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QUANTIFICATION OF QUININE IN MEDICINAL PLANTS FROM EDE, NIGERIA USING HPLC

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ABSTRACT

Nigeria, a West African country, is rich in medicinal plants traditionally used to manage various ailments. Quinine, a well-established antimalarial drug, has been widely used as a chemotherapeutic agent for treating malaria. This study aimed to develop and validate a rapid, simple, accurate and precise high-performance liquid chromatography (HPLC) method for the quantitatively determine the quinine content in three selected medicinal plants – *Azadirachtai ndica* (neem), *Citrus aurantii folia* (lime), and *Mangifera indica* (mango). Cold extraction method with vortex agitator was employed to obtain plant extracts. The separation and quantification of quinine were performed using an Agilent 1260 Infinity HPLC system equipped with a diode-array detector (DAD). Separation of quinine from extract was achieved on a Phenomenex Gemini C18 column in less than 2 minutes using an isocratic mobile phase of 30% acetonitrile and 70% double-distilled water and a flow rate of 1.0 mL/min at 254 nm. The standard calibration curve obtained was found to be linear R^2 = 0.9975. The analytical figures of merits were also evaluated for the percentage extraction recovery of quinine was 99.5%, the intraday precision as percent relative standard deviation was 1.14, it proved excellent % RSD which is less than 2 and of limits of detection (LOD) and limits of quantification (LOQ) were found to be 0.14µg/mL and 0.42µg/mL respectively. This study showed that, with the use of HPLC, quinine levels in these plants were accurately measured, thus, the method used was highly reliable, with excellent accuracy and precision. This study helps confirm whether these plants contain quinine and could contribute to traditional malaria treatments, supporting their potential medicinal value.

Keywords: Medicinal plants, Quinine, High-performance liquid chromatography, Malaria, Nigeria

INTRODUCTION

Quinine, like many other antimalarial drugs, is a synthetic aromatic compound widely used as an antipyretic, antiarrhythmic, and antimalarial agent. Historically, quinine has been employed as a mild analgesic and an effective chemotherapeutic agent for treating malaria symptoms (Cheilane *et al.*, 2021). The chemical structure of quinine is shown in Figure 1.

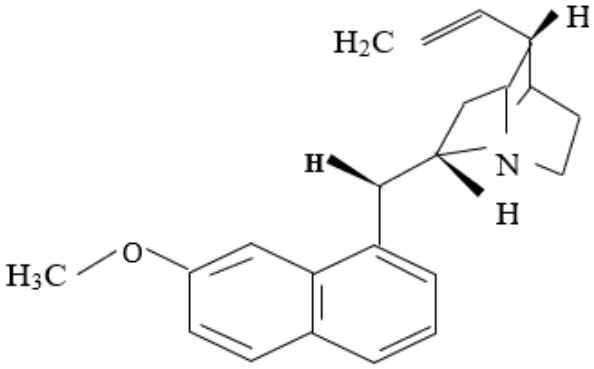

Figure 1: The structure of quinine

Salisu, (2018) reported that malaria is a life-threatening disease caused by protozoan parasites of the *Plasmodium* genus, transmitted through the bite of infected female Anopheles mosquitoes. The disease remains a major public health challenge, particularly in Asia, sub-Saharan Africa, and Nigeria, where malaria incidence is among the highest globally. In Nigeria, malaria accounted for approximately 26.6% of reported cases in 2021 (WHO, 2022; Giovane de Jesus Gomes Ribeiro *et al.*, 2023). Young children, the elderly and immune-compromised individuals are particularly vulnerable to the disease. Among the five Plasmodium species known to infect humans, *Plasmodium falciparum* is responsible for the majority of malaria-related deaths, while *Plasmodium vivax* and *Plasmodium knowlesi* can also cause severe infections. *Plasmodium ovale* and *Plasmodium. malariae* generally lead to milder forms of the disease (Rovira-Vallbona *et al.*, 2020).

For centuries, synthetic quinine has been the standard treatment for malaria, available in both oral and intravenous formulations. Recently, Walter and John (2022) described quinine as a highly effective and relatively non-toxic treatment for malaria. However, traditional medicine remains widely practiced in Nigeria, particularly in rural communities, where medicinal plants play a crucial role in healthcare. Several studies (Mainasara*et al.*, 2011) have investigated the efficacy of plants used by indigenous healers, providing scientific validation for their medicinal properties.

