

Original Research Article

Potential anti-plasmodial constituents of *Terminalia pennyana* stem bark

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Sent for review: 15 September 2025

Revised accepted: 18 April 2026

Abstract

Purpose: To evaluate the *in vivo* antiplasmodial activity of *Terminalia pennyana* stem bark and employ vacuum liquid chromatography (VLC) and high-performance liquid chromatography-mass spectrometry (HPLC–MS) to fractionate the extract and dereplicate its bioactive constituents.

Methods: *Terminalia pennyana* stem bark was macerated in 96% methanol (3 L) for 48 h to obtain crude extract. The extract was separated using liquid–liquid partitioning and further fractionated on silica gel using vacuum liquid chromatography with a dichloromethane–methanol gradient to yield six subfractions (TPMEv1–TPMEv6). Based on bioactivity, selected fractions were dereplicated by C18–HPLC–MS technique. A four-day Rane's curative model in *P. berghei*–infected mice was used to evaluate *in vivo* antiplasmodial activity.

Results: Cold maceration yielded 3.89 %w/w methanol extract (TPM). Partitioning of TPM afforded 6.65 g (2.16 %) ethyl acetate (TPME), while the VLC of TPME generated six subfractions, including the most active TPMEv2 (0.21 %) and TPMEv3 (0.16 %). The dereplication of TPMEv2 and TPMEv3 using the HPLC–MS technique detected two flavan-3-ols: catechin/epicatechin (C₁₅H₁₄O₆) at *m/z* 291.0865 {*M* + *H*}⁺ and a lignan, pinoresinol (C₂₀H₂₂O₆) at *m/z* 359.1024 {*M* + *H*}⁺. In an *in vivo* bioassay study, TPM, TPME, TPMEv2 and TPMEv3 at a dose of 200 mg/kg inhibited parasitaemia by 45.3, 58.6, 63.2, and 79.4%, respectively, with mean survival times of 21.4, 25.2, > 28, and > 28 days (*p* < 0.01), when compared with the effect of artesunate (83.4 % inhibition, > 28 days).

Conclusion: *Terminalia pennyana* extract and TPMEv2 and TPMEv3, which are rich in catechins and pinoresinol, exhibit strong, dose-dependent antiplasmodial effects.

Keywords: Antiplasmodial, *Plasmodium berghei*, *Terminalia pennyana*, Vacuum liquid chromatography

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INTRODUCTION

Ethnomedicine is an important aspect of traditional medical practices in different cultures,

such as Yoga, Ayurveda, and African traditional medicine, with a significant contribution to the improvement of health outcomes of developing countries [1]. The demand for herbal medicines

has recorded an upsurge across cultures despite the advances in science and the widespread use of semi-synthetic and synthetic pharmaceuticals. Africa is endowed with rich medicinal flora that has contributed significantly to the management of tropical diseases such as malaria and tuberculosis [2].

Malaria, caused by *Plasmodium* parasites, has remained a major public health risk globally in tropics and subtropics, with an estimated 249 million cases and 608,000 deaths, according to the World Health Organisation (WHO) data in 2023 [3]. Nigeria has maintained the lead in malaria epidemiology in sub-Saharan Africa, accounting for 27 % and 31 % of global malaria cases and deaths, respectively [4]. There have not been significant improvements in global malaria cases over the decades despite continued intervention strategies such as the recent deployment of the pyrethroid-chlorfenapyr insecticide-treated mosquito nets and the rollout of R21/Matrix-M and RTS, S/AS01 malaria vaccines [5]. The limited number of effective antimalarial drugs and the rapid emergence of *Plasmodium* strains resistant to existing antimalarial therapies also create barriers for malaria chemotherapy [6].

