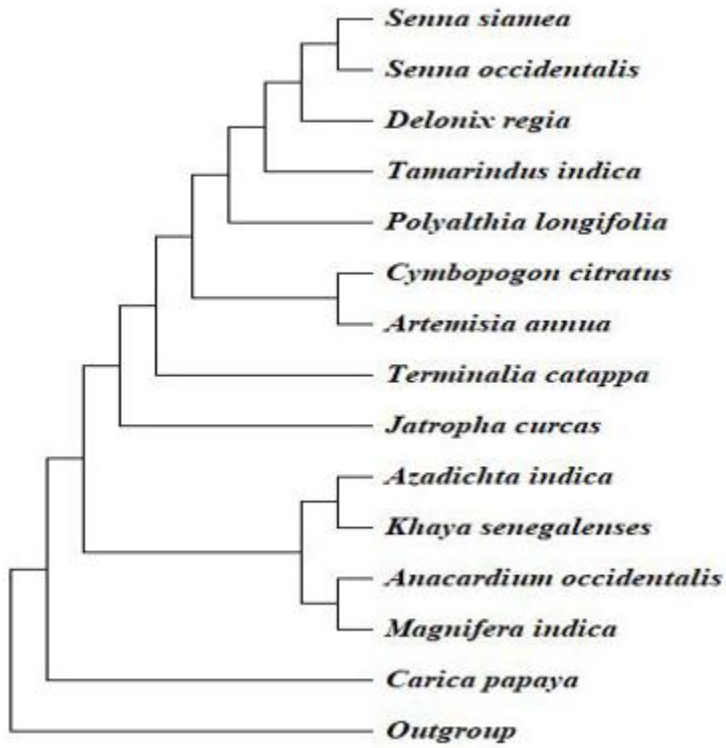

Figure 3: Phylogenetic Relationship of different antimalarial plant species

The phylogenetic tree grouped *Cassia occidentalis* with *Senna siamea, Delonix regia,* and *Tamarindus indica* into a distinct clade, reflecting their close evolutionary relationships within the Fabaceae family. The tree topology showed four major clades: A clade comprising Fabaceae species *(Cassia occidentalis*, *Senna siamea, Delonix regia,* and *Tamarindus indica).* A clade containing *Azadirachta indica, Khaya senegalensis* (Meliaceae family). Another clade consisting of *Mangifera indica, Anacardium occidentale,* and *Terminalia catappa* (order Sapindales). A clade with more distantly related species, such as *Cymbopogon citratus, Artemisia annua,* and others. *Cassia occidentalis* shares a recent common ancestor with *Senna siamea*, indicating close evolutionary relationships. Distantly related species were placed in separate clades, reflecting greater divergence (Figure 3).

DISCUSSION

The qualitative screening of fourteen medicinal plants, *Azadirachta indica* (AI), *Anacardium occidentalis* (AO), *Artemisia annua* (AS), *Cymbopogon citratus* (CC), *Cassia occidentalis* (CO), *Carica papaya* (CP), *Delonix regia* (DR), *Jatropha curcas* (JC), *Khaya senegalensis* (KS), *Mangifera indica* (MI), *Polyalthia longifolia* (PL), *Senna siamensis* (SS), *Terminalia catappa* (TC), and *Tamarindus indica* (TI) reveals considerable variability in phytochemical profiles. *A. indica* (AI) exhibited strong presence of saponins and steroids and moderate to strong presence of tannins, glycosides, flavonoids, and alkaloids, consistent with prior reports of its rich secondary-metabolite content (e.g., alkaloids, flavonoids, saponins, tannins) (Dash *et al*., 2017; Khanal, 2021). Other taxa, such as *Carica papaya* (CP) and *Jatropha curcas* (JC), also showed broad phytochemical presence (flavonoids, tannins, glycosides, saponins, alkaloids), suggesting their multipotent bioactivity potential (Zunjar, 2017; Rahu *et al*., 2021). In contrast, *Mangifera indica* (MI) lacked detectable flavonoids but possessed moderate levels of tannins and other constituents, indicating selective secondary metabolite accumulation in that species. Similarly, *Senna siamensis* (SS) was negative for saponins in your screening despite showing flavonoids and tannins, which underscores the species-specific distribution of phytochemicals. The wide distribution of tannins across almost all species often suggests that phenolic-based defense systems are common among these taxa (Rubert-Nason and Lindroth, 2021), while the relative scarcity or absence of anthraquinones and steroids in some species (absence in TI) suggests more restricted biosynthetic pathways (Wang *et al*., 2024).

