RESEARCH PAPER

Phylogenetic, Phytochemical and Antimicrobial Evaluation of Quisqualis Indica L. to Explore Its Drug Delivery Potential as a Nutraceutical

Bindu Rajaguru[1] and Seema Kokitkar[2]*

[1,2]*Department of Biotechnology, Changu Kana Thakur College of Arts, Commerce and Science, New Panvel, Raigad, Maharashtra, India*

Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 12th Feb, 2026; Available Online: 28th Feb, 2026

ABSTRACT

Quisqualis indica commonly known as Rangoon creeper, is a medicinal and ornamental plant belonging to the family Combretaceae. It is widely distributed across tropical and subtropical regions and is traditionally used for its pharmacological properties. The plant is rich in bioactive compounds such as flavonoids, tannins, saponins, and alkaloids, which contribute to its diverse therapeutic potentials. Extracts of Q. indica have demonstrated significant antioxidant, antimicrobial, anti-inflammatory, anthelmintic, and anticancer activities in various studies. In the present study the molecular and phylogenetic analysis were performed using DNA barcoding for establishing the authenticity of the plant sample. Further HPTLC and GC-MS were conducted for phytochemical profiling followed by determining the antimicrobial efficacy of the plant extracts. The DNA barcoding with gene markers ITS, matK, rpoB, trnH- psbA and rbcL) showed >99% genetic similarity. Qualitative assays and HPTLC analysis revealed a predominant phytochemical signature dominated by phenols, tannins, and saponins under the experimental conditions. The gas chromatography–mass spectrometry (GC–MS) analysis revealed solvent-dependent differences in chemical composition between the extracts. The antibacterial and antifungal assays significantly ($p = <0.005$) confirmed the efficiency of extracts. Overall, this study is an integrated approach employing DNA barcoding and antimicrobial assessment which lays a solid foundation for developing standardized, safe, and effective Q. indica-based nutraceutical and therapeutic formulations with broad-spectrum antimicrobial relevance.

Keywords: *Quisqualis indica, DNA barcoding, phytochemical analysis, HPTLC, GC-MS, antimicrobial analysis*

How to cite this article: Rajaguru B. and Kokitkar S. Phylogenetic, Phytochemical and Antimicrobial Evaluation of Quisqualis Indica L. to Explore Its Drug Delivery Potential as a Nutraceutical. Int J Drug Deliv Technol. 2026;16(5s): 20-32. DOI: 10.25258/ijddt.16.5s.3

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The global nutraceutical sector is experiencing sustained expansion as both consumers and healthcare systems increasingly prioritize preventive, food-adjacent interventions fortified by credible scientific validation. Growth is being driven by rising demand for functional botanicals, microbiome-targeted formulations, and clean-label products. The Current market analyses value of this sector is approximately at USD 591 billion in 2024, with projections of USD 636 billion in 2025 and a projected compound annual growth rate (CAGR) of 7–8% through 2030. These market trajectories highlight the dual commercial and public-health significance of authenticated botanicals, whose chemical composition and bioactivity must be rigorously characterized to ensure their safe translation into standardized, efficacious ingredients. Within this framework, many plant family members show a valuable application, one of the examples is, members of the *Combretaceae* family representing a valuable niche owing to their polyphenol-enriched chemotypes and extensive history of ethnomedical application. Some of the phenolic and tannin-based metabolites from genera such as *Terminalia* have been linked to antimicrobial and other bioactivities relevant in nutraceutical innovation, underscoring the importance of a "chemistry-first, authentication-first" paradigm in evaluating new candidates[1,2]. In addition, *Quisqualis indica* (syn. *Combretum indicum*), a plant of this family and a perennial woody climber native to tropical Asia, holds a prominent position in South and Southeast Asian traditional medicine, particularly for gastrointestinal and inflammatory disorders [3]. Historically, its seeds and fruits were employed as anthelmintics, with activity partly attributed to quisqualic acid, an amino acid derivative localized in the seeds. Contemporary ethnopharmacological reviews additionally record its use in managing diarrhoea, abdominal discomfort, and dermatological conditions, although rigorous clinical validation of these applications remains limited[4].

Quisqualis indica (QI) has been increasingly examined in recent times for its phytochemical and antimicrobial potential [5]. Modern surveys and reviews have consistently reported that its tissues including leaves, flowers, and seeds harbour diverse secondary metabolites such as flavonoids, saponins, tannins, alkaloids, and a range of phenolic compounds, associated with antimicrobial

**Author for Correspondence: seemaskk11@gmail.com*

activity [6] across a spectrum of target organisms. A focused investigation on QI flower extracts, fractionated with solvents of varying polarity, demonstrated notable antibacterial activity in agar-well diffusion and MIC assays against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. Strikingly non-polar extracts of petroleum ether showed comparatively stronger inhibition, suggesting the contribution of lipophilic phytoconstituents to the observed activity[7]. The richer bioactive fractions of leaves and flowers, despite methodological variation across studies, provides evidence supporting the antimicrobial potential of *Q. indica*. This justifies systematic, solvent-stratified exploration for antibacterial leads.

The *Q indica* plant species continues to attract attention as a phytochemical and pharmacological diverse species. Integrating GC–MS analyses, for volatile and semi-volatile constituents indicating solvent polarity which strongly modulates the extractable metabolite profile[8,9] followed by instrumental fingerprinting techniques such as HPTLC to map class distribution across extracts before progressing to quantitative and qualitative microbiological assessments[10] is a suitable method to explore the untapped potentials of phytochemicals. Canonical phytochemical classes in *Q. indica* include flavonoids, tannins, saponins, and alkaloids, standardized through qualitative assays across solvents of varying polarity[11]. Phenolics, especially tannins, exhibit antibacterial activity via protein precipitation, ion chelation, and membrane disruption, while saponins enhance effects through detergent-like interactions. Seeds contain quisqualic acid, an excitatory amino acid with anthelmintic potential but neurological risks, highlighting the need for organ- and solvent-specific selectivity to balance efficacy and safety[12]. In parallel, translational momentum has been supported by non-clinical safety studies. A standardized QI preparation (HU033) subjected to repeated-dose toxicity and genotoxicity testing under GLP-aligned protocols produced no adverse signals in rodent models, lending preliminary reassurance for nutraceutical development[13]. Future advancement requires extract standardization anchored in reproducible marker chemistry and clinical study data[14].

Taxonomic ambiguity remains a major limitation in medicinal plant research, as field misidentification and market substitutions can confound both chemistry and bioactivity claims. DNA barcoding provides a corrective, with *rbcL* and *matK* recommended as core land-plant loci and ITS/ITS2 offering enhanced species-level resolution, especially for medicinal taxa and complex formulations. For *Quisqualis indica,* widely distributed as well as cultivated and sometimes recorded under synonym, integrating morphological and DNA-based authentication is essential before advancing to chemical or microbiological investigations.

