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# BIOACTIVITY EVALUATION OF THE SCHOLAR TREE (*Echites scholaris* L.) BARK EXTRACTS BASED ON CHEMICAL PROFILING, *in vitro* AND *in silico* ASSAYS AGAINST MALARIAL PARASITE

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## ABSTRACT

**In Mizo traditional medicine, the scholar tree (*Echites scholaris*) is a therapy for various illnesses including blood disorders and malarial infection. To evaluate its antimalarial property, the bark extracts were prepared using solvents (chloroform and petroleum ether) of differing polarities. The plant extracts were tested on two strains of human malarial parasite, a drug-sensitive *Plasmodium falciparum* (Pf3D7) and a multidrug-resistant strain (PfK1). In a notable finding, the plant extracts were effective against both the strains. The chloroform-based extract exhibited high activity showing a half-maximal inhibitory concentration ($IC_{50}$) of 14.0 µg $mL^{-1}$ against Pf3D7 and 10.0 µg $mL^{-1}$ against PfK1. The petroleum ether-based extract was slightly less potent with $IC_{50}$ of 22.0 µg $mL^{-1}$ against Pf3D7 and 26.7 µg $mL^{-1}$ against PfK1. In terms of potentiality of drug resistance, the petroleum-based extract exhibited extremely low level of resistance ($R_i$ = 1.2), while chloroform-based extract at all showed no resistance ($R_i$ < 1). Cytotoxicity test against VERO C1008 cells revealed that the plant extracts were highly safe, having half-maximal cytotoxic concentrations ($CC_{50}$) far exceeding the toxicity threshold. From gas chromatography-mass spectrometric analysis, *cis*-3-hexenyl acetate and 3-decyn-2-ol were identified as major phyto-compounds in chloroform-based extract, while linoelaidic acid, phthalic acid, and 2-ethylbutyl nonyl ester were predominant in petroleum ether-based extract. Computational binding of compounds against vital proteins such as *Plasmodium falciparum* erythrocyte membrane protein 1 (VAR2CSA) and S-adenosyl-L-homocysteine hydrolase (SAHH) indicated high ligand-binding capacity. This study validates the antimalarial application of the plant and the rationale for further molecular and pharmacological investigations.**

**Keywords:** Antimalarial, drug resistance, medicinal plant, phytocompound, *Plasmodium falciparum*

## INTRODUCTION

Malaria is one of the deadliest infectious diseases in humans. Its high mortality is due to a protozoan species, *Plasmodium falciparum*. Despite global efforts on the prevention, treatment and eradication of vector mosquitos since the last century, little progress has been achieved to curtail the infection and associated death in the most endemic regions of the world. Development of new antimalarials and new treatment regimes, specifically artemisinin-based combination therapies, resulted in extensive decline of clinical cases in recent years, with records of reduction in millions in the past decade (Weiss *et al*., 2019). However, the rampant infections are on the surge again as the World Health Organization

reported 263 million cases accounting for 0.6 million deaths in 83 countries during 2023 (WHO, 2024). The dilemma is not entirely on the failure of medical and policy interventions, but largely due to a natural phenomenon, antimalarial resistance (Feachem *et al*., 2019; Thellier *et al.*, 2024). The intricate parasitic adaptations in *P. falciparum* had endowed the parasites of cunning molecular strategies to acquire drug resistance to the level of multidrug resistance (Lalchhandama, 2017). The historically champion antimalarials, quinine and the quinolines have been rendered almost useless and are no more the primary prescription medications. The reports of resistance to the currently prescribed first-line medication, artemisinins, are critically terrifying (Ward *et al.*, 2022). The urgency to gather complete understanding of parasite evolution is as challenging and vital as developing novel therapies.

Medicinal plants remain the mainstay of antimalarial sources from which the most important medications have been derived. Quinine from *Cinchona pubescens* was the first-known antimalarial compound, most effective treatment of any infectious disease (Miller *et al.*, 2022). By the early 20$^{th}$ century, chemical exploitations of quinine yielded novel quinoline compounds and their synthetic derivatives (Woodland and Chibale, 2022). The availability of a number of quinoline drugs almost heralded at the complete control of malaria (Van Der Hoogte and Pieters, 2016). However, the celebrated success of malaria management was on hold by the end of the 20$^{th}$ century as quinoline resistance became a health crisis of utmost concern (Bruce-Chwatt, 1990; Ursos and Roepe, 2002). By the late 20$^{th}$ century, a new group of highly effective antimalarials were developed from a herb *Artemisia annua*. The artemisinins and their derivatives became the primary malarial treatments ever since (Ma *et al.*, 2020). However, the emergence of artemisinin-resistance became sporadic, and a global health issue (Feng *et al.*, 2019; White and Chotivanich, 2024). The rise and fall of these phyto-compounds in malaria therapy have fostered zeal for new search on other traditionally used medicinal plants for alternative and new molecules (Ceravolo *et al.*, 2021; Habibi *et al.*, 2022).

The scholar or blackboard tree, *Echites* (synonym *Alstonia* R.Br.) *scholaris* L. is a deciduous evergreen tree belonging to the family Apocynaceae. It is native to Asia and Australia, where it is used as a traditional therapy for various ailments. It is acclaimed to be an efficacious medicine for the remedy of haematological diseases, blood infections and non-specific febrile fever (Willcox, 2011; Zhao *et al.*, 2023). It is also a well-known herbal medicine in India with several applications in asthma, cancer, helminthiasis, jaundice, leprosy, rheumatoid arthritis and ulcers (Gadekar *et al.*, 2010; Banik *et al*., 2020; Laksemi *et al*., 2022). The plant and related species are characterized by multiple leaves arising from a single node so the leaves are arranged in circular whorls, and there can be few to several leaves per whorl due to geographical variation. For example, a seven-leafed whorl is most common variety that is used in Indian traditional medicine (Singh *et al.*, 2017). A unique variety with specific eight-leafed whorls is found in Mizoram, the remotest northeastern state in India that lies at the heart of the Indo-Burma biodiversity hot spot. In the Mizo folk medicine, the leaf and bark extracts are used in the treatment of asthma, diarrhoea, dysentery, cardiac problems, hypertension, malaria, snake bites and typhoid fever (Sharma *et al.*, 2001; Ralte *et al.*, 2024). The traditional claim as an effective antimalarial agent makes the species interesting in the light of parasite evolution and drug resistance. Therefore, it was conceived experimentally worthwhile to test its bioactivity against malarial parasite and analyze its bioactive constituents in an attempt to identify the possible antimalarial compounds.