Plants are valuable sources of medicine for household particularly in rural communities of Nigeria (Salisu, 2018). In Ede, Osun State, Nigeria, neem (*Azadirachta indica*), lime (*Citrus aurantii folia*), and mango (*Mangifera indica*) are among the most commonly used plants in traditional medicine. These plants are valued for their multifunctional medicinal properties and are widely utilized for their potential antimalarial effects. In many African and Asian countries, up to 80% of the population relies on herbal medicine for primary healthcare needs (Oyebode*et al.,* 2016). According to a literature survey, approximately 25% of modern pharmaceuticals are derived from plant-based sources (Mittal *et al.,* 2014). Integrating traditional knowledge with modern scientific techniques presents a promising approach for developing novel antimalarial and antimicrobial drugs (Yuan *et al.,* 2016).

Given the increasing interest in natural sources of quinine, the need for accurate and efficient analytical methods for its detection has become essential. Plant extracts contain a diverse range of phytochemicals, making quinine analysis particularly challenging due to stereoisomeric complexity and matrix interferences. This study aims to quantitatively determine quinine in three medicinal plant extracts commonly used in Ede, Osun State, Nigeria, using high-performance liquid chromatography (HPLC). HPLC is widely recognized for its selectivity, sensitivity, and efficiency in detecting bioactive compounds at low concentrations (Costa *et al.,* 2015). The technique provides a reliable, rapid, and precise method for quinine quantification, facilitating further research into the therapeutic potential of these medicinal plants.

MATERIALS AND METHODS

Chemicals and Reagents

Analytical-grade reagents and HPLC-grade solvents were used throughout this study. Hydrochloric acid, n-hexane, and methanol were obtained from Sigma-Aldrich (USA), while acetonitrile was supplied by J.T. Baker (Netherlands). The quinine standard was purchased from Rochelle Chemicals (Johannesburg, South Africa). Teflon membrane filters (0.45 µm) were obtained from Restek (USA), and double-distilled water was prepared using a Millipore purification system (Millipore, France). A Phenomenex Synergi C18 column was used for chromatographic separation.

Instrumentation and Chromatographic Conditions

Quinine detection and quantification were performed using an Agilent Technologies 1260 Infinity Series HPLC system (Mainz, Germany). The system consisted of:

i. A G1312C binary pump,
ii. A degasser,
iii. A manual injector (Agilent S6020) with a 20 µL loop,
iv. A UV-Vis diode array detector (DAD), and
v. A G1316A column thermostat set at 25°C.

Chromatographic separation was carried out on a Phenomenex Synergi C18 column (150 × 4.6 mm, 4 µm particle size). The mobile phase consisted of 30% acetonitrile (solvent A) and 70% double-distilled water (solvent B), with an isocratic flow rate of 1 mL/min. The injection volume was 20 µL, and detection was performed at 254 nm, corresponding to quinine's maximum absorbance. HPLC analysis was conducted at the Central Science Laboratory, Obafemi Awolowo University (O.A.U.), Ile-Ife, Nigeria.

Wavelength Selection

The maximum absorption wavelength for quinine was determined by scanning solutions over a 200–800 nm range. The highest absorbance for quinine was observed at 254 nm, which was selected for HPLC analysis.

Preparation of Standard Solutions

A quinine stock solution (1 mg/mL) was prepared in double-distilled water. Serial dilutions were then made to obtain working standard solutions at concentrations of 1, 2, 5, 10, 15, and 20 µg/mL. The absorbance of each dilution was measured at 254 nm using a 10 mm quartz cuvette, and a calibration curve was constructed by plotting absorbance against concentration.

Sample Collection and Preparation

Three medicinal plant samples—neem (*Azadirachta indica*), lime (*Citrus aurantii folia*), and mango (*Mangifera indica*) - were collected from the botanical garden of Federal Polytechnic, Ede (North Campus).

Fresh, healthy, and mature leaves were:

i. Plucked, sorted, and washed with distilled water to remove dust and impurities.
ii. Air-dried in a laboratory setting for several days.
iii. Crushed into fine powder using an electric blender, ensuring thorough cleaning between samples to prevent cross-contamination.
iv. Sieved and stored in airtight containers for further analysis.