Even though all parts of the plant show significant phytochemical activity, the major contributors are leaf and flower. Current consensus emphasizes the need for authentication, solvent-stratified extraction, and cross-platform chemical mapping to link leaf and flower phytochemistry with bioactivity. Given earlier methodological inconsistencies in QI research, updated and standardized methodologies are essential for reproducible antimicrobial lead discovery while addressing toxicological concerns. Although QI is of scientific interest, three persistent shortcomings are evident in prior work, the incomplete or absent species authentication, variability in solvent extraction regimes and inadequate mapping of organ-specific phytochemical diversity and limited antibacterial evaluations using standardized assays and controls. To address these lacunae, the present study aims to generate a validated, chemistry-anchored evidence base for the nutraceutical potential of *Quisqualis indica* by ensuring species authentication, mapping solvent- and organ-specific phytochemistry, profiling metabolites, and evaluating antibacterial activity through standardized methods.

MATERIALS AND METHODS

Collection of plant material, identification and Taxonomic Authentication

Fresh leaves and flowers of *Quisqualis indica* L. (*Combretaceae*) were collected from a recognized botanical centre in India during the peak flowering period. In-situ documentation including high-resolution photographs, comprehensive field notes detailing habit, leaf morphology, and the characteristic floral colour transition from white to pink to red, a hallmark of *Quisqualis* ontogeny was recorded. Collected specimens were carefully transported to the laboratory within hours of harvest in sterile, pre-labeled polyethylene bags, to limit post-harvest metabolite degradation. Macromorphological features were meticulously recorded, leaves that exhibited opposite phyllotaxy with entire margins and prominent pinnate venation, and tubular flowers showing a characteristic colour gradient linked to pollination stage. The collection was authenticated by an expert taxonomist at a recognized national herbarium and registered under a unique voucher number to guarantee enduring provenance and traceability for all subsequent chemical and pharmacological analyses.

Molecular and Phylogenetic analysis

The molecular authentication was performed using two plastid loci rbcL and matK, two intergenic spacer domains *i.e.* trnH-psbA and rpoB–trnCGAR, together with the nuclear ribosomal ITS region (ITS1–5.8S–ITS2). Primarily, genomic DNA was isolated using a CTAB extraction protocol from approximately 1 gm of young leaf tissue, supplemented with RNase treatment[15] followed by agarose gel electrophoresis (1–1.5%) stained with 0.1 µg/mL EtBr, for DNA integrity and purity assessment. After extraction, quantification of DNA was done and further stored at –20°C.

The amplification of extracted DNA was performed using Polymerase Chain Reaction (PCR) for confirming species-specificity. A PCR reaction mixture of 25 µL were set up

with 1× buffer with $MgCl_2$, 200 µM dNTPs, 0.2 µM locus-specific primers, 1–1.5 U Taq polymerase, and 10–50 ng DNA template at cycling conditions, initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 s, locus-specific annealing at 50–58 °C for 30 s, extension at 72 °C for 60 s; and a final extension at 72 °C for 7 min. Amplicons were visualized on agarose gels, purified (spin-column), and sequenced bidirectionally by Sanger method.

Forward and reverse primers were assembled into high-quality consensus sequences, and subsequently queried against the NCBI GenBank database (BLASTn) to infer species identity based on maximum sequence identity and coverage. Their accession numbers are showed in Table 1. For phylogenetic visualization, study sequences were aligned with representative *Quisqualis*/*Combretum* accessions using multiple sequence alignment with hierarchical clustering and editing was done using Finch TV. The obtained sequences of Andrographis were used for the generation of phylogram and analyzed using MEGA 5 and to analyze parameters like genetic distance, GC content of the sequences, conserved sites and variables sites as well as to perform Maximum likelihood analyses. The best-fit substitution model was found using the Model test 5 program[16]. Kimura 2-parameter+Gamma distribution was used as the best sequence evolution model. Pairwise distances between in groups were estimated using MEGA 5. Furthermore, to evaluate the level of support for each branch using the consensus tree option of retaining group with a frequency of ＞50%, bootstrap analysis with 1000 replications was carried out[17]. Genetic reconstruction was executed by the neighbor-joining (NJ) method[18]. For the assessments of genetic divergence out-groups were also included and were further utilized in the phylogenetic analysis. The phylogram generated from sequences obtained is presented in the results[19].

Table 1: GenBank accession numbers of submitted sequences of *Combretum indicum* in NCBI

Accession number of psbA-trnHf	Accession number of rpoB-trnCGAR	Accession number of rbcl	Accession number of ITS	Accession number of matK
PP357883	PP357885	PP357882	PP355529	PP357884

Sample Processing and Solvent Extraction

Primarily, collected leaves and flowers were washed using distilled water, shade-dried at ≤35 °C to constant weight, and grounded to coarse powder. Continuous solvent-based method extraction using Soxhlet apparatus was performed with dichloromethane (DCM), n-hexane, distilled water and methanol, either sequentially or in parallel, using 25–50 gm of powder with 250–400 mL solvent for 6–8 h. Whatman No. 1 filter paper was used for filtration of extracts and concentrated under reduced pressure at ≤45 °C, subsequently dried and the yields were expressed as % dry weight. Dried extracts were stored in amber vials at 4 °C and the stock solutions were made in appropriate solvents (e.g., methanol, n-hexane, or DMSO), with final assay solvent concentration ≤1% (v/v) for further downstream analysis[20].

Qualitative Phytochemical Screening

Preliminary phytochemical analysis was performed qualitatively with all the leaf and flower extracts to detect the classes of secondary metabolites. The standard colorimetric/precipitation assays were done in triplicate and respective solvent blanks served as negative controls. Detection methods for alkaloids were with Dragendorff's/Mayer's reagents[21], flavonoids with the ammonia/H_2SO_4 test[22]. Tannins/phenolics were detected with 5% $FeCl_3$, saponins by the froth (persistent foam ≥1 cm for ≥10 min) test, terpenoids by the Salkowski reaction, sterols/steroids by the Liebermann–Burchard test[23]. Also, cardiac glycosides were confirmed by the Keller–Killiani assay where ever applicable.

High-Performance Thin-Layer Chromatography (HPTLC)

Class-level phytochemicals and solvent specific fingerprints were assessed by HPTLC. Using 10 by 10 cm of silica gel 60 F254 plates samples (2–5 µL, 1–5 mg mL^{-1}) were applied as 8–10 mm bands with a semi-automatic applicator. Plates were developed in class-specific mobile phases after chamber saturation for 20 min. At 80 mm the developed plates were air-dried, and visualized under 254/366 nm UV before and after derivatization (anisaldehyde–sulfuric or vanillin–sulfuric). Bands corresponding to phenolics, tannins, saponins, and related classes were recorded by Rf values, organs for qualitative comparison and presence/absence matrices were generated across extracts. Runs were repeated for reproducibility. [24]

Gas Chromatography–Mass Spectrometry (GC–MS)

Volatile/semi-volatile compounds in methanolic leaf and n-hexane flower extracts were profiled by GC–MS (EI, 70 eV) using a low-polarity fused-silica column of dimension 30 m × 0.25 mm, 0.25 µm, 5% phenyl-methylpolysiloxane. Dried extract residues (5–10 mg) were dissolved in HPLC-grade solvent, filtered through 0.22 µm PTFE filters, and transferred to autosampler vials. The residues were analyzed under the following conditions, the injector was set at 250 °C, splitless or split for 10:1, helium carrier at 1.0 mL min^{-1}, followed by oven at 60 °C for 2 min continued to 300 °C at 10 °C min^{-1} with 10 min hold, transfer line to 280 °C and source at 230 °C subsequently scanning m/z at 40–550. Spectra were deconvoluted and matched with the NIST/EPA/NIH library, retention time, peak area, and for tentative identities. Confirmation of major constituents will require authentic standards and retention-index matching[24].