## MATERIALS AND METHODS

### *Specimen identification*

Herbaria of the leaves and flowers of *E. scholaris* were prepared from specimens collected at a forest in Lungdai, Mizoram, India (23°52' N 92°44' E). The specimens were validated (BSI/ERC/Tech/2023-24/102-17-05-23) at the Eastern Regional Centre of Botanical Survey of India, Shillong, India. Further, validation was done with voucher specimens preserved at the Royal Botanic Gardens Edinburgh (catalogue No. E00043906) and the Royal Botanic Gardens Kew (catalogue No. K000227695).

***Extract preparation***

*E. scholaris* barks were collected from the trees, washed in distilled water, cut into fine pieces and dried under ambient condition for one month. The dried samples were loaded in batches of 350 g into a 5 L Soxhlet apparatus. To ensure maximum extraction of bioactive compounds, the bark samples were extracted with solvents of opposing polarities: petroleum ether (an extremely non-polar solvent with 0.1 polarity index), and chloroform (polar solvent with high polarity index of 4.1). Extraction was processed for each solvent continuously for 4 days. The crude extracts were concentrated in vacuum-pressurised Buchi Rotavapor® R-100 (Flawil, Switzerland) and extracts preserved at 4°C till use.

***In vitro cultivation of malarial parasites***

Human malarial parasite, *Plamodium falciparum* was chosen as test model for antimalarial susceptibility. Two different strains used were: Pf3D7 (an antimalarial-sensitive strain) and PfK1 (a multidrug-resistant strain). The blood-stage (erythrocytic) parasites (trophozoites) were obtained from Biodefense and Emerging Infections (BEI) Research Resources Repository of the National Institute of Allergy and Infectious Diseases, U.S. National Institutes of Health. The parasites were cultivated in 6-8% parasitaemia and 2% haematocrit in complete RPMI-1640 medium (Sigma-Aldrich, USA) following the standardised culture method of Trager and Jensen (1976). The culture medium was supplemented with 0.5% AlbuMAX™ II lipid-rich bovine serum albumin, 0.2% D-glucose, 0.25 mg $L^{-1}$ fungizone, 50 mg $L^{-1}$ gentamycin, HEPES, 45 mg $L^{-1}$ hypoxanthine and 0.2% $NaHCO_3$. Incubation was done at 37±1°C in a humidified 5% $CO_2$ incubator (Forma™ series II, Thermo Fisher Scientific, USA).

***Antimalarial assay***

The plant extract was tested for antimalarial susceptibility against two strains of *P. falciparum* based on Sybr® Green 1-based fluorescence (MSF) assay (Johnson *et al.*, 2007). Erythrocytes were maintained in 8% parasitaemia and 1% haematocrit in RPMI medium. The ring-stage trophozoites were treated with serially diluted (from 50 µg $mL^{-1}$) solutions of plant extract in a 96-well plate and incubated at 37±1°C in a humidified 5% $CO_2$ incubator. After 72 h incubation, untreated (infected-cells without drug treatment) and sulfinylbismethane treated parasites were used as positive controls, whereas non-infected erythrocytes served as negative controls. A quinoline antimalarial, chloroquine (C6628, Sigma-Aldrich) was used as a reference drug. After 72 h incubation, 100 µL lytic buffer composed of 1X Sybr Green was added to each sample and further incubated for 2 h at room temperature in dark. The fluorescence intensity of Sybr Green-bound cells was measured at wavelengths of 485±20 nm excitation and 530±20 nm emission using fluorescence reader (BioTek Synergy™, Agilent Technologies, USA). The half-maximal inhibitory concentration ($IC_{50}$) was calculated from dose-response curves using non-linear regression analysis following standard statistical analysis (Agarwal *et al.*, 2017). The value of resistance index ($R_i$) was calculated from the equation:

$$R_i = [IC_{50} \text{ of drug-resistant strain}] \div [IC_{50} \text{ of drug-sensitive parent strain}]$$

***Cytotoxicity assay***

Cellular effects in terms of cytotoxicity of plant extracts were evaluated against VERO C1008 (normal monkey kidney epithelial cell) obtained from the American Type Culture Collection (ATCC) following the 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The cells were maintained in RPMI media supplemented with 0.2% D-glucose, 10% foetal BSA, 0.25 mg $L^{-1}$ fungizone, 50 mg $L^{-1}$ gentamycin, HEPES and 0.2% $NaHCO_3$ at 37±1°C in a humidified $CO_2$ incubator. The cells were seeded in a 96-well plate at $10^4$ cells per well. After 18 h incubation, the cells were treated with different dilutions of plant extracts (from 50 µg $mL^{-1}$). Cells exposed to podophyllotoxin (P4405, Sigma-Aldrich) were used as positive control. After incubating for 72 h, 25 µL MTT (M2128, Sigma-Aldrich) stock solution was added to each well and incubated for another 2 h. MTT stock solution was prepared in 5 mg $mL^{-1}$. The absorbance of samples was taken at 570 nm from which the half-maximal cytotoxic concentrations ($CC_{50}$) was calculated (Singh *et al.*, 2018).