The WHO has continued to advocate for traditional medicine, considering its enormous contribution to the healthcare needs and socioeconomic development of the resource-limited global populations [7]. Several studies have evaluated the potential of some plants as herbal remedies or sources of lead compounds for the chemotherapy of malaria [8]. *Terminalia pennyanana* Anozie (family Combretaceae), closely related to *T. glaucescens* and *T. ivorensis*, is indigenous to Nigeria. It is used as a mosquito repellent in sub-Saharan Africa and serves as a complement to orthodox medicines where access to standard antimalarial drugs is limited, considering that some *Terminalia* species such as *T. mantaly* (H. Perrier), *T. superba*, *T. brownie* and *T. avicennioides* possess antiplasmodial property [9]. Apart from its traditional use for management of malaria symptoms, *T. pennyanana* has also been investigated for antimicrobial and antioxidant properties as well as its ethnomedicinal benefits and uses in the management of flu, pneumonia and other related diseases [10].

There has been growing interest in ethnomedicine, especially among resource-limited populations, because medicinal plants are widely available, affordable, and accessible [8]. The therapeutic properties of these plants have been attributed to their diverse phytochemical

constituents [11]. However, although many studies document antiplasmodial activity in crude extracts, only a limited number of studies have progressed to isolating, characterising, or identifying the molecules underlying those effects [9]. This represents a significant gap: without isolating and characterising the bioactive compounds, it is impossible to understand the mechanisms of action, optimise the potency, or assess the safety profiles. Despite the strong ethnopharmacological relevance of *Terminalia* species in traditional medicine, the phytochemical data from these plants remain fragmented, and correlations with antiplasmodial activity are inconclusive [10].

To address these shortcomings, the current study aims to evaluate the *in vivo* antiplasmodial activity of *T. pennyanana* extract. It also aimed to identify the compounds responsible for its antiplasmodial effects through a series of chromatographic separations.

EXPERIMENTAL

Plant sample

The stem bark was harvested from the *Terminalia pennyanana* tree at the Botanical Garden (Global Positioning System: 6° 51' 28.19" N; 7° 23' 44.77" E) of the University of Nigeria, Nsukka, Nigeria, on June 28, 2023. It was identified and authenticated by Mr Felix Nwafor of the Department of Pharmacognosy and Environmental Medicine of the same university. The voucher specimen was assigned a voucher number UNH/04/0233 and deposited at the herbarium of the Department. The taxonomic profile was further verified at <https://www.theplantlist.org> on August 3, 2024. The sample was chopped into smaller pieces, dried in shade at 25 °C for 6 weeks and pulverised into a coarse powder before extraction.

Animal model

Thirty Swiss albino mice weighing 39 ± 10 g and within 9 ± 3 weeks of age were used for the study. The mice were housed in aluminium cages in bedding made of softwood shavings at 25 °C temperature and 25.25 ± 0.75 % humidity, with free access to drinking water and pelleted poultry feed whenever necessary. The mice were acclimatised to normal laboratory conditions for 7 days before the study. Ethical clearance for the use of mice was reviewed and granted by the ethical committee on the use of animals of the Enugu State University of Science and Technology (approval no. ESUTH/23/0074).

Parasites

A chloroquine-sensitive culture of *Plasmodium berghei* (NK47 strain) was obtained from the University of Nigeria Veterinary Teaching Hospital. The *P. berghei*, previously preserved at 4 °C, were injected into a recipient mouse intraperitoneally. Four days after infection, blood was collected from the tail vein and the parasitaemia was monitored daily under oil immersion microscopy using a Giemsa-stained thin smear. The parasites were regularly passaged in a new mouse via injection of 200 µL of infected erythrocytes (containing about 10⁸ parasites) interperitoneally.

Extraction and Solvent partitioning of the extract

A 650 g coarse sample was macerated in 3.0 L 96 %v/v methanol at 25 °C, agitated at intervals for 48 h and filtered [12]. The marc was further macerated for 24 h, and the combined extract was concentrated under vacuum at 40 °C to obtain the methanol crude extract of *T. pennyana* (TPM). Thereafter, 12 g of TPM, dispersed in 1000 mL of 10 % aqueous methanol, was successively partitioned in 1 L (2 x 1000 mL) each of *n*-hexane, ethyl acetate and *n*-butanol using a 2 L separating funnel. The solvent partitions were evaporated to dryness to obtain *n*-hexane (TPMH), ethyl acetate (TPME) and *n*-butanol (TPMB) fractions.