The agarose gel electrophoregram shows distinct amplification bands corresponding to the *rbcL* gene, a chloroplast-encoded marker widely used in plant DNA barcoding and phylogenetic studies (Patwardhan *et al*., 2014). Amplified products for most of the samples were observed between 600 and 900 bp, consistent with the expected fragment size of the ther*bcL* region (Maloukh *et al.,* 2017). Clear and intense bands were obtained in species such as *Azadirachta indica* (AI), *Cassia occidentalis* (CO), *Carica papaya* (CP), *Delonix regia* (DR), *Jatropha curcas* (JC), and *Mangifera indica* (MI), indicating efficient DNA extraction and successful amplification. Slightly faint bands in species *Khaya senegalensis* (KS) and *Senna siamensis* (SS) suggest variations in DNA template quality or the presence of secondary metabolites that may have partially inhibited the PCR reaction (Ghawana *et al*., 2011).

The absence of any band in the negative control confirms that there was no contamination during PCR setup, validating the reliability of the amplification results. The presence of single, distinct bands without smearing indicates high-quality DNA and specific primer annealing to the *rbcL* locus, making these samples suitable for sequencing and downstream phylogenetic analysis. The consistency of band sizes across different taxa supports the conserved nature of the rbcL gene across angiosperms (Leaks *et al*., 2025). Overall, the results confirm that the *rbcL* marker is a reliable and universal barcode for assessing genetic diversity and phylogenetic relationships among these medicinal plant species.

The phylogenetic tree constructed from the rbcL gene sequences reveals evolutionary relationships among the fourteen medicinal plant species, showing clear clustering that reflects both taxonomic and genetic affinities. Species within the same genus, such as *Senna siamea* and *Senna occidentalis*, clustered closely together, indicating a high degree of genetic similarity and supporting their shared evolutionary lineage. *Delonix regia* and *Tamarindus indica*, both members of the Fabaceae family, are also grouped together, further confirming the conserved nature of the *rbcL* gene in tracing familial relationships (Leaks *et al*., 2025). Similarly, *Azadirachta indica* and *Khaya senegalensis* formed a distinct clade, consistent with their placement in the Meliaceae family (Nie *et al*., 2025). The grouping of *Mangifera indica* with *Anacardium occidentale* also aligns with their known classification within the Anacardiaceae family, reflecting the reliability of *rbcL* as a molecular marker for phylogenetic inference (Patwardhan *et al*., 2014). The relatively basal positioning of *Carica papaya* suggests a greater evolutionary divergence from the other taxa, consistent with its placement in a separate family (Caricaceae).

This study successfully combined molecular phylogenetic analysis and phytochemical screening to characterize fourteen medicinal plants traditionally used for malaria treatment within Kaduna State University. The qualitative phytochemical results revealed that most of the plant species contained diverse classes of secondary metabolites, particularly flavonoids, tannins, saponins, glycosides, and alkaloids, which are known for their antimalarial and therapeutic properties. The successful amplification and sequencing of the *rbcL* gene confirmed its reliability as a universal molecular marker for assessing genetic diversity and evolutionary relatedness among plant taxa. The phylogenetic analysis grouped the species into distinct clades corresponding to their known taxonomic families, demonstrating clear genetic affinities among closely related species such as *Cassia occidentalis* and *Senna siamea* (Fabaceae), as well as *Azadirachta indica* and *Khaya senegalensis* (Meliaceae).

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