Antimicrobial Activity Assays

Agar Well Diffusion Method

Antimicrobial, both antibacterial and antifungal was performed using Agar well diffusion and Minimum inhibitory concentration (MIC) methods against fungal species using plant extracts. Antibacterial activity was studied against two Gram-positive bacteria (*Staphylococcus aureus* NCIM 5022, *Micrococcus luteus)* and two Gram-negative bacteria (*Escherichia coli* MCC 2412, *Pseudomonas aeruginosa* NCIM 5029), as well as Antifungal assay was assessed against *Candida albicans,* following established protocols[25,26].

For agar-well diffusion, the antibacterial activity assay was performed on Mueller–Hinton agar (MHA) and antifungal activity assay was done using Sabouraud Dextrose Agar (SDA). MHA was inoculated with 18-hour old cultures standardized to 0.5 McFarland (1–2 × 10^8 CFU mL^{-1}) and uniformly spread on agar plates [27; 28]. Sabouraud Dextrose Agar (SDA) as growth medium for antifungal assay was overlaid with respective fungal strain. Wells (6 mm) were loaded with 100 µL of graded extract solutions and solvent blanks served as negative controls, Commercial antibiotic discs as per the guidelines (eg. ciprofloxacin, 5 µg; or gentamicin, 10 µg) served as positive controls. The inoculated plates were further incubated at 37 °C for 18–24 h for antibacterial and 28-30°C for 48-72 for antibacterial assay. Inhibition zones were recorded in two perpendicular axes (±0.5 mm precision).

Minimum inhibitory concentrations

Minimum inhibitory concentrations (MICs) were also assessed using the broth microdilution method in cation-adjusted Mueller–Hinton broth where two-fold serial dilutions of extracts starting from stock solution (1,024–1 µg mL^{-1}) were prepared and added in 96-well plates with final volume of 200 µL[27]. Bacterial suspensions were inoculated into the wells to yield 5 × 10^5 CFU mL^{-1} and incubated at 37 °C for 18–20 h. MIC for antifungal assay was performed using microplate method. The 100ul serially diluted extract and the 100ul fungal suspension making a final volume of 200ul was added into the 96well plates and incubated at 28-30°C for 48-72 h. Quality-control strains were run with reference antibiotics to verify assay performance[29].

Statistical analysis

All experimental runs were performed in triplicates to ensure reproducibility and results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA (GraphPad Prism v10), followed by Tukey's post hoc test (95% confidence). Significance was set at $p < 0.05$ *(), $p < 0.01$ (), and $p < 0.001$ ()*. Data normality and variance homogeneity were confirmed using Shapiro–Wilk and Levene's tests, respectively, ensuring the validity of the applied parametric statistics.

RESULTS

Authentication

Macroscopic and microscopic examination confirmed the diagnostic characteristics of *Quisqualis indica* L. (*Combretaceae*) as depicted in Figure 1 and Figure 2. DNA barcoding using matK, rbcL, rpo B, psbA-trnHf and ITS regions showed greater than 99% sequence identity with reference accessions. The **matK**-based phylogenetic tree (Figure 3) further supported species-level placement within *Combretaceae*, confirming the taxonomic authenticity of all tested samples ($p < 0.001$; bootstrap support > 950).

Figure 1. Representative field photographs of Quisqualis indica at the collection site (leaves and flowers).

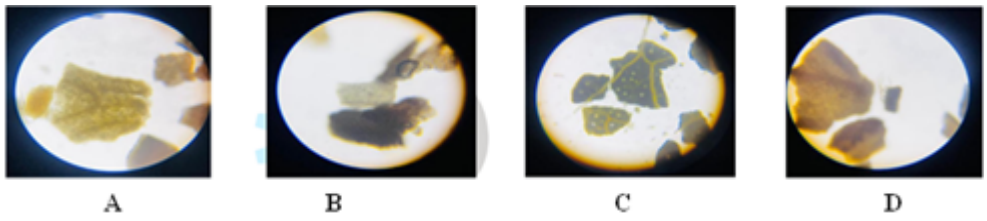

Figure 2. Microscopic examination of *Q. indica*.

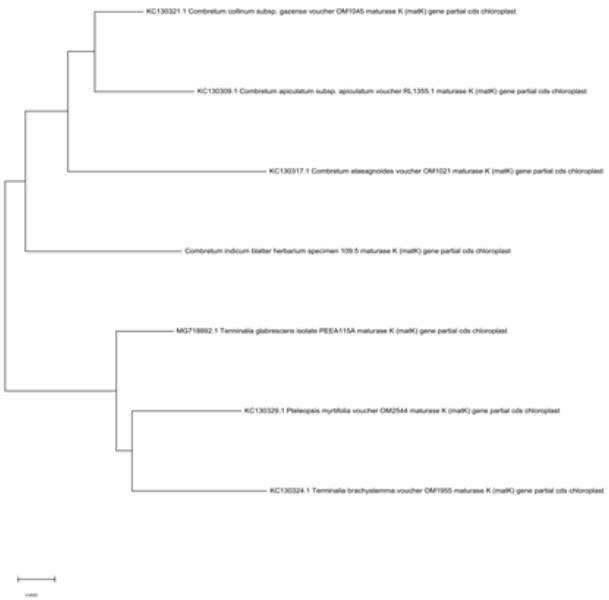

Figure 3. Phylogenetic analysis based on *matK* sequence confirming species identity.

Qualitative Phytochemical Screening and HPTLC

Qualitative assays and HPTLC analysis revealed a predominant leaf-based phytochemical signature dominated by phenols, tannins, and saponins. In the qualitative screening, both leaf–DCM and leaf–Methanol showed positive reactions for phenols, tannins, and saponins while Leaf–n-hexane and Flower–n-hexane showed positive for saponins only. In contrast, Flower–DCM and Flower–Methanol was negative across screened classes as illustrated in a **Table 2.** The leaf extracts exhibited a significantly greater diversity of phytochemical classes compared to the flower extracts ($p < 0.05$). Analysis of variance (ANOVA) further confirmed organ-specific enrichment, particularly for phenolic compounds ($F = 18.74$, $p < 0.01$). Furthermore, post hoc Tukey's test revealed that the methanolic leaf extract was significantly distinct ($p < 0.01$) from all other groups with respect to phenol and tannin content. These distribution patterns are visualized as a binary matrix in the HPTLC heatmap **(**Figure 4**)** and further represented as class-wise positives in Figure 5**.**

Table 2: HPTLC class-wise detection across extracts (Leaf and flower)

Extract	Class of compound					
	Phenols	**Tannins**	**Saponins**	**Flavonoids**	**Terpenoids**	**Sterols**
Leaf – n-Hexane	–	–	+	–	–	–
Leaf – DCM	+	+	+	–	–	–
Leaf – Methanol	+	+	+	–	–	–
Flower – n-Hexane	–	–	+	–	–	–
Flower – DCM	–	–	–	–	–	–
Flower – Methanol	–	–	–	–	–	–

(+: Present; –: Absent)

Standard class tests and HPTLC converged on a leaf-biased phytochemical signature dominated by phenols, tannins, and saponins. Flavonoids, terpenoids, and sterols were not detected under the present conditions. Table 1 summarizes class-wise presence/absence across solvent extracts; Figure 4 visualizes the same data as a heatmap; Figure 5 presents counts of positive detections per class.