Sample Identification

The collected plant samples were botanically identified by Dr. Israel Ogunsumi, a botanist from the Department of Biological Sciences, Federal Polytechnic Ede, Nigeria. The local names of the plants are:

i. Neem (*Azadirachta indica*) – "Dongoyaro",
ii. Lime (*Citrus*aurantii *folia*) – "Osan-wewe",
iii. Mango (*Mangifera indica*) – "Mangoro".

Preparation of Sample Extracts

For each plant sample:

i. 0.50 g of powdered material was accurately weighed and transferred into a 50 mL centrifuge tube.
ii. 8 mL of methanolic acid was added, and the mixture was vortex-agitated for 10 minutes.
iii. The sample was then centrifuged at 400 rpm for 10 minutes, and the supernatant was collected.
iv. Filtration was carried out using Whatman No. 1 filter paper to remove larger suspended particles.
v. The extraction process was repeated twice, and the two filtrates were combined.
vi. 1 mL of n-hexane was added to the combined filtrate to remove lipids, carbohydrates, and proteins, which could interfere with HPLC detection.
vii. The sample was vortexed for 5 minutes, then centrifuged at 200 rpm for 5 minutes.
viii. The resulting supernatant was filtered through a 0.45 µm Teflon membrane filter.
ix. To concentrate the extract, it was placed in a water bath, reducing the volume to one-third while evaporating n-hexane.
x. The purified extract was transferred into a sealed bottle and stored in a laboratory refrigerator before HPLC analysis.
xi. The procedure was repeated for all selected medicinal plant samples.

Method Validation

To assess the accuracy, precision, and matrix effects of the developed HPLC method, a spike-and-recovery experimentwas conducted. The methodology was adapted from Abibu *et al*. (2019) with slight modifications.

i. A control standard solution was prepared by dissolving 100 µg of quininein900 mL ofdouble-distilled water, yielding a 1000 µg/mL standard solution.
ii. The solution was injected into the HPLC system, and its chromatographic peak area was recorded.
iii. To evaluate recovery, quinine standard solution was spiked into powdered plant samples, followed by extraction as described in Section 2.6.
iv. The extracted solution was analyzed, and its chromatographic peak area was compared to the control standard solution.
v. The percentage recovery was calculated using the formula:

$$\text{Recovery (\%)} = \frac{\text{Peak area of quinine spiked before extraction}}{\text{Peak area of control quinine standard without matrices}} \times 100 \quad (1)$$

Additionally, linearity was evaluated by constructing a calibration curve, and plotting concentration against peak area.

Detection and Quantification Limits

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the signal-to-noise (S/N) ratio method, where S/N = 3 for LOD and S/N = 10 for LOQ. Calculations followed ICH guidelines, using the formulas:

$$\text{LOD} = 3.3 \times \frac{Sy}{S} \quad (2)$$

$$\text{LOQ} = 10 \times \frac{Sy}{S} \quad (3)$$

Where Sy is the standard deviation of the response of the curve and S is the slope of the calibration curve.

Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel (Version 19).

RESULTS AND DISCUSSION

Determination of Quinine by RP-HPLC

In this study, a simple, rapid, and practical RP-HPLC method was developed to accurately quantify free quinine in neem, lime, and mango leaf extracts. This method ensures reliable detection and measurement of quinine in these plant samples. The key parameters and specifications of the developed method are summarized in Table 1.

Table 1: Developed method parameters

Parameters	Specifications
Matrices	Neem, lime and mango leaf extracts
Analyte	Quinine
Mobile phase composition	30% acetonitrile & 70% doubled distilled water
Flow rate	1mL/minute
Column	Silica C18, 150 × 4.6, 4 µm particle size Phenomenex

The qualitative analysis was carried out by matching retention times in the chromatogram of the quinine standard solution Figure 2 with the chromatogram of spiked mango leaves extract Figure 3 in a single base line RP-HPLC run while Figure 4 shows poor chromatogram of lime leaf extracts respectively.