***Phytocompound profiling***

Bioactive phytocompounds were analysed by gas chromatography-mass spectrometry (GC-MS) using

a single quadrupole TRACE™ 1300 ISQ™ LT (Thermo Scientific™, USA). Chromatographic-grade methanol (Sigma-Aldrich) was used to dissolve the plant extracts to make 50 mg $mL^{-1}$ of a working solution. 1 µL of each sample was allowed to pass through a stationary phase, a non-polar column TR-5MS (30 m length × 0.25 mm width × 0.25 µm film thickness). Helium as a carrier gas was injected at 1 mL $min^{-1}$ and split into a ratio of 1:50. The temperatures of transfer line and ion source were set at 220°C, while the oven temperature was initially set at 70°C and incrementally raised by 10°C every 10 min to a maximum of 250°C. The mass spectrometry was scanned for 33 min within a spectral range of 10 to 1,100 amu. Chromatogram and spectral data were generated from Thermo Scientific™ Xcalibur™ software. Compound identification was done by chemical comparison in the database of the National Institute of Standards and Technology (U.S. Department of Commerce).

***In silico binding assay***

The identified predominant phytocompounds, the probable sources of bioactivity in plant extract, were selected for modelling the molecular binding to the key receptors in *P. falciparum* that are involved in antimalarial effects. Their three-dimensional structures were retrieved from PubChem (*https://pubchem.ncbi.nlm.nih.gov*), NCBI, in structured data file formats. The chemical structures were optimised and their cumulative energies minimised using ChemBio3D Ultra 12.0 (CambridgeSoft, Cambridge, USA) with a force field MMFF94. The compounds were docked to *P. falciparum* proteins available at the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (RCSB-PDB) (*www.rcsb.org*) such as *P. falciparum* erythrocyte membrane protein 1 (VAR2CSA; PDB code: 7JGD) (Ma *et al.*, 2021), and S-adenosyl-L-homocysteine hydrolase (SAHH; PDB code: 1V8B) (Tanaka *et al.*, 2004). To obtain an unhindered chemical configuration, molecules adhering to the proteins such as co-factors, vehicle ligands and water were removed using Molegro Molecular Viewer software. Ligand binding was simulated on the AutoDock Vina v1.2.4 platform (Molecular Graphics Lab, Scripps Research, La Jolla, USA) (Trott and Olson, 2010). Polar hydrogens and Kollman charges were incorporated to the proteins in Molecular Graphics Lab biosoftware MGLTools 1.5.6. The final configurations were saved in PDBQT files. Ligand binding and analysis were done with BIOVIA Discovery Studio Visualizer 2016 v16.1.0.15350 (Dassault Systèmes, Vélizy-Villacoublay, France).

***Statistical analysis***

The statistical analyses of treatments against control were performed using Student's *t*-test. Comparison of mean differences was done using analysis of variance (ANOVA) and Tukey's honest significant difference test. The level of significance was taken at *p* value less than 0.05. Statistical data and graphs were prepared in GraphPad Prism 10.4.1 of 2024 (Dotmatics, Boston, USA).

## RESULTS AND DISCUSSION

***Antimalarial activity***

*E. scholaris* bark extracts exerted substantial antimalarial activity against both the drug-sensitive and multidrug-resistant strains of *P. falciparum* (Table 1). The chloroform-based extract was more potent

**Table 1: Antimalarial activity (in $IC_{50}$) and cytotoxicity (in $CC_{50}$) of *E. scholaris* bark extracts in comparison to standard drugs**

| Treatments | $IC_{50}$ (µg $mL^{-1}$) against *P. falciparum* | | $CC_{50}$ (µg $mL^{-1}$) against VERO (cytotoxicity) |
|---|---|---|---|
| | Pf3D7 | PfK1 | |
| Chloroform extract | 14.00 | 10.00 | >100 |
| Petroleum ether extract | 22.00 | 26.70 | >100 |
| Chloroquine | 2.05 | 159.94 | NA |
| Podophyllotoxin | NA | NA | 1.45 |

Chloroquine = positive control for antimalarial test; NA = not applicable; Pf3D7 = chloroquine-sensitive strain; PfK1 = Multidrug-resistant strain; podophyllotoxin = positive control for cytotoxicity test; VERO = C1008 strain of epithelial cell line from the kidney of an African green monkey.

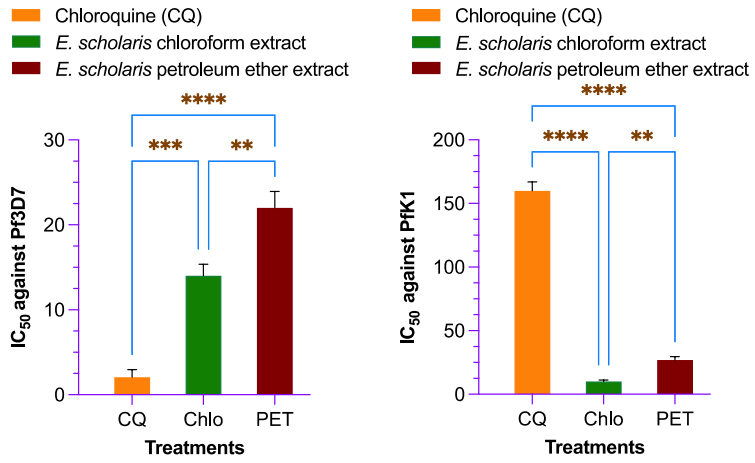


**Fig. 1: Antimalarial susceptibility of two strains of *P. falciparum*, drug-sensitive Pf3D7 and multidrug-resistant PfK1, to different extracts of *E. scholaris* and the standard drug, chloroquine. The treatment groups were compared using ANOVA and Turkey's multiple comparison test. Values are in means ± standard error of means ($n$ = 3); **** $p < 0.0001$, *** $p < 0.002$, and ** $p < 0.001$.**

with $IC_{50}$ values of 14.0 µg $mL^{-1}$ against drug-sensitive Pf3D7 and 10.0 µg $mL^{-1}$ against drug-resistant PfK1. The efficacy was classified as high activity. The petroleum ether-based extract showed good activity but less than that of chloroform-based extract, with $IC_{50}$ values of 22.0 µg $mL^{-1}$ against Pf3D7 and 26.7 µg $mL^{-1}$ against PfK1. Comparison of treatment groups showed that the standard antimalarial, chloroquine, was more active than plant extracts, and chloroform-based extract showed significantly higher efficacy than petroleum ether-based extract (Fig. 1). The most remarkable finding was that the plant extracts were active against PfK1, upon which chloroquine had no activity.