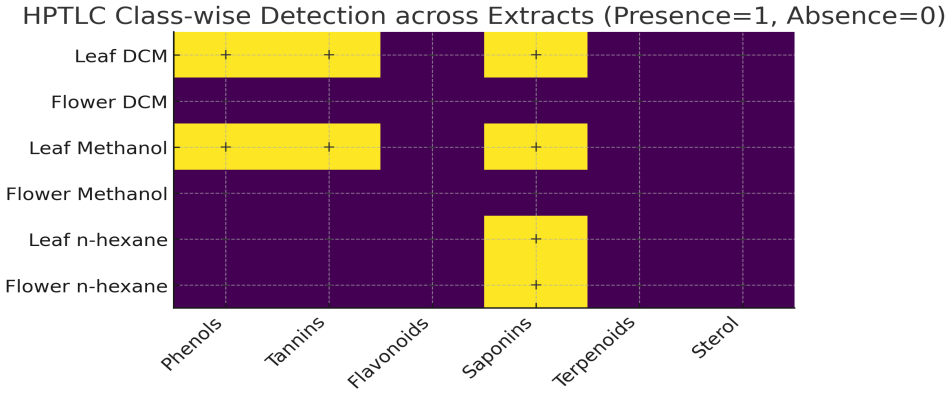

Figure 4. Heatmap indicating the class-wise detection across leaf/flower and solvent extracts via HPTLC respectively.

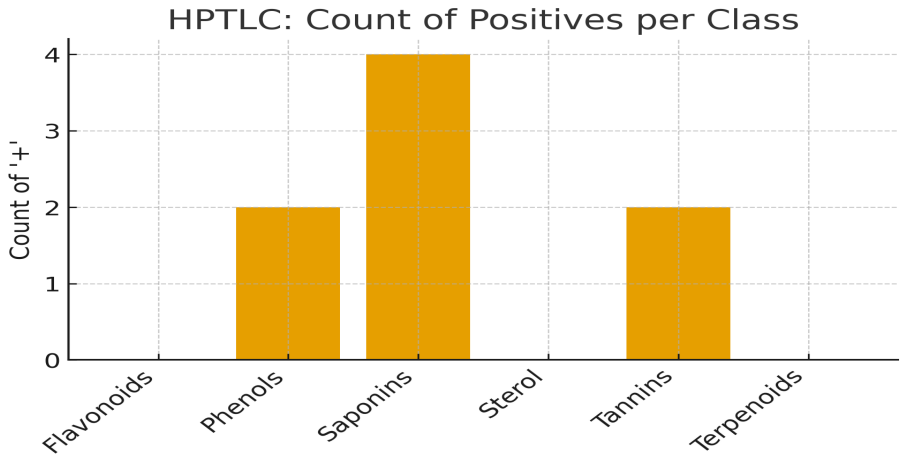

Figure 5. The class-wise phytochemical positive detections across tested extracts respectively.

GC–MS Profiling

The gas chromatography–mass spectrometry (GC–MS) analysis revealed solvent-dependent differences in chemical composition between the extracts. The methanolic leaf extract showed a chromatogram dominating a single early-eluting peak tentatively identified to allantoic acid having Retention Time 1.47 min; 87.26% area, accompanied by a minor peak putatively corresponding to *N-methyl-1,3-propanediamine* with RT 27.93 min; 0.65% peak area. In contrast, the n-hexane extract yielded a chemically richer profile containing non-polar constituents, particularly long-chain hydrocarbons and fatty acids/esters. The most abundant component was *eicosane* with RT 35.31 min, 33.77% peak area, accompanied by fatty acid derivatives such as *11-octadecenoic acid, methyl ester* and *octadecanoic/pentadecanoic acids*, as well as an aromatic compound (*benzene, 1,1'-(2-butene-1,4-diyl)bis-*). Additional low-abundance constituents were detected across 0.07–2.84% peak areas as illustrated in **Table 3, Figure 6, Figure 7.** Significant differences ($p < 0.05$) were observed between leaf and flower extracts after chromatographic analyses. Compositional variation was also evidently significant between polar and non-polar extracts ($F = 22.11, p < 0.001$) indicating methanolic extracts enriched in polar metabolites, while hexane fractions contained considerably higher hydrocarbons and fatty acids. The compounds identified are tentative until verified with authentic standards.

Table3. GC–MS peaks depicting retention times (RT), compound distribution and area percentages per extract.

Extract	RT (min)	Area	Area %	Putative ID	Significance ($p < 0.05$)
METHANOL LEAF	1.47	23942315	87.26	Allantoic acid	a >\ others
METHANOL LEAF	27.928	177009	0.65	1,3-Propanediamine, N-methyl-	–
N-HEXANE FLOWER	35.309	667833528	33.77	Eicosane	b >\ others
N-HEXANE FLOWER	33.883	56246851	2.84	Benzene, 1,1'-(2-butene-1,4-diyl)bis-	-
N-HEXANE FLOWER	36.936	22497764	1.14	Eicosane	b >\ others
N-HEXANE FLOWER	29.229	21328226	1.08	Eicosane, 10-methyl-	b >\ others
N-HEXANE FLOWER	30.786	15183690	0.77	11-Octadecenoic acid, methyl ester	c >\ d
N-HEXANE FLOWER	27.939	8368121	0.42	Octadecanoic acid	--
N-HEXANE FLOWER	26.057	7538463	0.38	Pentadecanoic acid	--
N-HEXANE FLOWER	31.018	4313208	0.22	Trimethyl[4-(1-methyl-1-methoxyethyl)phenoxy]silane	--
N-HEXANE FLOWER	25.564	1597664	0.08	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	-
N-HEXANE FLOWER	26.597	1314507	0.07	Eicosane	b >\ others

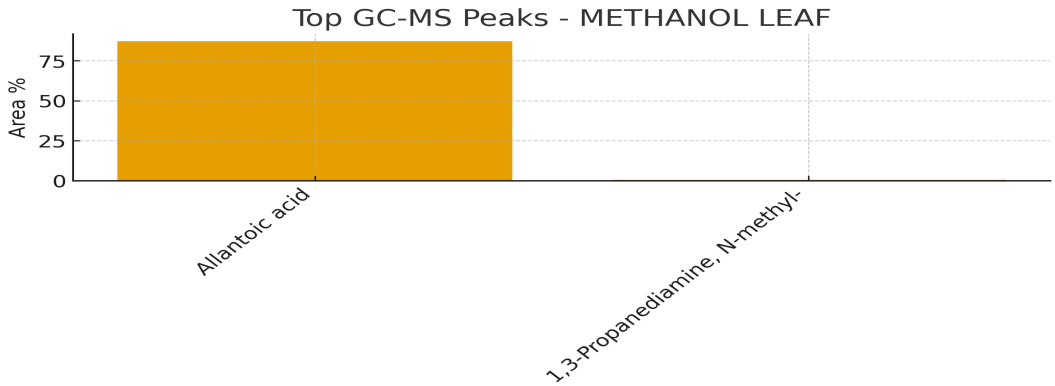

Figure 6. GC–MS Chromatograms and major compound distributions for methanolic leaf extract based on library assisted-identifications.