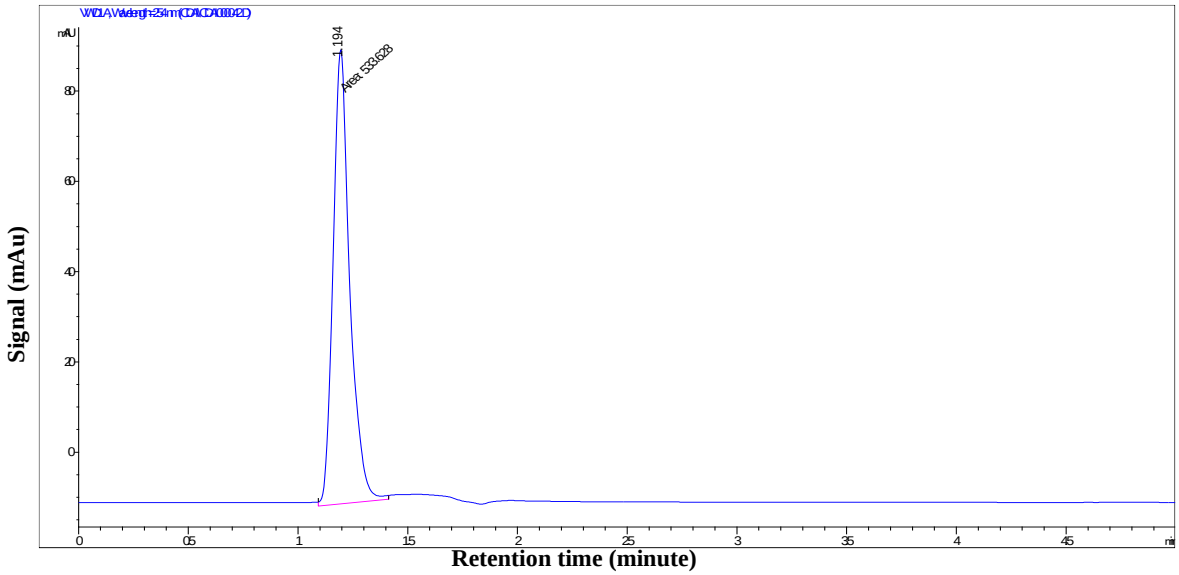

Figure 2: Representative chromatogram of quinine standard- 2 ug/mL

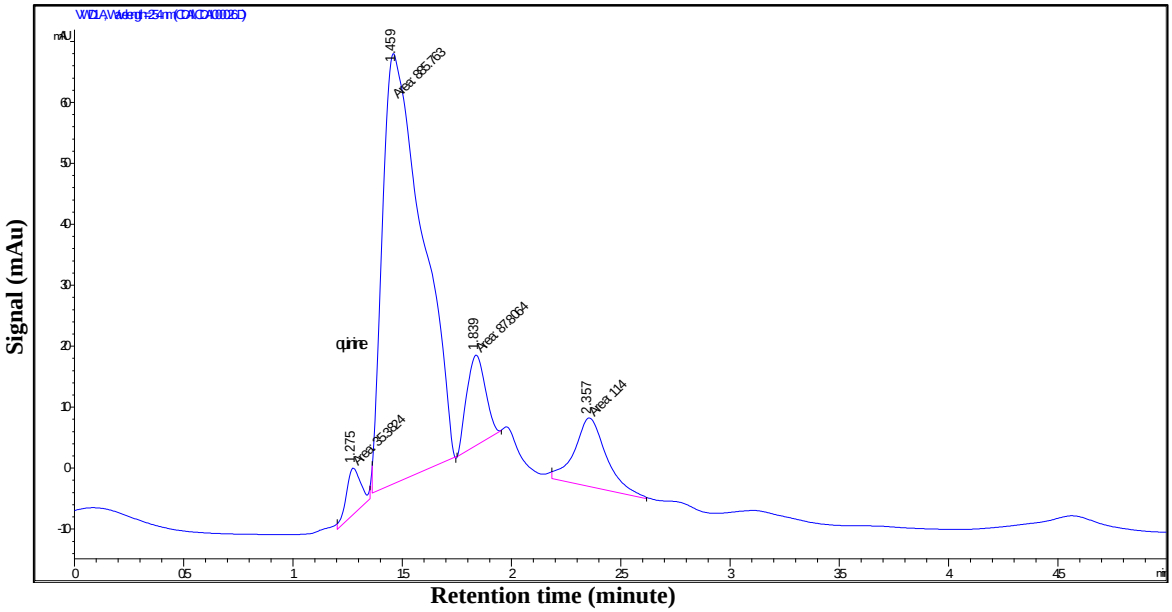

Figure 3: Representative chromatogram of Mango (*mangifera indica)* leaf extract

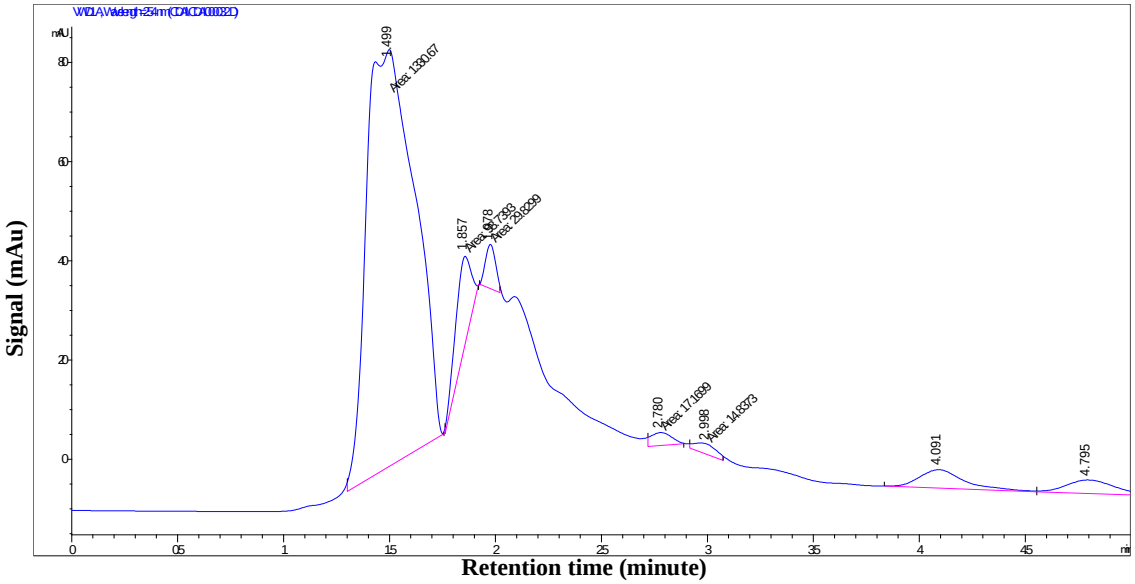

Figure 4: Representative chromatogram of Lime (*Citrus*aurantii *folia*) leaf extract

Validation report

Linearity and range

Calibration curve is used to understand the instrument response to an analyte and predict the concentration in an unknown sample. The standard calibration curve was plotted using concentration against average peak areas obtained by quinine. Analyte showed linear response between the concentration range of 1, 2, 5, 10, 15 and 20 µg/mL with linear regression equationof R^2 = 0.9975. Linearity data is reported in Table. 2 and standard calibration curve is presented in Figure 5.

Table 2: Calibration data

Conc. µg/mL	Peak area 1	Peak area 2	Average Peak area
1	24	25.6	24.8
2	60.1	58	59.05
5	140.2	141	140.6
10	292.7	290	291.35