The level of antimalarial resistance estimated from resistance index ($R_i$) indicated that *E. scholaris* bark extracts had negligible potential of resistance in *P. falciparum*. The petroleum extract with $R_i$ of 1.2 exhibited extremely low resistance, while chloroform extract showed no resistance at all with $R_i$ < 1. For comparison, chloroquine had $R_i$ of 78.02 indicating that it has extremely high resistance level.

*P. falciparum* is a hard parasite to crack in terms of medication and evolutionary adaptation, so remains the deadliest single species of parasite. The conundrum to its notorious success as a parasite lies in its relative ease to acquire genetic mutations to evade antimalarials thus giving rise to an astonishing adaptation. The development of synthetic chloroquine, an analogue of natural quinine, as first-line treatment of falciparum malaria in 1940's, was considered a medical revolution, only to be beset by the dismal news of chloroquine resistance, with first reports appearing in 1957 in Thailand (Packard, 2014; Severini and Menegon, 2015). It was subsequently established that the parasite developed multiple mutations in its transporter protein aptly called '*Plasmodium falciparum* chloroquine resistance transporter' (PfCRT) that rendered invincibility to the parasite by effective dispelling any chloroquine molecule it encounters (Ecker *et al*., 2012). The parasite does not simply deploy its PfCRT arsenal but allows the protein to act in concert with complex network of other co-factors by which the resistance becomes an irreversible phenomenon. The PfCRT is not just a chloroquine-resistance protein but a true multi-drug resistance molecular device (Summers *et al.*, 2012). These distressing revelations were compounded by the discoveries of a cohort of other specific- and multi-drug resistance genes and their products (Gil and Fançony, 2021). Mutations in these proteins confer antimalarial resistance, even to the currently prescribed artemisinin groups (Ward *et al.*, 2022). The search for new improved antimalarial is a foreseeable medical venture, and medicinal plants will always play a pivotal role and promising impetus in the search for the lead molecules.

In present study *E. scholaris* bark extract exhibited commendable antimalarial activity against *P. falciparum*. The chloroform- and petroleum ether-based extracts showed $IC_{50}$ of 14 and 22 µg $mL^{-1}$ against drug-sensitive strain (Pf3D7), and 10.0 and 26.7 µg $mL^{-1}$ against drug-resistant strain (PfK1), respectively. Rasoanaivo *et al.* (1999) suggested the threshold for $IC_{50}$ with a value < 5 µg $mL^{-1}$ indicating high activity, < 50 µg $mL^{-1}$ indicating activity, between 50 and 100 µg $mL^{-1}$ indicating weak activity, while >100 µg $mL^{-1}$ is inactive for antimalarial agents. Our findings fall well within the values of antimalarial activity. Several phytocompounds from antimalarial plants have been documented with similar or high activity against *P. falciparum*. Among the promising sources, poly-

syphorin, roridin E and rhaphidecurperoxin isolated from the leaves and stems of *Rhaphidophora decursiva*, and verrucarin L acetate from the leaves of *Ficus fistulosa* reportedly showed high activity at $IC_{50}$ < 5 µg $mL^{-1}$ (Zhang *et al.*, 2002; Frausin *et al.*, 2015). Two novel flavonoids, 3′-formyl-2′,4′ dihydroxy-6′-methoxychalcone and 8-formyl-7-hydroxy-5-methoxyflavanone from the leaves of *Friesodielsia discolor* exhibited $IC_{50}$ of 9.2 and 9.3 µg $mL^{-1}$, respectively (Prawat *et al.*, 2012). 5-Hydroxy-6-methoxyonychine isolated from *Mitrephora diversifolia* roots showed $IC_{50}$ values of ~5 µg $mL^{-1}$ against different strains of *P. falciparum* (Mueller *et al.*, 2009). Miliusacunines A and B from the leaves and twigs of *Miliusa cuneatas* indicated $IC_{50}$ values of ~ 15 µg $mL^{-1}$ against different strains (Promchai *et al.*, 2016). High activities are also documented for the bioactive compounds of the stems of *Cogniauxia podolaena*, *Glossocalyx brevipes*, *Psorospermum glaberrimum*, *Rourea minor* and *Strychnos icaja*; the leaves of *Ageratum conyzoides*, *Cornus florida*, *Diospyros quaesita*, *Fuerstia africana*, *Ludwigia erecta*, *Piptadenia pervillei*, *Prosopis glandulosa*, and *Strophioblachia fimbricalyx*; the roots of *Cecropia pachystachya*, *Citropsis articulata*, *Echinops hoehnelii*, *Ocimum sanctum*, and *Zanthoxylum chiloperone* (Schwikkard and van Heerden, 2002; Guchait *et al.*, 2023).

### *Cytotoxicity*

The cytotoxicity property of *E. scholaris* bark extracts evaluated against VERO C1008 cells is given in Table 1. The exact values of $CC_{50}$ of both chloroform- and petroleum ether-based extracts could not be determined since they crossed the maximum threshold detectable in the assay, i.e., $CC_{50}$ of 100 µg $mL^{-1}$. VERO cells are considered ideal in antiviral and antiparasitic tests as they represent the general epithelial cells of body which are at the direct interface of chemicals and anatomical environments. The maximum concentration of 100 µg $mL^{-1}$ was targeted because the optimal level of $CC_{50}$ is normally 20 µg $mL^{-1}$, above which molecules are considered non-toxic and safe for interaction with normal mammalian cells like VERO (Freire *et al.*, 2009; Njeru and Muema, 2021). Taking this value as a benchmark, VERO cells are ideal and staple target models in antiviral and antimalarial tests (de Castro Barbosa *et al.*, 2022; Passarini *et al.*, 2022). In comparison, the reference toxin, podophyllotoxin showed high toxicity with $CC_{50}$ of 1.45 µg $mL^{-1}$. In other words, the *E. scholaris* bark extracts are highly non-toxic and safe to be used as nutraceutical source.