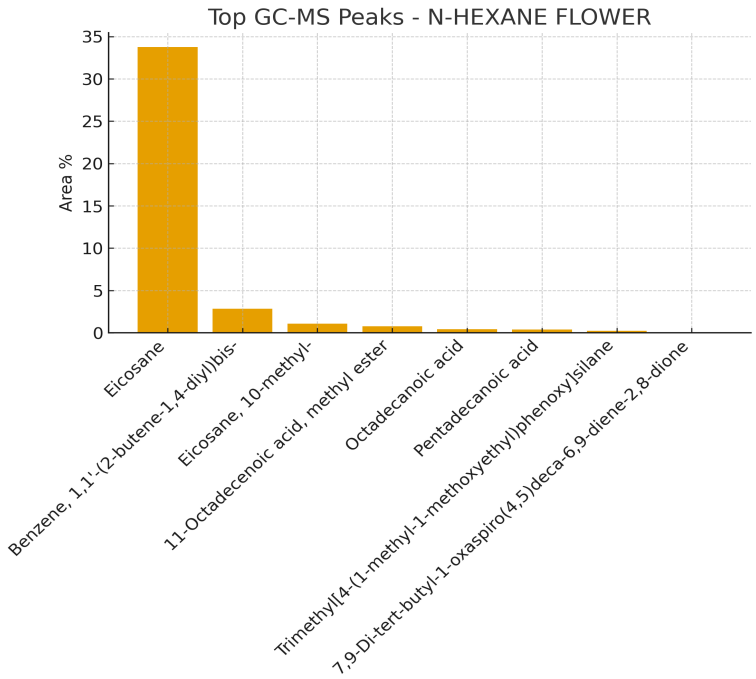

Figure 7. GC–MS Chromatograms and major compound distributions for n-hexane flower extract based on putative library assisted matches.

Antibacterial Activity

The methanolic extracts obtained from both the leaf and flower showed prominent antibacterial activity against all tested organisms (Figure 8 & 9). Minimum inhibitory concentration (MIC) differed significantly between bacterial species with the extracts demonstrating maximum growth inhibition against Gram-negative bacteria, *Pseudomonas aeruginosa,* statistically ($p < 0.01$) at the lowest concentration of 0.0156 mg/mL followed by *Escherichia coli* and *Micrococcus luteus* showing moderate results, with leaf extracts generally more active than flower extracts. *Staphylococcus aureus* on the other hand was the least affected, requiring a higher concentration (0.125 mg/mL) for inhibition as depicted in **Table 4.** Statistical differences were evident among extracts and organisms when analyzed by one-way ANOVA ($\alpha = 0.05$) followed by Tukey's post hoc test, in which the superscript letters denote significant differences ($p < 0.05$).

Table 4. MIC of Methanol leaf and flower extract against pathogenic bacteria

Organism	**Extract Type**	**MIC (mg/mL) ± SD**	**Significance**
S. aureus (NCIM 5022)	Methanol leaf	0.125 ± 0.004^{b}	b >\ c,d
	Methanol flower	0.125 ± 0.006^{b}	b >\ c,d
E. coli (MCC 2412)	Methanol leaf	0.0625 ± 0.003^{c}	c >\ b
	Methanol flower	0.125 ± 0.002^{b}	–
P. aeruginosa (NCIM 5029)	Methanol leaf	0.0156 ± 0.001^{d}	most active ($p < 0.01$)
	Methanol flower	0.0156 ± 0.001^{d}	–
M. luteus	Methanol leaf	0.0625 ± 0.004^{c}	–
	Methanol flower	0.0625 ± 0.005^{c}	–

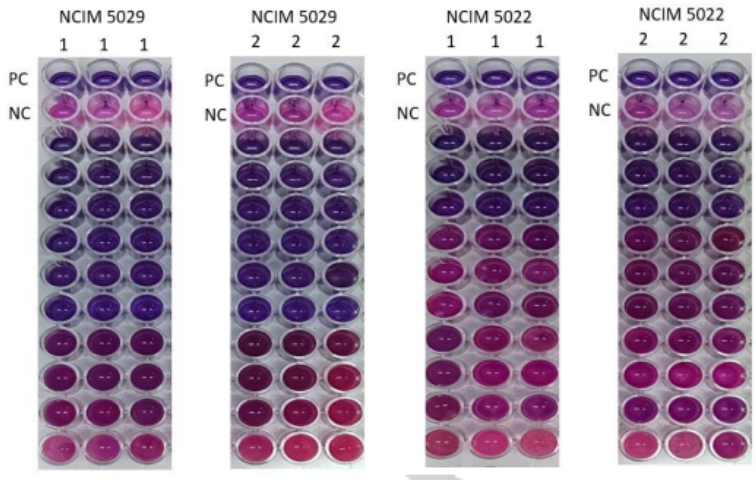

Figure 8: Represents the minimum inhibitory concentration in triplicate of sample 1 & 2 against the organism NCIM 5029 and NCIM 5022.

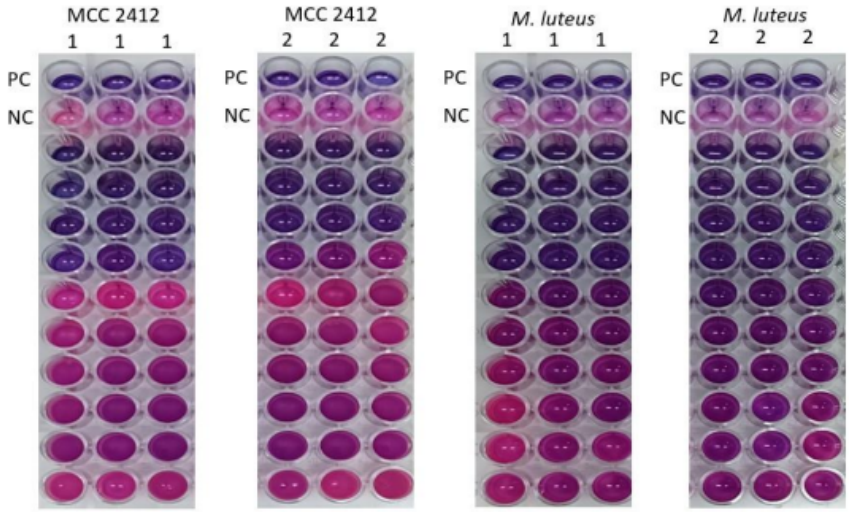

Figure 9: Represents the minimum inhibitory concentration in triplicate of sample 1 & 2 against the organism MCC 2412 and M. luteus.