Chromatographic separation of a fraction

The active solvent fraction (TPME) was subjected to silica gel-packed vacuum liquid chromatographic (VLC) separation using a 200 x 4 cm glass column [13]. A 5 g sample was dry-loaded on the glass column and eluted sequentially with 500 mL (2 x 250 mL) each of 80, 60, 50, 40, and 20 %v/v of dichloromethane in methanol and 100%v/v methanol under an ES50 Speedivac high-pressure vacuum pump. The combined eluates of 500 mL each were concentrated to dryness to yield six VLC fractions of TPMEv1 to TPMEv6, respectively.

HPLC-MS dereplication of bioactive fraction

A 5 mg/mL sample of each of TPEv2 and TPEv3 was subjected to the high-performance liquid chromatographic-mass spectrometric (HPLC-MS) analysis [14]. A Dionex RSLC system on a C18 column in a binary of water/acetonitrile was used for the liquid chromatographic system at a 1 mL/min flow rate. The eluted compounds were identified using a mass spectrometer (Bruker Daltonics micrOTOF-QII) at 200 - 400 nm

wavelength. The dereplication of the peaks was carried out by peak-matching the mass-charge (*m/z*) and retention times and automatic querying of the masses of molecular ions and common adducts after peak deconvolution.

Parasitaemia induction and mice inoculation

A mouse infected with *Plasmodium berghei* (~ 34 % parasite level) was used as the parasite donor in the study. Aliquots of 0.20 mL of blood (10⁸ parasites) obtained from the donor mouse via ocular puncture were transferred into a small volume of trisodium citrate (0.50% w/v) in a heparinised capillary tube, and thereafter, the sample was made up to 0.40 mL using normal saline (0.9 % v/v) to adjust to a 0.50 x 10⁸ parasites load. To inoculate the experimental mice, 0.20 mL of the suspension of the parasite was injected intraperitoneally on the first day (day 1) of the study, monitored for 2 h before randomly grouping the mice [4].

Grouping and dosing of mice

The mice for the antiplasmodial study were randomly assigned to fourteen experimental groups (*n* = 5) as follows: Group 1 mice received the vehicle (0.5 mL/kg), while groups 2 - 3 received 100 and 200 mg of TPM/kg mouse. Groups 4-5 received 100 and 200 mg of TPME/kg mouse, respectively. Groups 6 - 7 were treated with 100 and 200 mg of TPMEv2/kg mouse, respectively. Mice in groups 8 - 9 received 100 and 200 mg of TPMEv1 to TPMEv6/kg mouse, respectively. Group 10 received the standard drug (artesunate, 5 mg/kg mouse). All treatments were prepared in 10% aqueous Tween 80 as a vehicle and administered once daily for four days.

Antiplasmodial activity assay

The *in vivo* Rane's curative assay model was adopted [4]. The infected mice were monitored for 72 h to allow for the establishment of infection. In this study, 0.5 mL of blood sample from each mouse on day 4 post-parasitaemia induction was smeared into a thin film for the baseline parasite count. Thereafter, the mice were given oral treatments of the vehicle, extract (TPM), solvent partitions (TPMH, TPME, TPMB) or VLC fractions (TPMEv1 to TPMEv6) once daily as specified in the dosing schedules from day 4 to 7 post-inoculation. On the 7th day (or 24 h after the last treatment), the parasitaemia in each mouse was reassessed and the inhibition calculated based on the baseline values using equation 1. The mean survival time (MST) of the

mice in groups 1-10 over 0-27 days post-infection was also estimated [4].

Determination of parasitaemia

A methanol-fixed thin blood film, prepared on microscopic slides, was stained with Giemsa dyes. Ten random fields were selected from the microscope to count the red blood cells containing *P. berghei* as a mean percentage of the total number of RBCs in each field. The percentage inhibition or clearance of *P. berghei* (pi) was expressed by comparing the average number of parasites in the RBCs of the treated (PbT) and negative control (PbC) from the ten random fields using Eq 1 [4,8].

$$pi = \{(PbC - PbT) / PbC\} 100 \dots\dots\dots (1)$$

Analysis of data

All the data generated are expressed as mean $\pm$ standard error of the mean (SEM). The treated groups were compared with the control groups using one-way analysis of variance, followed by Dunnett's Post-hoc analysis using GraphPad Prism v.5. Results were considered statistically significant at $p < 0.05$.