### *Compound identification*

GC-MS chromatograms and mass spectra of *E. scholaris* bark extracts screened to NIST library showed abundance of alcohol derivatives in chloroform-based extract while petroleum ether-based extract mostly composed of fatty acids and their derivative. The gas chromatograms identified several compounds in chloroform- and petroleum ether-based extracts (Fig. 2 & 3). In chloroform extract, the principal phytocompounds were allyl alcohols like *cis*-3-hexenyl acetate and 3-decyn-2-ol (Table 2).

**Table 2: Identification of bioactive compounds from NIST chemical database using GC-MS in the chloroform extract of *E. scholaris* bark**

| Peak No. | Retention time (min) | Relative abundance (%) | Compounds identified | Formula | Molecular weight |
|---|---|---|---|---|---|
| 1 | 3.47 | 23.3 | 2-Pentadecyn-1-ol | $C_{15}H_{28}O$ | 224 |
| 2 | 4.88 | 99.8 | *cis*-3-Hexenyl acetate | $C_8H_{14}O_2$ | 142 |
| 3 | 5.36 | 91.6 | 3-Decyn-2-ol | $C_{10}H_{18}O$ | 154 |
| 4 | 7.43 | 27 | 2-(1-Nitropropan-2-yl)cyclohexan-1-one | $C_9H_{15}NO_3$ | 185 |
| 5 | 8.41 | 20.3 | Dodecyl acrylate | $C_{15}H_{28}O_2$ | 240 |
| 6 | 10.83 | 22.2 | 4,7,7-Trimethylbicyclo[2.2.1]heptan-2-one | $C_{11}H_{19}N_{30}$ | 209 |
| 7 | 13.56 | 52.9 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 |
| 8 | 16.72 | 31.2 | Trans-13-octadecenoic acid | $C_{18}H_{36}O_2$ | 282 |
| 9 | 17.11 | 29.8 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 |
| 10 | 26.46 | 16.4 | 1-Heptatriacotanol | $C_{37}H_{76}O$ | 536 |
| 11 | 27.84 | 51.5 | Cycloeucalenol acetate | $C_{32}H_{52}O_2$ | 468 |
| 12 | 29.17 | 42.7 | Lup-20(29)-en-3-ol | $C_{30}H_{50}O$ | 426 |
| 13 | 31.49 | 48.7 | 24-Noroleana-3,12-diene | $C_{29}H_{46}$ | 394 |
| 14 | 31.58 | 53.6 | 24-Noroleana-3,12-diene | $C_{29}H_{46}$ | 394 |

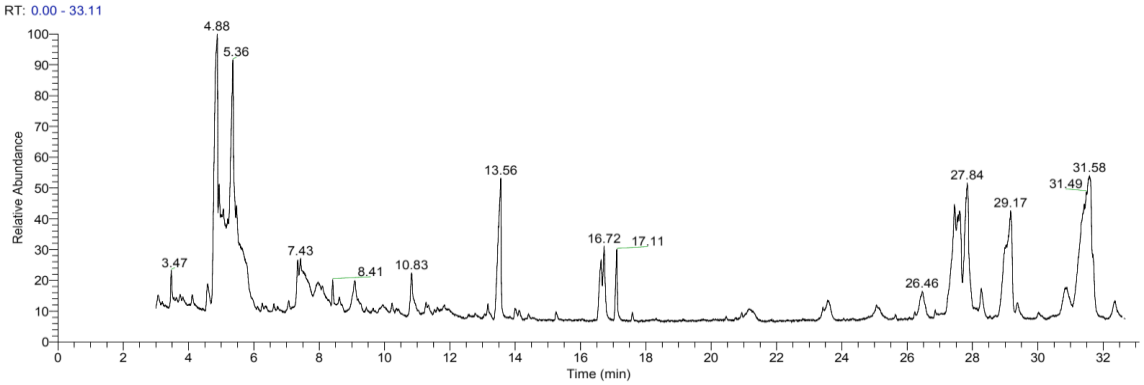


**Fig. 2: Gas chromatogram of the chloroform-based extract of *E. scholaris* bark for identification of bioactive compounds from NIST chemical library**

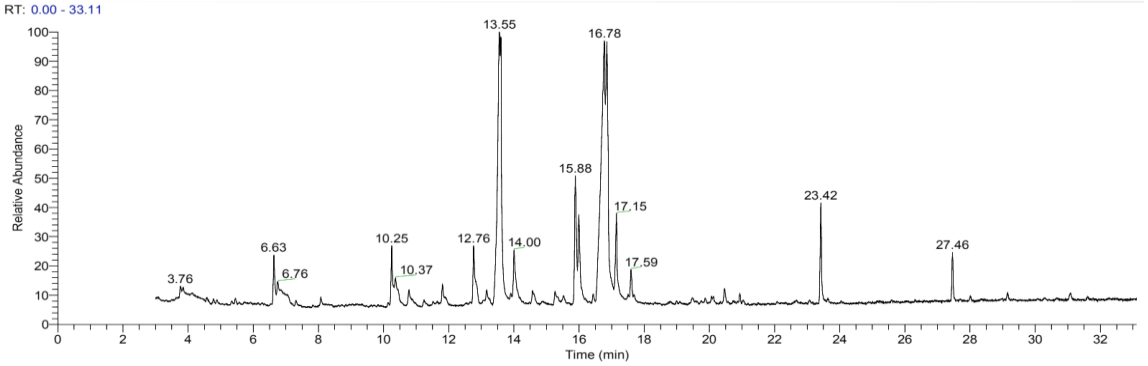


**Fig. 3: Gas chromatogram of the petroleum ether-based extract of *E. scholaris* bark for identification of bioactive compounds from NIST chemical library.**