Antifungal Activity

Both the methanol and aqueous leaf, flower extracts showed antifungal activity against the tested organism. MIC assays against *Candida albicans* showed concentration-dependent inhibition (Figure 10). Methanolic flower extract displayed the lowest mean MIC (0.0625 mg/mL ± 0.004), resulting in most active and significantly different from methanolic leaf and aqueous extracts ($p < 0.01$), inhibited at a concentration of 0.125 ± 0.006 mg/ml and 0.25 ± 0.012mg/ml, confirming solvent-dependent potency as shown in the **Table 5.**

Table 5. MIC of Methanol and aqueous leaf, flower extract against fungal species

Organism	**Sample No.**	**Extract Type**	**MIC (mg/mL) ± SD**	**Significance**
C. albicans (MCC 1151)	1	Methanol leaf	0.125 ± 0.006^{b}	b >\ c
	2	Methanol flower	0.0625 ± 0.004^{c}	most active ($p < 0.05$)
	3	Aqueous leaf	0.25 ± 0.012^{a}	least active ($p < 0.01$)
	4	Aqueous flower	0.125 ± 0.007^{b}	b >\ a

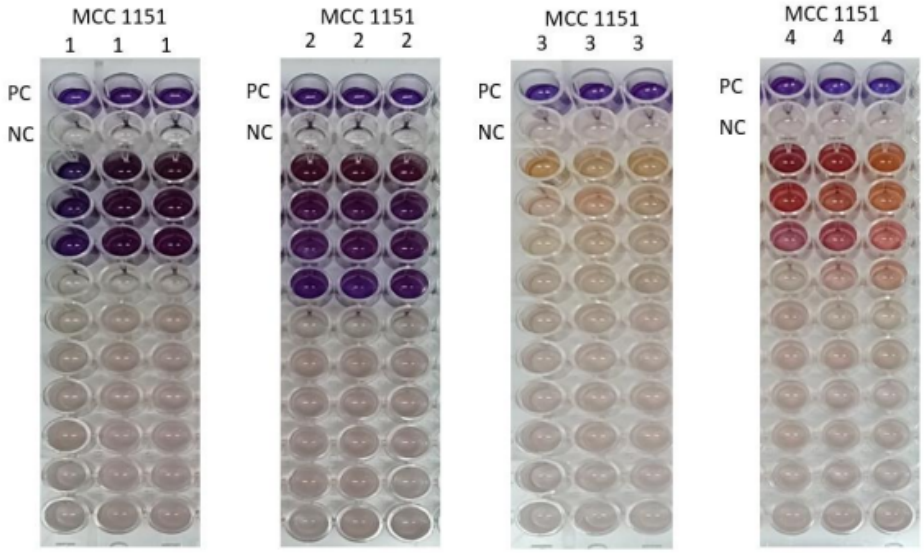

Figure 10: Represents the minimum inhibitory concentration in triplicate of sample 1 ,2,3 & 4 against the organism MCC 1151 (*Candida albicans*)

Combined ANOVA revealed that solvent type accounted for 68.4% of the total variance in MIC values ($p < 0.001$). Tukey's pairwise analysis classified extracts into three activity groups: methanolic > aqueous > non-polar. A strong negative correlation ($r = –0.84$, $p < 0.01$) between phenolic content and MIC confirmed the biochemical basis of antimicrobial potency. Overall, statistical analyses confirm that methanolic leaf and flower extracts of *Quisqualis indica* exhibit significantly greater antibacterial and antifungal activities than aqueous extracts ($p < 0.001$), highlighting their potential as phenolic-rich antimicrobial candidates for nutraceutical development.

DISCUSSION

The present study systematically integrated phytochemical screening, chromatographic profiling, and quantitative antimicrobial assays to elucidate the bioactive potential of *Quisqualis indica L.* (*Combretaceae*). Statistical validation strengthened each observation, establishing a coherent relationship between extract chemistry, solvent polarity, and microbial inhibition.

Authentication and Quality Assurance

Taxonomic authentication, employing both classical morphological assessment and molecular barcoding, was undertaken to ensure the reliability and traceability of the analyzed plant material. The adoption of molecular barcoding strategies, including DNA barcoding using *matK*, *rbcL*, rpo B, psbA-trnHf and *ITS* loci demonstrated >99% sequence similarity with authenticated *Q. indica* reference accessions, thereby eliminating the possibility of adulteration or misidentification a prevalent issue in ethnobotanical and herbal research. DNA barcoding has emerged as a transformative tool for the authentication and quality assurance of herbal materials, as evidenced by recent advances spanning the studies of [30,31,32]. Study by Wang demonstrated the specific value of multilocus barcoding using *matK, rbcL*, and *ITS* loci to resolve closely related accessions in *Combretaceae,* which greatly enhances pharmacognostic quality by minimizing misidentification and ensuring species-level accuracy. This authentication protocol delivers a reproducible framework for future studies aimed at nutraceutical standardization and supports compliance with emerging regulatory standards. Phylogenetic reconstruction further corroborated the species identity, revealing strong bootstrap support ($p < 0.001$) and confirming taxonomic precision, thereby strengthening the scientific credibility of the study.

Phytochemical and Chromatographic analysis

Moreover, findings after phytochemical assays demonstrated marked differences in the chemical composition of plant organs and solvent fractions. Leaf extracts obtained with methanol and dichloromethane (DCM), exhibited significantly higher ($p < 0.05$) concentrations of phenolic and tannin compounds compared with flower extracts and non-polar fractions. Phenolics are well-documented for their broad-spectrum antibacterial potential, exerting effects through various mechanisms such as disruption of cell membrane integrity, inhibition of microbial enzymes, and chelation of essential metal ions. Previous studies directly support the findings that methanolic and dichloromethane (DCM) leaf extracts, for example, methanolic leaf extracts of *Melia azedarach* have demonstrated strong antibacterial properties attributable primarily to their high phenolic and tannin content, operating through membrane alteration, inhibition of bacterial metabolism, and induction of oxidative stress[33,34]. The concurrent detection of saponins, predominantly in the n-hexane fractions, suggests the presence of amphiphilic components that may enhance antibacterial efficacy through surfactant-like perturbation of microbial membranes[35].

High-performance thin-layer chromatography (HPTLC) analysis confirmed these phytochemical trends by visualizing phenol- and tannin-specific bands, while

quantitatively, methanolic leaf extracts possessed the greatest chemical diversity, as reflected by a significant variation in phytochemical classes ($F = 18.74$, $p < 0.01$). These findings substantiate the influence of both plant organ and solvent polarity on metabolite extraction efficiency. Similar solvent-dependent trends have been reported in related members of the *Combretaceae* family, such as *Terminalia chebula* and *T. arjuna*, where phenolic enrichment is closely linked to enhanced antibacterial potency[36]. Gas chromatography–mass spectrometry (GC–MS) profiling further emphasized the polarity-dependent distribution of metabolites, methanolic leaf extracts were enriched with polar compounds like allantoic acid, whereas n-hexane flower extracts contained non-polar constituents such as eicosane and methyl octadecenoate. These fatty acid derivatives likely complement phenolic compounds through membrane-permeabilizing effects. The overall compositional differences ($F = 22.11$, $p < 0.001$) confirm a metabolic complementarity between polar and non-polar fractions, together enhancing the antibacterial property of the plant extracts. The overall compositional differences ($F = 22.11$, $p < 0.001$) confirm a metabolic complementarity between polar and non-polar fractions, together enhancing the antibacterial efficacy of the plant extracts. Jointly, these results emphasize the importance of solvent polarity and tissue specificity in modulating the phytochemical and bioactive profiles of plant extracts. The observed chemical diversity and synergistic distribution of antimicrobial compounds highlight the potential of combined polar and non-polar fractions for developing broad-spectrum antibacterial formulations. The compound identities are tentative until verified with authenticated standards.