RESULTS

Extraction and separation of *T. pennyanus*

The extraction of coarsely powdered stem bark of *T. pennyanus* in methanol yielded 25.3 g (3.89 %) of TPM. Exhaustive partitioning of 12 g of TPM in solvents of different polarities furnished 1.1 (0.36 %), 6.65 (2.16 %) and 2.75 (0.89 %) grams of TPMH, TPME and TPMB, respectively.

VLC fractionation of the active fraction

Further fractionation of the most active TPME using VLC resulted in six fractions: 0.25 g of TPMEv1 (0.05 %), 1.15 g of TPMEv2 (0.21 %), 0.80 g of TPMEv3 (0.16 %), 0.65 g of TPMEv4 (0.12 %), 0.90 g of TPMEv5 (0.18 %), and 0.55 g of TPMEv6 (0.11 %). All yields are expressed relative to the 650 g of powdered sample material used.

Dereplication of antiplasmodial VLC fraction

The HPLC-MS dereplication of TPMEv2 and TPMEv3 (Figure 1) revealed the presence of two flavan-3-ols: catechin (1) and/or epicatechin (2; $C_{15}H_{14}O_6$), alongside the lignan pinoresinol (3; $C_{20}H_{22}O_6$; Figure 2). In positive-mode electrospray ionisation {ESI(+)}, catechins produced two molecular-ion peaks at m/z 291.1965 $\{M + H\}^+$ and m/z 313.0684 $\{M + Na\}^+$. A diagnostic A-ring fragment appeared at m/z 139.0285 $\{C_8H_7O_2\}^+$ (Figure 2 A). Pinoresinol ESI(+) spectrum (Figure 2 B) showed: m/z 359.1024 $\{M + H\}^+$ (molecular ion), m/z 309.0918 (loss of H_2O and CH_3) and m/z 179.0712 (cleavage of the tetrahydrofuran bridge, yielding two guaiacyl fragments).

Antiplasmodial activity of *T. pennyanus*

Across all treatment groups, a clear dose-dependent antiplasmodial effect against *P. berghei*-infected mice was evident (Table 1). Compared to the negative control (group 1), all doses of TPM, TPME, TPMEv1, and TPMEv2 significantly ($p < 0.01$) reduced parasitaemia and extended mean survival time (MST).

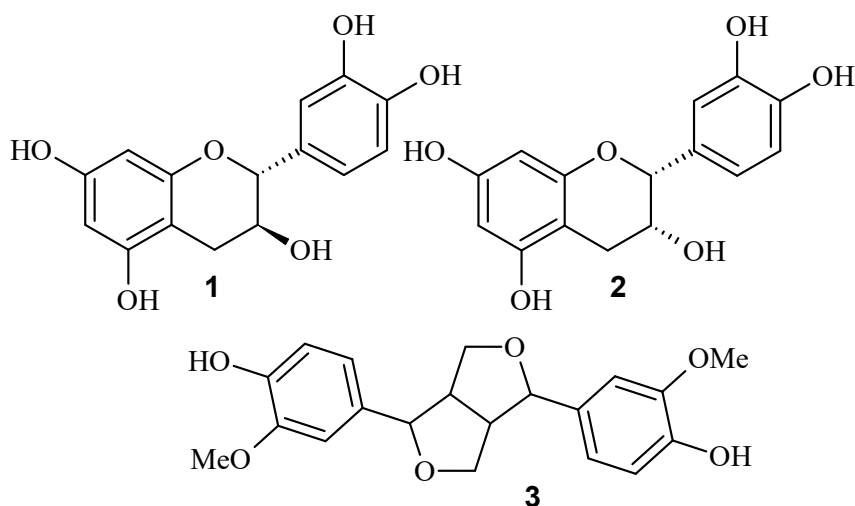


Figure 1: Major compounds identified from the HPLC-MS dereplication of TPMEv2 and TPMEv3