15	395.5	397	396.25
20	533.6	540.5	537.05

Where: Conc.µg/mL stands for Concentration in micrograms per milliliter (µg/mL). It represents the amount of a substance (e.g., quinine) dissolved in a given volume of solution.
PA1 (Peak Area 1) is the first recorded peak area in chromatographic analysis. The peak area is proportional to the concentration of the compound detected.
PA2 (Peak Area 2) is the second recorded peak area, usually from a duplicate or replicate measurement.
Average PA (Average Peak Area) is the mean of PA1 and PA2, providing a more reliable estimate of the peak area for quantification.

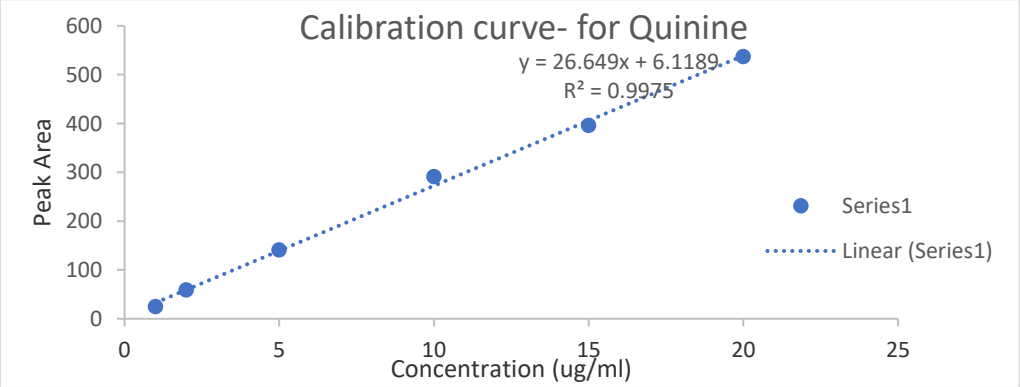

Figure 5: Standard calibration curve plot of quinine in µg/mL

Limit of detection (LOD) and limit of quantification (LOQ)
The detection limits, measured by serial dilution of the standard of quinine at 254 nm (n=2). LOD value of quinine was found to be 0.210±0.24 µg/mL and LOQ value was found to be 0.636±0.12 µg/mL, respectively. Values obtained are similar to those reported in literature.