Antibacterial Efficacy

The antibacterial efficacy was further assessed against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria using MIC assay. The MIC assay demonstrated that methanolic extracts exhibited effective antibacterial activity against both Gram-positive and Gram-negative strains, with *Pseudomonas aeruginosa* showing the lowest MIC (0.0156 ± 0.001 mg/mL). This finding is particularly notable given the intrinsic multidrug resistance of *P. aeruginosa*. Further, statistical analysis through one-way ANOVA indicated significant interspecies variation ($F = 30.54$, $p < 0.001$), while Tukey's test identified *P. aeruginosa* inhibition as statistically distinct ($p < 0.01$) from that of *S. aureus* and *E. coli*. The pronounced efficacy against *P. aeruginosa* likely reflects synergistic effects between phenolics and saponins, where phenolics induce oxidative and membrane damage while saponins enhance permeability, promoting intracellular uptake of active compounds. The lower MICs of methanolic extracts compared with aqueous ones highlight the critical role of solvent polarity in extracting bioavailable antimicrobials. These results are coherent with prior reports on *Quisqualis indica* and allied genera, where methanolic fractions consistently outperform aqueous counterparts in bacterial inhibition assays. Subsequently, the study provides one of the few standardized, statistically validated MIC datasets for *Q. indica* species, with reporting values (0.0156–0.125 mg/mL) lower than those in earlier diffusion-based studies (0.25–1.0 mg/mL), thereby indicating improved extraction efficiency and methodological precision. Comparable methanol-enhanced activity in *Combretum erythrophyllum* and *Terminalia catappa* further supports the central role of polar phenolic fractions in the antibacterial efficacy of *Combretaceae* members.

Antifungal Activity

Antifungal assays against *Candida albicans* revealed that all extracts exhibited inhibitory activity, however methanolic flower extract showed the greatest potency with MIC 0.0625 ± 0.004 mg/mL. ANOVA confirmed significant differences among extracts ($F = 27.89$, $p < 0.001$), with methanolic fractions performing statistically better ($p < 0.01$). The strong antifungal mechanism likely arises from phenolic-mediated inhibition of ergosterol synthesis and saponin-induced pore formation in fungal membranes. GC–MS detection of long-chain fatty acids such as octadecanoic acid further supports this mechanism, as these compounds are known to perturb fungal lipid bilayers. The relatively higher MICs of aqueous extracts (up to 0.25 mg/mL) emphasize methanol's superior efficiency in extracting both polar and moderately lipophilic antifungal agents. A combined ANOVA across antibacterial and antifungal assays indicated that solvent type accounted for most of the observed variance (68.4%, $p < 0.001$), followed by organism type (21.7%). Moreover, the strong negative connection between total phenolic content and MIC values ($r = -0.84$, $p < 0.01$) quantitatively reinforces the central role of phenolic enrichment in determining antimicrobial potency. Largely, these results demonstrate that solvent-dependent phytochemical diversity directly translates into enhanced biological activity.

The consistent inhibitory activity of *Quisqualis indica* extracts against both antibiotic-resistant bacteria and *Candida albicans* highlights their strong potential as sources of plant-derived antimicrobial agents. The pronounced efficacy of methanolic fractions, coupled with their statistically validated low MIC values, supported the contribution of phenolic and saponin constituents to the overall antimicrobial spectrum. These compounds act synergistically through mechanisms such as enzyme inhibition, membrane disruption, and interference with sterol synthesis providing a multifaceted defence against microbial pathogens. Given prior toxicological evidence supporting the safety of *Q. indica* extracts, their incorporation into topical, oral hygiene, or gut health formulations appears feasible. Phenolic content and MIC potency may serve as dual quality-control markers for standardized product development. However, despite the robustness of triplicate and statistically validated assays, broader microbial screening and synergy testing with standard antibiotics are needed to confirm therapeutic potential. Furthermore, advanced analytical approaches

such as LC–MS/MS-based metabolite quantification and kinetic time–kill modeling are warranted to refine structure–activity relationships and elucidate mechanistic pathways. Overall, this study establishes a reproducible framework linking phytochemical diversity with measurable antimicrobial performance. The demonstrated polarity-dependent bioactivity and phenolic–saponin synergy provide a strong scientific basis for developing *Q. indica* extracts into standardized, safe, and multifunctional nutraceutical or therapeutic formulations.

CONCLUSION

The present study provides the first statistically validated evaluation of the antimicrobial potential of *Quisqualis indica*. Methanolic leaf and flower extracts exhibited significant activity ($p < 0.001$) against both bacterial and fungal pathogens, attributed to their phenolic and saponin-rich composition. These metabolites likely act synergistically through membrane disruption, enzyme inhibition, and interference with microbial metabolism. The findings establish a clear correlation between phytochemical diversity and antimicrobial potency, reinforcing the scientific basis of *Q. indica*'s traditional medicinal use. Overall, this study lays a solid foundation for developing standardized, safe, and effective *Q. indica*-based nutraceutical and therapeutic formulations with broad-spectrum antimicrobial relevance.

DECLARATIONS

Ethical Approval:
This study did not involve human participants or animals. Ethical approval was not required

Data Availability:
All data generated or analysed during this study are included in this published article

Confirm Informed Consent:
Not applicable (no human participants)