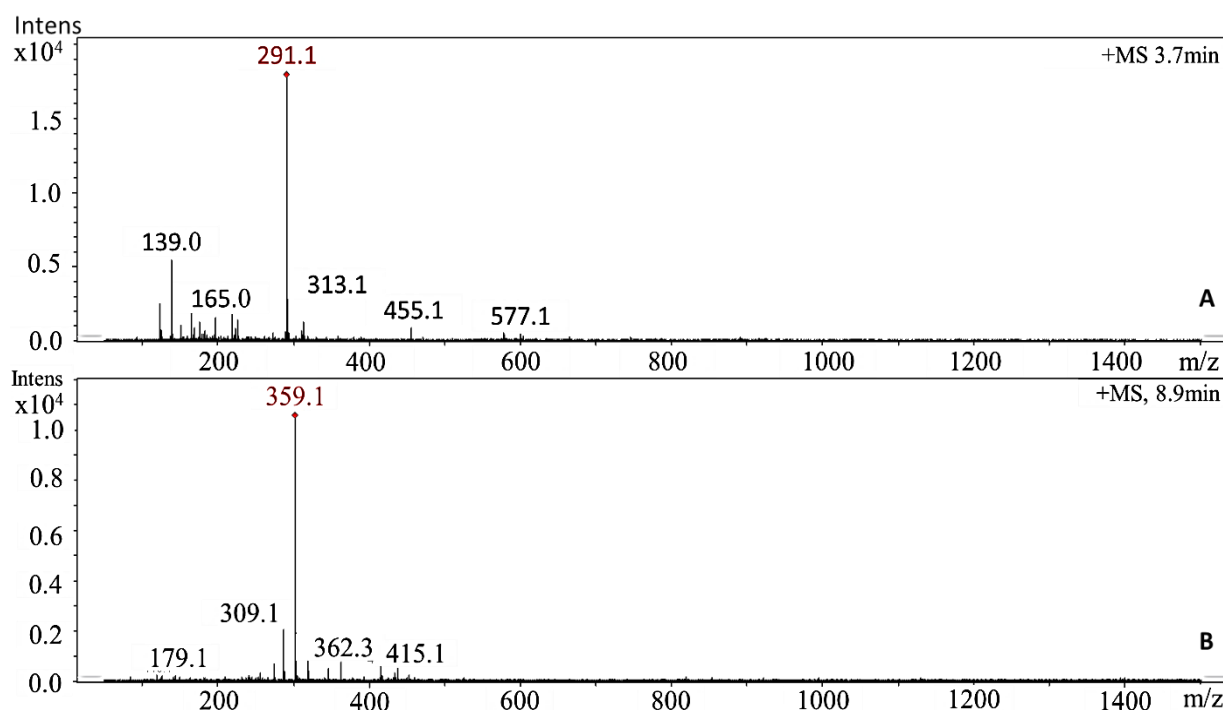


Figure 2: +ESI-qToF MS spectrum of identified major compounds in TPMEv2 (A) and TPMEv3 (B). ($C_{15}H_{14}O_6$, calculated m/z : 290.2806 Da, R_t = 3.7 min; $C_{20}H_{22}O_6$, calculated m/z : 358.3811 Da, R_t = 8.9 min)

Table 1: Effect of *T. pennyana* on parasitaemia in infected mice

Groups/Treatment, dose (mg/kg)	Mean parasitaemia (%)			MST (day)
	Day 4	Day 7	Inhibition	
1 (5 mL, Tween 80)	15.3±0.1	18.4±2.4 ^b	–	9.2±3.6
2 (100, TPM)	17.8±0.6	12.4±1.1 ^b	30.3±3.5	13.4±2.8
3 (200, TPM)	14.6±1.0	9.9±2.5 ^{a,b}	32.2±3.1	12.8±1.6
4 (100, TPME)	15.7±0.9	10.1±0.8 ^{a,b}	35.7±1.1	19.3±5.2
5 (200, TPME)	15.3±0.4	7.6±1.3 ^{a,b}	50.3±1.2	20.2±3.3
6 (100, TPMEv2)	14.7±1.8	6.4±1.0 ^{a,b}	56.5±3.8	27.8±2.1
7 (200, TPMEv2)	15.2±1.1	5.6±2.0 ^{a,b}	63.2±2.2	>28.0
8 (100, TPMEv3)	13.1±1.5	4.8±0.6 ^{a,b}	63.4±3.7	26.9±3.8
9 (200, TPMEv3)	16.5±0.8	3.4±0.5 ^a	79.4±0.8	>28.0
10 (5, Artesunate)	15.9±2.7	2.6±0.9 ^a	83.4±4.8	>28.0