Fatty acid derivatives like n-hexadecanoic acid, cycloeucalenol acetate and 24-noroleana-3,12-diene were present in appreciable amounts. A fatty acid ester, phthalic acid, 2-ethylbutyl nonyl ester, and a saturated fatty acid, linoelaidic acid are predominant compounds in petroleum ether extract (Table 3). The chemical analysis identified 3-decyn-2-ol in chloroform-based extract, and linoelaidic acid and phthalic acid in petroleum ether-based extract as major bioactive compounds of *E. scholaris* bark. 3-Decyn-2-ol is a secondary allylic alcohol, found in many plant species and cyanobacteria, acts

**Table 3: Identification of bioactive compounds from NIST chemical database using GC-MS in the petroleum ether extract of *E. scholaris* bark**

| Peak No. | Retention time (min) | Relative abundance (%) | Compounds identified | Formula | Molecular weight |
|---|---|---|---|---|---|
| 1 | 3.76 | 12.4 | (*E*)-Hexadec-9-en-1-ol | $C_{16}H_{32}O$ | 240 |
| 2 | 6.63 | 23.1 | 1,9-Nonadecane | $C_{19}H_{38}$ | 266 |
| 3 | 6.76 | 14.6 | 2-Hexyldecan-1-ol | $C1_6H_{34}O$ | 242 |
| 4 | 10.25 | 26.5 | (*E*)-icos-3-ene | $C_{20}H_{4O}$ | 280 |
| 5 | 10.37 | 15.7 | 2-Hexyldecan-1-ol | $C_{16}H_{34}O$ | 242 |
| 6 | 12.76 | 26.4 | 14-Methylpentadecanoic acid | $C_{17}H_{34}O_2$ | 270 |
| 7 | 13.55 | 99.8 | Phthalic acid, 2-ethylbutyl nonyl ester | $C_{23}H_{36}O_4$ | 376 |
| 8 | 14 | 46.6 | Heptacos-1-ene | $C_{27}H_{54}$ | 378 |
| 9 | 15.88 | 50.7 | Methyl lineoleate | $C_{19}H_{34}O_2$ | 294 |
| 10 | 16.78 | 97.1 | Linoelaidic acid | $C_{18}H_{32}O_2$ | 280 |
| 11 | 17.15 | 37.5 | Otadecanoic acid | $C_{18}H_{36}O_2$ | 284 |
| 12 | 17.59 | 18.4 | Nonacos-1-ene | $C_{29}H_{58}$ | 406 |
| 13 | 23.42 | 41.3 | Diisooctyl phthalate | $C_{24}H_{38}O_4$ | 390 |
| 14 | 27.46 | 24.2 | Squalene | $C_{30}H_{50}$ | 410 |

as a defensive molecule against pathogens (Perveen and Alwathnani, 2013; El Mohammady *et al.*, 2024). Its specific antifungal property reportedly has shown high activity against human fungal pathogen, *Fusarium oxysporum* (Perveen *et al.*, 2022). Linoelaidic acid, an omega-6 trans fatty acid, is present in many organisms shows anticancer and antimicrobial activities (Dutta *et al.*, 2023; Salman *et al.*, 2024). Phthalic acid, an aromatic dicarboxylic acid, exhibits a range of biological activities and is used in chemical synthesis to produce bioactive compounds that show analgesic, anti-inflammatory, anti-depressant antimicrobial and insecticidal activities (Huang *et al.*, 2021; Jelali *et al.*, 2022).

***Computational ligand-receptor interaction***

The vital proteins of *P. falciparum*, namely VAR2CSA and SAHH, were chosen as targets to simulate the probable binding efficiency and binding sites for the compounds identified from *E. scholaris* bark extracts. The grid boxes were prepared for the target proteins to cover all the possible binding sites at the coordinates (Table 4). With an exhaustiveness of 8, the ligands were docked to the receptors and the results were saved for visual analysis. The binding scores are tabulated in Table 5. Moderately high interaction was observed for 3-decyn-2-ol which showed a binding energy of −4.8 kcal mol$^{-1}$ against VAR2CSA and −4.7 kcal mol$^{-1}$ against SAHH with single amino acid interaction at Tyr1044 and Asp134, respectively (Fig. 4). *cis*-3-Hexenyl acetate showed weak affinity for the target proteins,

**Table 4: Molecular data set up for docking in AutoDock Vina, showing the grid coordinates and protein data bank (PDB) identification codes**

| Target | Size x | Size y | Size z | Centre x | Centre y | Centre z | PDB code | Reference |
|---|---|---|---|---|---|---|---|---|
| VAR2CSA | 62 | 68 | 68 | 37.859 | 32.362 | 56.095 | 7JGD | Ma *et al.* (2021) |
| SAHH | 92 | 104 | 126 | 157.763 | 159.146 | 151.655 | 1V8B | Tanaka *et al.* (2004) |

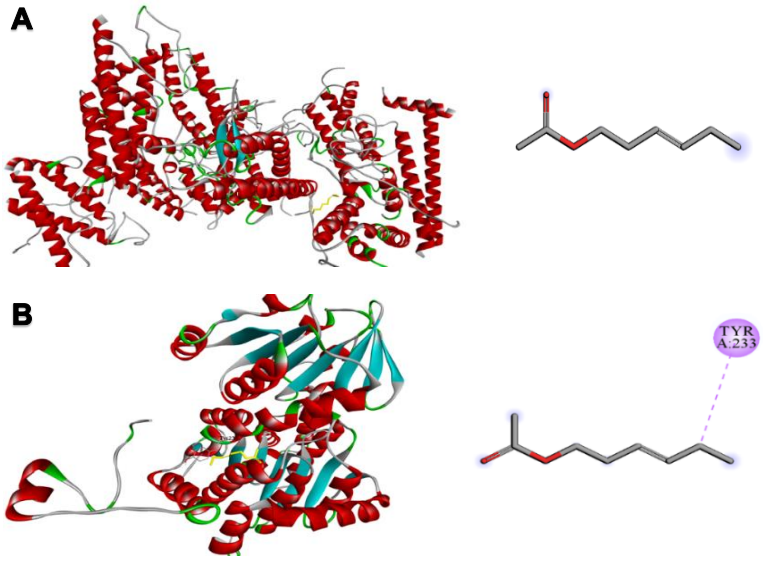


**Fig. 4: Molecular binding between 3-decyn-2-ol identified from *E. scholaris* bark with (A) *P. falciparum* erythrocyte membrane protein 1 (VAR2CSA) and (B) S-adenosyl-L-homocysteine hydrolase (SAHH)**