Recovery
The accuracy of the extraction procedure was evaluated using spike-and-recovery experiments (external matrix matching standard). This approach assesses the efficiency of the extraction method by comparing recovered quinine levels to known spiked concentrations. Recovery for quinine was performed using dried *Mangifera indica* leaves. The average relative recovery was determined to be 99.5%, confirming the high accuracy and reliability of both the extraction method and the instrumental analysis for quinine quantification.

Discussion
The detection of quinine exclusively in mango leaf extract (*Mangifera indica*) raises critical questions regarding the phytochemical differences between the studied plants and the factors influencing quinine biosynthesis. Quinine is primarily associated with *Cinchona species (*Mekonnen *et al., 2025)*, known for their quinoline alkaloids, and its presence in other plant species is not well documented. The absence of quinine in neem (*Azadirachta indica*) and lime (*Citrus aurantiifolia*) leaf extracts could be attributed to several factors, including differences in genetic pathways, metabolic precursors, and environmental influences (Trenti *et al.*, 2021).
One possible explanation for the presence of quinine in mango leaves is the evolutionary development of secondary metabolites. Some plants share biosynthetic pathways for alkaloid production, while others lack the necessary enzymatic machinery. Quinine biosynthesis involves the shikimate and mevalonate pathways (Carrington *et al.*, 2018), which may be more active in *Mangifera indica* than in *Azadirachta indica* and *Citrus aurantii folia*. Additionally, previous studies have not quite reported that quinine and related alkaloids are commonly found in neem and citrus species, suggesting that these plants may not be natural reservoirs of quinine but may contain structurally similar compounds that require further characterization.
Another key factor that could explain these results is the potential degradation or modification of quinine in certain plant matrices. The poor chromatographic and peak tailing separation observed for neem and lime leaf extracts suggests the presence of interfering compounds that could mask or alter quinine detection Gosetti *et al.,* 2010). This highlights the need for additional phytochemical profiling using techniques such as mass spectrometry or nuclear magnetic resonance (NMR) to clarify the structural composition of these extracts

Comparison with Previous Studies
Previous research on quinine quantification in medicinal plants has primarily focused on Cinchona bark, where high quinine concentrations are well-documented (Canales *et al*., 2019). In contrast, studies on other plant species have yielded mixed results, with some detecting trace amounts of quinine or structurally related alkaloids. The current study contributes to this body of knowledge by identifying quinine in mango leaves, suggesting that certain non-Cinchona plants may serve as alternative sources of this bioactive compound (Cosenza *et al.,* 2013). However, further studies are required to confirm whether the detected quinine originates from endogenous biosynthesis or external contamination.

Study Limitations and Future Directions
While this study successfully identified and quantified quinine in mango leaf extract, several limitations must be acknowledged. Firstly, the research relied solely on in vitro assays without validating the findings through in vivo studies. The bioavailability and pharmacokinetics of plant-derived quinine in animal models or human subjects remain unexplored, warranting further investigation. Additionally, the limited number of plant species analyzed restricts the generalizability of the findings. Future research should expand the scope to include a broader range of medicinal plants to assess quinine distribution more comprehensively.
Moreover, the chromatographic challenges encountered with neem and lime extracts underscore the need for method optimization. Employing alternative extraction techniques, such as solid-phase extraction (SPE) or liquid-liquid partitioning could improve compound isolation and reduce matrix interference. Further, advanced spectroscopic

techniques could aid in identifying potential quinine analogs or precursors within these plants.

CONCLUSION

This study provides insight into quinine distribution in medicinal plants, with mango leaves emerging as a potential source. However, the findings emphasize the necessity for further phytochemical and pharmacological research to validate the medicinal significance of these observations and to explore their therapeutic applications beyond in vitro settings.

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