Results are expressed as Mean ± SEM (n = 5). Significant at $p < 0.01$ compared with ^agroup 1 and ^bgroup 10. Inhibition (%) calculated relative to the treatment group baseline parasitaemia on day 4. Methanol crude extract of *T. pennyana* (TPM), *n*-hexane (TPMH) fraction, ethyl acetate (TPME) fraction, *n*-butanol (TPMB) fraction, VLC fractions (TPMEv1 to TPMEv6)

The crude TPM at a dose of 200 mg/kg elicited a 45.3 % parasitaemia inhibition and a mean survival time (MST) of 21.4 days, indicating moderate activity. Upon solvent partitioning, TPME showed enhanced efficacy, yielding 58.6 % inhibition and an MST of 25.2 days at the same dose. The VLC-derived fraction, TPMEv1, demonstrated even stronger activity, with 63.2 % inhibition and MST exceeding 28 days. Notably, the most refined fraction, TPMEv2, exhibited the highest potency: at a dose of 200 mg/kg, a 79.4 % inhibition was attained and MST >28 days, showing no significant difference ($p > 0.01$) compared to the positive control, artesunate, with 83.4 % inhibition and MST >28 days.

DISCUSSION

Recent findings have shown the promising antiparasmodial potential of *Terminalia* species. For instance, extracts from *T. mantaly* and *T. superba* demonstrated potent activity against *P. falciparum*, with $IC_{50} < 2 \mu\text{g/mL}$ and selectivity index values > 100 , indicating strong efficacy and low cytotoxicity. The findings validated the traditional use and complemented other pharmacological activities of *Terminalia* species [10]. The extracts elicited strong *in vitro* activity against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains, with an IC_{50} of 0.26 $\mu\text{g/mL}$. These extracts also demonstrated high selectivity index values (SI >

158), indicating low cytotoxicity to human cells and strong parasite-specific action [10]. Further fractionation using chloroform and ethyl acetate yielded even more potent fractions with $IC_{50} < 2 \mu\text{g/mL}$ and $SI > 109$. This study investigated *T. pennyanana* to further explore the significance of *Terminalia* species in the management of protozoal infections [10].

Extraction technique and extracting solvents play vital roles in herbal therapies [15]. Sequential maceration in methanol yielded 3.89%w/w crude methanol extract, and solvent partitioning of the extract yielded 0.36, 2.16, and 0.89 % for TPMH (n-hexane), TPME (ethyl acetate), and TPMB (n-butanol), respectively. The extraction yield in this study was significantly lower than the 23.6 % yielded in ethanolic extraction of *T. arjuna* and comparable with the 3.5%w/w in methanolic extraction of *T. catappa*. However, subsequent fractionation of *T. arjuna* extract yielded a lower amount (0.4 – 0.8 %w/w) of nonpolar fractions [10], highlighting the influence of solvent polarity on phytochemical distribution. Vacuum liquid chromatography of the active TPME yielded six fractions (TPMEv1–TPMEv6) with individual yields from 0.05 to 0.21 %, relative to the initial plant material. The most abundant and active fractions, TPMEv2 (0.21 %) and TPMEv5 (0.18 %), could contain high concentrations of bioactive metabolites. Vacuum liquid chromatographic fractionation of *T. catappa* acetone extract yielded eight subfractions in a previous study, with comparable percentage yields of 0.04 – 0.25 %w/w [10]. The narrow yield distribution further underscores the role of solvent polarity gradients in selectively enriching active constituents for possible targeted isolation and bioactivity evaluation. The dereplication of TPMEv2 and TPMEv3 identified two flavan-3-ols: catechin and/or epicatechin, and the lignan, pinoresinol. The ESI(+) spectra displayed the expected $\{M + H\}^+$ and $\{M + Na\}^+$ ions at m/z 291.0865 and 313.0684 for catechins, with a diagnostic A-ring fragment at m/z 139.0285. Pinoresinol showed $\{M + H\}^+$ at m/z 359.1024 and fragment ions at m/z 309.0918 and 179.0712. These findings corroborate recent LC-MS analyses of red rice catechins by Kim *et al*, who reported identical ion pairs under ESI(+) for catechins [16]. Moreover, fragmentation pathways for tetrahydrofuran-containing lignans align with mechanisms summarised by Cioanca *et al* in the tea extracts [17]. This further supports that efficient dereplication of extracts could provide insights into bioactivity-guided fractionation, isolation and characterisation of bioactive molecules. Recent studies have demonstrated that catechin and pinoresinol inhibited *P. falciparum* in an *in vitro* assay with