**Table 5: Molecular binding scores of bioactive compounds as ligands identified from *E. scholaris* bark extracts on proteins associated with drug susceptibility in *P. falciparum***

| Ligand | CID code | Target | Binding energy (kcal mol$^{-1}$) | Amino acid interaction |
|---|---|---|---|---|
| 3-Decyn-2-ol | 536504 | VAR2CSA | −4.8 | Tyr1044 |
| | | SAHH | −4.7 | Asp134 |
| *cis*-3-Hexenyl acetate | 5363388 | VAR2CSA | −3.8 | - |
| | | SAHH | −4.2 | Tyr233 |
| Linoelaidic acid | | VAR2CSA | −4.7 | - |
| | | SAHH | −4.7 | Glu27 |
| Phthalic acid, 2-ethylbutyl nonyl ester | 91719594 | VAR2CSA | −5.0 | Pro73, Pro144, Glu282, Asp286 |
| | | SAHH | −6.4 | His54, Asp134, Phe346, Leu389, Asn391, Leu392, Met403 |

CID = PubChem compound identification; SAHH = S-adenosyl-L-homocysteine hydrolase; VAR2CSA = *Plasmodium falciparum* erythrocyte membrane protein 1 specific for placental malaria.

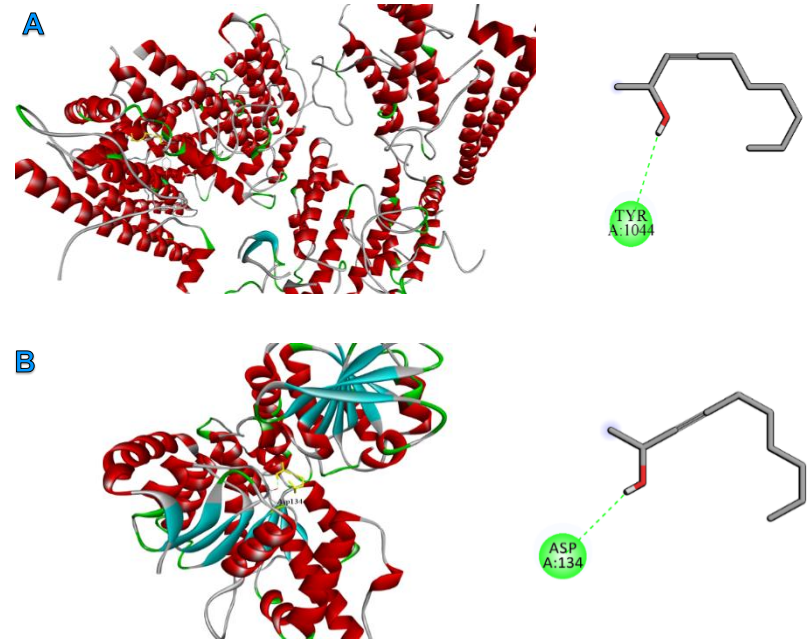


**Fig. 5: Molecular binding between *cis*-3-hexenyl acetate identified from *E. scholaris* bark with (A) *P. falciparum* erythrocyte membrane protein 1 (VAR2CSA) and (B) S-adenosyl-L-homocysteine hydrolase (SAHH)**

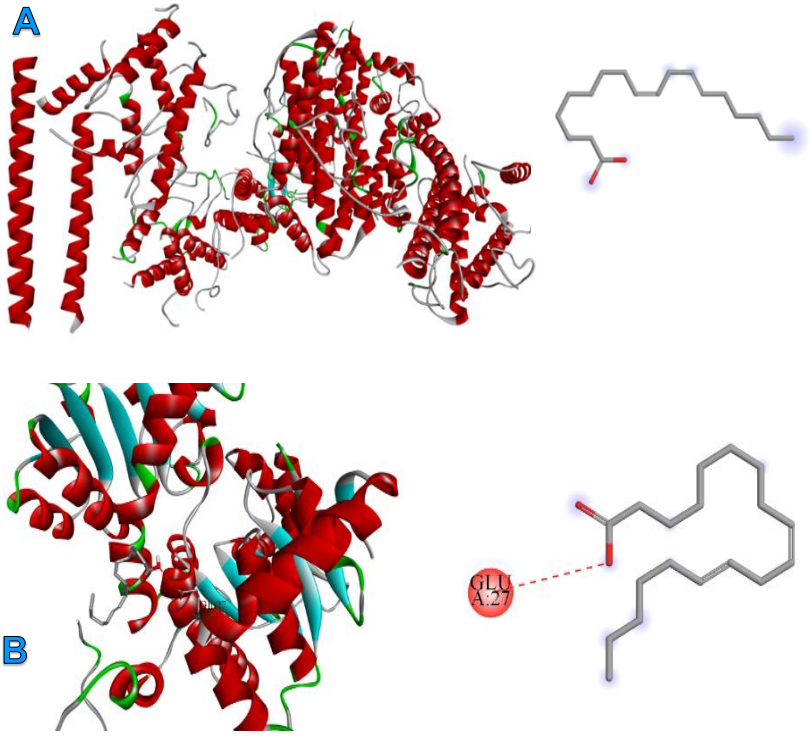


**Fig. 6: Molecular binding between linoelaidic acid identified from *E. scholaris* bark with (A) *P. falciparum* erythrocyte membrane protein 1 (VAR2CSA) and (B) S-adenosyl-L-homocysteine hydrolase (SAHH)**