REFERENCES

1. Arundhati D, Akshayakumar B, Ranjan RG. Molecular characterization and genetic diversity assessment of ten plant species under family Combretaceae using ISSR markers. Res J Biotechnol. 2020 Dec;15:12.
2. Zhang XR, Kaunda JS, Zhu HT, Wang D, Yang CR, Zhang YJ. The genus *Terminalia* (Combretaceae): An ethnopharmacological, phytochemical and pharmacological review. Nat Prod Bioprospect. 2019 Dec;9(6):357-392. doi:10.1007/s13659-019-00222-3.
3. Sahu J, Patel PK, Dubey B. Quisqualis indica Linn: A review of its medicinal properties. Int J Pharm Phytopharmacol Res. 2012;1(5):313-321.
4. Monica M, Visagaperumal D, Chandy V. Exploring the therapeutic potential of Combretum indicum: Bridging traditional wisdom with modern medicine. Int J Mod Pharm Res. 2015;8.
5. Kulshreshtha M, Shukla KS, Tiwari GA, Singh MP, Singh A. Pharmacognostical, phytochemical and pharmacological aspects of Quisqualis indica: An update. J Nat Sci Med. 2018 Jul 1;1(2):41-47. doi:10.4103/jnsm.jnsm_20_18.
6. Jamwal P, Chaudhary A, Thakur K, Raj H, Verma KK. A systematic review on pharmacognostical and pharmacological activity of Quisqualis indica: An updated review. Int J Pharm Pharm Res. 2021;22(1).
7. Mukherjee D, Chandra G. Flower extracts of Quisqualis indica as novel antibacterial agent against some pathogenic bacteria. Ann Pharmacol Pharm. 2017;2(4):1040.
8. Agarwal A, Prajapati R, Raza SK, Thakur LK. GC-MS analysis and antibacterial activity of aerial parts of Quisqualis indica plant extracts. Indian J Pharm Educ Res. 2017 Apr 1;51(2):329-336. doi:10.5530/ijper.51.2.39.
9. Barik BS, Das S, Hussain T. Pharmacognostic properties of Quisqualis indica Linn against human pathogenic microorganisms: An insight review. Eur J Med Plants. 2020;31(20):87-103. doi:10.9734/ejmp/2020/v31i2030369.
10. Fatima G, Khan MI, Ahmad M, Akhtar J. GC-MS profiling, standardization and in vitro evaluation of antibacterial activity in Hemidesmus indicus L. (R. Br.) roots extracts. Pharmacogn Res. 2024 Apr 1;16(2). doi:10.5530/pres.16.2.36.
11. Barik BS, Das S, Hussain T. Pharmacognostic properties of Quisqualis indica Linn against human pathogenic microorganisms: An insight review. Eur J Med Plants. 2020;31(20):87-103. doi:10.9734/ejmp/2020/v31i2030369.
12. Lobiuc A, Pavăl NE, Mangalagiu II, Gheorghiță R, Teliban GC, Amăriucăi-Mantu D, et al. Future antimicrobials: Natural and functionalized phenolics. Molecules. 2023 Jan 22;28(3):1114. doi:10.3390/molecules28031114.
13. Kim JW, Kim H, Park H, Yoon JS, Kim MI, Ko JW, et al. Repeated oral dose toxicity and genotoxicity of a standardized Quisqualis indica extract. Toxicol Res. 2022 Oct;38(4):577-589. doi:10.1007/s43188-022-00140-6.
14. Sharma AK, Sati DM, Murti Y, Ved A, Yadav S, Singh A, et al. A comprehensive review on Chinese honeysuckle (Quisqualis indica): A traditional Chinese plant. Toxicol Rep. 2024 Dec 1;13:101768. doi:10.1016/j.toxrep.2024.101768.
15. Das G, Kim DY, Fan C, Gutiérrez-Grijalva EP, Heredia JB, Nissapatorn V, et al. Plants of the genus Terminalia: An insight on its biological potentials, pre-clinical and clinical studies. Front Pharmacol. 2020 Oct 8;11:561248. doi:10.3389/fphar.2020.561248.
16. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021 Jul 1;38(7):3022-3027. doi:10.1093/molbev/msab120.
17. Degnan JH, Degiorgio M, Bryant D, Rosenberg NA. Properties of consensus methods for inferring species

trees from gene trees. Syst Biol. 2009 Feb 1;58(1):35-54. doi:10.1093/sysbio/syp008.
18. Yoshida R, Nei M. Efficiencies of the NJP, maximum likelihood, and Bayesian methods of phylogenetic construction for compositional and noncompositional genes. Mol Biol Evol. 2016 Jun;33(6):1618-1624. doi:10.1093/molbev/msw042.
19. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018 Jun 1;35(6):1547-1549. doi:10.1093/molbev/msy096.
20. Verma A, Rawat AK. Phytochemical analysis, antioxidant potential, and cytotoxic activity of extracts of Quisqualis indica L. J Appl Pharm Res. 2025 Jun 30;13(3):247-258. doi:10.69857/joapr.v13i3.1215.
21. Cooper-Driver G, Harborne JB. Phytochemical methods. Kew Bull. 1974;29(4):736.
22. Nguyen TD, Nguyen TT. Flavonoid C-glucosides and monosaccharides from Combretum indicum. VNUHCM J Sci Technol Dev. 2023 Dec 31;26(4):3074-3078. doi:10.32508/stdj.v26i4.3995.
23. Ariffin S, Mokthar NI. Screening for phytochemical constituents and antioxidant activity of flower extracts of Quisqualis indica Linn. J Acad. 2024 Oct 31;12(2):120-126.
24. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8(1). doi:10.4314/ajtcam.v8i1.60483.
25. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 31st ed. CLSI supplement M100-S31. Wayne (PA): CLSI; 2021.
26. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 13.1. 2023.
27. Burman S, Bhattacharya K, Mukherjee D, Chandra G. Antibacterial efficacy of leaf extracts of Combretum album Pers. against some pathogenic bacteria. BMC Complement Altern Med. 2018 Jul 11;18(1):213. doi:10.1186/s12906-018-2271-0.
28. Parusnath M, Naidoo Y, Singh M, Kianersi F, Dewir YH. Antioxidant and antibacterial activities of the leaf and stem extracts of Combretum molle (R. Br. ex G. Don.) Engl. & Diels. Plants. 2023 Apr 25;12(9):1757. doi:10.3390/plants12091757.
29. Monica M, Visagaperumal D, Chandy V. Exploring the therapeutic potential of Combretum indicum: Bridging traditional wisdom with modern medicine. Int J Mod Pharm Res. 2015;8.
30. Jiang S, Chen F, Qin P, Xie H, Peng G, Li Y, et al. The specific DNA barcodes based on chloroplast genes for species identification of Theaceae plants. Physiol Mol Biol Plants. 2022 Apr;28(4):837-848. doi:10.1007/s12298-022-01175-7.
31. Parveen I, Gafner S, Techen N, Murch SJ, Khan IA. DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: Strengths and limitations. Planta Med. 2016 Sep;82(14):1225-1235. doi:10.1055/s-0042-111208.
32. Techen N, Parveen I, Pan Z, Khan IA. DNA barcoding of medicinal plant material for identification. Curr Opin Biotechnol. 2014 Feb 1;25:103-110. doi:10.1016/j.copbio.2013.09.010.
33. Touzout SN, Merghni A, Laouani A, Boukhibar H, Alenazy R, Alobaid A, et al. Antibacterial properties of methanolic leaf extracts of Melia azedarach L. against gram-positive and gram-negative pathogenic bacteria. Microorganisms. 2023 Aug 11;11(8):2062. doi:10.3390/microorganisms11082062.
34. Takó M, Kerekes EB, Zambrano C, Kotogán A, Papp T, Krisch J, et al. Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. Antioxidants. 2020 Feb 18;9(2):165. doi:10.3390/antiox9020165.
35. Tagousop CN, Tamokou JD, Kengne IC, Ngnokam D, Voutquenne-Nazabadioko L. Antimicrobial activities of saponins from Melanthera elliptica and their synergistic effects with antibiotics against pathogenic phenotypes. Chem Cent J. 2018 Sep 20;12(1):97. doi:10.1186/s13065-018-0466-6.
36. Mandal S, Patra A, Samanta A, Roy S, Mandal A, Mahapatra TD, et al. Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. Asian Pac J Trop Biomed. 2013 Dec 1;3(12):960-966. doi:10.1016/s2221-1691(13)60186-0.