IC_{50} values in the range of 10 – 50 μM , suggesting the involvement of oxidative stress, mitochondrial dysfunction, and hemozoin formation blockade mechanisms [18]. In an *in vivo* assay model, catechin- or pinoresinol-enriched subfractions at doses of 50 mg/kg elicited 60 – 80 % parasitaemia suppression and extended the MST with minimal host toxicity. Structure–activity studies have shown that the introduction of galloyl groups to catechins enhances antiplasmodial potency, whereas glycosylation diminishes the potency [19]. In addition, encapsulation of catechins in lipid nanocarriers enhances oral bioavailability and synergises with chloroquine or artemisinin to lower effective doses and potentially overcome resistance. The antiplasmodial potential of catechins provides strong prospects in lead optimisation via synthetic flavan-3-ol and lignan analogues, combination regimens against resistant *Plasmodium* strains and targeted delivery systems to maximise parasite uptake.

The dose-dependent antiplasmodial efficacy observed across TPM and its fractions showed a similar pattern with the enrichment of catechin and pinoresinol in TPMEv2. As solvent partitioning increased the concentration of these flavan-3-ols and lignans, the parasitaemia inhibition rose from 45.3 % (TPM) to 79.4 % (TPMEv2), with corresponding MST improvements. This trend corroborates *in vitro* IC_{50} data demonstrating the mechanisms of action of catechin and pinoresinol, such as oxidative stress induction and hemozoin formation blockade [20]. The comparable potency of TPMEv2 to artesunate in this study highlights the significance of VLC in enrichment of bioactive constituents necessary for further development of antimalarial drugs. Despite promising antiplasmodial activity and HPLC-MS evidence of catechin and pinoresinol enrichment, this study is limited by a lack of direct comparison with commercial reference standards. Furthermore, no single compounds were fully isolated, and structural assignments remain tentative without nuclear magnetic resonance (NMR) or complementary spectroscopic validation. Future work will be focused on preparative isolation of individual flavan-3-ols and lignans, followed by comprehensive NMR characterisation and standard-based quantification to confirm chemical identities and refine activity correlations.

CONCLUSION

Terminalia pennyanana extract and VLC fractions, particularly TPMEv2 enriched in catechin and

pinoresinol, demonstrate potent dose-dependent antiplasmodial activity comparable to that of artesunate. These findings support the use of VLC for enriching bioactive constituents and provide chemical templates for the discovery and development of antimalarial therapies. Further isolation, NMR-based characterisation, standard comparisons, pharmacokinetic profiling, and comprehensive preclinical evaluation are essential for antimalarial drug development ultimately.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

None provided.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Use of research reporting tools

ChemSketch (ACD Inc., 2021) and GraphPad Prism (v.5) were used for drawing chemical structures of molecules, while Grammarly was used for English language editing.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article, and all liability and claims relating to the content of this article will be

borne by the authors. All authors contributed equally to this study and approved the final version of the manuscript.

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