with higher binding efficiency for SAHH with a binding energy of −4.2 kcal $mol^{-1}$ and a single amino acid interaction at Tyr233 (Fig. 5). A slightly more efficient binding was indicated by linoelaidic acid with a binding energy of −4.7 kcal $mol^{-1}$ against both the proteins, but with one amino acid interaction at Glu27 on SAHH (Fig. 6). The highest binding efficiency was shown by phthalic acid. It had a binding energy of −5.0 kcal $mol^{-1}$ against VAR2CSA with four amino acid binding sites at Pro73, Pro144, Glu282 and Asp286. Its binding capacity was even higher with SAHH showing a binding energy of −6.4 kcal $mol^{-1}$ against VAR2CSA with multiple binding sites such as at His54, Asp134, Phe346, Leu389, Asn391, Leu392 and Met403 (Fig. 7). The results suggest for the first time an implication of the compounds, identified in *E. scholaris* bark extracts, as potential antimalarials. This reveals their ability to interact with the two most critical proteins of *P. falciparum*. S-adenosyl-homo-cysteine hydrolase (SAHH) is a regulatory enzyme specific for methylations and crucial for parasite survival during the blood-feeding stage. Developing SAHH inhibitor has been one of the major goals in novel drug discovery (Leela *et al.*,

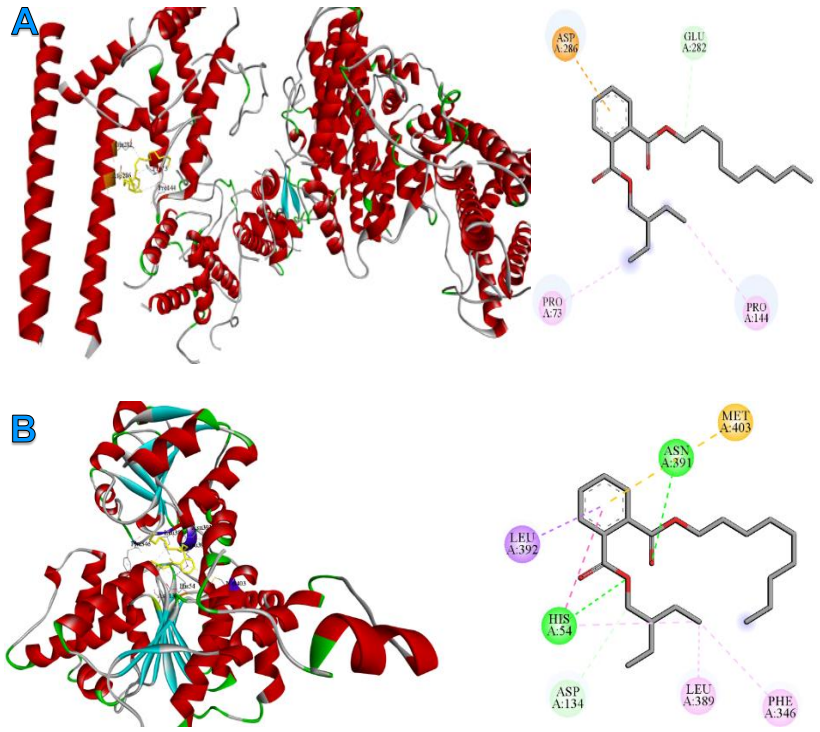


**Fig. 7: Molecular binding between phthalic acid, 2-ethylbutyl nonyl ester identified from *E. scholaris* bark with (A) *P. falciparum* erythrocyte membrane protein 1 (VAR2CSA), and (B) S-adenosyl-L-homocysteine hydrolase (SAHH).**

2024). A protein receptor used in present analysis, VAR2CSA, is a member of *Plasmodium falciparum* erythrocyte membrane proteins (PfEMPs), which are recognised as the molecular armaments of parasite for cellular invasion of erythrocytes, and are lead targets for vaccine and drug development (Lalchhandama, 2017; Su *et al.*, 2023). Our findings that *E. scholaris* compounds showed high binding affinities to the SAHH and VAR2CSA suggest their importance in anti-malarial investigation. However, the results by no means established that these compounds could be directly used, but suggest that their chemical nature that *E. scholaris* is an would help in the development of related or other compounds with higher safety and potency.

**Conclusion:** The study revealed antimalarial plant as used in Mizo and other traditional systems. *In vitro* assay confirmed that the bark extracts had high activity against both drug- sensitive and drug-resistant strains of *P. falciparum.* The chloroform-based extract was more efficacious with $IC_{50}$ values of 14.0 and 10.0 µg $mL^{-1}$ against drug-sensitive and drug-resistant strains respectively, compared to the petroleum-based extract which exhibited $IC_{50}$ values of 22.0 and 26.7 µg $mL^{-1}$, respectively. Both plant extracts were effective against multidrug-resistant strain, while standard drug, chloroquine, was utterly ineffective. The plant extracts are highly non-toxic upon normal cell lines, suggesting its possible safety as a medication. The major compounds identified using GC-MS included *cis*-3-hexenyl acetate and 3-decyn-2-ol in chloroform-based extract, and phthalic acid, 2-ethylbutyl nonyl ester and linoelaidic acid in petroleum ether extract. Computation modelling of chemical interaction of these compounds on vital proteins revealed high ligand-receptor binding capacity. These findings foster the medicinal application of plant and further enquiry into the molecular mechanisms of action and clinical aspects of the plant and its compounds.

**Conflict of interest:** The authors declare that they have no conflict of interest.

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**Ethical consent:** The study was approved by the Institutional Animal Ethics Committee of Pachhunga University College (vide PUC-IAEC-2022-Z02 of 15/03/2022).

**Authors' contribution:** All authors listed have made a substantial, direct and intellectual contribution to the work, and approved the manuscript for publication. PBL performed chemical analysis and experiments. LNM performed field works, plant identification and extraction. BLR performed computation studies and data generation. LND conceived the project and supervised the research. KLC conceptualised the experiments, acquired funds, interpreted the data, and prepared the draft. All authors read and approved the final manuscript.

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