



RESEARCH ARTICLE

# Evaluation of antimalarial potential of *Artemisia nilagirica* against chloroquine resistant and sensitive strains of *Plasmodium falciparum*

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## Abstract

Malaria represents a major vector borne global health problem. The greatest challenge in the malarial treatment is due to the increasing resistance of parasite to antimalarial drugs. The rise of drug-resistant malaria parasites is undermining the effectiveness of more potential drugs. Consequently, there is an urgent requirement for novel antimalarial compounds to manage this disease. Therefore, the current investigation is designed to analyze the antimalarial properties of *Artemisia nilagirica* against chloroquine (CQ) resistant K1 strain and chloroquine-sensitive 3D7 strain through different solvent extracts of various plant parts (root, stem, leaf and flower). Among the multiple extracts tested, the methanolic flower extract exhibited the highest antiplasmodial activity against CQ resistant K1 strain (IC<sub>50</sub>= 5.76 µg/mL) and CQ-sensitive 3D7 strain (IC<sub>50</sub>= 6.24 µg/mL) respectively. The lowest values of antimalarial activity were reported in aqueous extract of root against CQ resistant K1 strain (IC<sub>50</sub>= 68.83 µg/mL) and CQ sensitive 3D7 strain (IC<sub>50</sub>= 70.02 µg/mL). However, moderate activity was reported in chloroform, n-hexane, petroleum ether and ethanol extracts. The GC-MS investigation of methanol extracts of flower confirmed the availability of specific bioactive compounds like bicyclo (2.2.1) heptane-2-one 1,7,7-trimethyl, bicyclo (3.1.1) heptanes 2,4,6-trimethyl, 1,6-cyclodecadiene 1- methyl-5- methylene-8-(1-methyl ethyl) and 3,3-iminosprolamine, 3- methyl-3,5 (cyanoethyl) tetrahydro-4-thiopyranone which are responsible for antiplasmodial activity. The present study's findings confirm the potential antimicrobial activity of flower methanolic extracts of *A. nilagirica* against CQ resistant and sensitive strains of *P. falciparum*.

## Keywords

antimalarial activity; *Artemisia nilagirica*; chloroquine resistant; chloroquine sensitive; *Plasmodium falciparum*

## Introduction

Malaria imposes large socioeconomic impact on population throughout the world and is being considered as a major global public health challenge (1, 2). Mostly 608000 deaths and 249 million infections are recorded globally in 2022 whereas 5511 deaths and 33.8 lakh infections registered in India (3). In India, the highest numbers of malarial cases are reported from the state Odisha, contributing 25% of a total of 1.5-2 million reported annually. Moreover, the malaria infection in Odisha due to *P. falciparum* is 39.5%, which needs serious attention as these species are

gradually becoming predominantly resistant to various antimicrobial drugs (4, 5). Therefore, plant bioactive compounds with effective and controlling efficiency against malaria parasites may be an alternative and safe option to control this disease (6, 7).

Despite various efforts to reduce morbidity and mortality, the eradication of this disease is still at risk, resulting in a socioeconomic burden throughout the globe. The rise in drug-resistant parasite strains undermines efforts to develop potent antimalarial medications, especially without clinically approved vaccines. Artemisinin-based combination treatments continue to be the primary and secondary treatment choices for managing malaria (8); however, a strong, effective product is required to completely cure of the vector-borne malaria (9). Malaria in pregnant women, young children and immune deficient persons are mainly susceptible to experiencing severe complications (10). However, the majority of deaths from malaria are attributed to infections caused by *P. falciparum*, which may be exacerbated by the emergence of resistance to chloroquine (11).

On the other hand, plant-based natural and/or traditional medicines are recognized due to diverse biologically active compounds. Some plant species have undergone screening for their anti-plasmodial properties, indicating their active potential sources for developing antimalarial drugs (12-14). Plant-derived natural products are used in mosquito management due to their effective treatment and nontoxic effect on hosts (15, 16). Synthetic quinine (derivatives of CQ) has been used for decades to treat malaria (17). However, it is now gradually acquiring resistance to the parasites that cause malaria (18) through alteration in PfCRT and PfMDR genes, accountable for the transport of chloroquine to food vacuole of *P. falciparum* (19-21) and hence CQ lost its effectiveness.

Additionally, the application of 2 or more drugs with different modes of action insist on developing resistance strategies against CQ (22). As per the WHO recommendation, artemisinin-based combination therapies (ACT) are the initial and most effective approach to treatment against normal *P. falciparum* malaria in different countries (10). The dissemination and resistance patterns of malaria have rendered it a critical vector-borne concern, thereby necessitating the development of innovative antimalarial pharmaceuticals.

*Artemisia nilagirica* (Clarke) Pamp (the Indian wormwood) is a medicinal, aromatic and endangered plant that belongs to the family Asteraceae (7). This species is prevalent across various countries, including India, China, Japan and Afghanistan. In India, it is found in Uttarakhand, Tamilnadu, Rajasthan, Himachal Pradesh and Sikkim (23). It is widely used as antimicrobial, insecticidal, anti-inflammatory, anti-cancer and repellent activity (24-27). However, few records are available about the comparative analysis of the antimalarial activity of *A. nilagirica* (7, 28, 29). This study evaluated the anti-plasmodial efficacy of various extracts from *A. nilagirica* against chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum*.

## Materials and Methods

### Plant materials collection

Plant collection, maintenance and identification were done as

mentioned in our previously published paper (7). Healthy selected plant parts like root, stem, leaf and flower were considered for the study.

### Plant extracts preparation

The collected root, stem, leaf and flower samples were washed under running tap water and then air-dried in the shade at a temperature range of 27-37 °C. 250 g of each sample was powdered mechanically and extracted using methanol, ethanol, chloroform, petroleum ether, n-hexane and distilled water in a Soxhlet apparatus at 60-80 °C for 72 hr. The extracts were concentrated to 0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL, using a pressure range of 22-26 mmHg at 45 °C. The resulting residues were weighed and stored at -4 °C.

### Serum collection and processing

AB-positive blood was obtained from the blood bank of Srirama Chandra Bhanja Medical College and Hospital, Cuttack, Odisha, India. The blood was centrifuged at 2500 rpm to segregate serum from RBCs. After collecting serum from at least seven donors, it was portioned into 40-50 mL aliquots and frozen at -20 °C. Subsequently, the serum was subjected to a 30 min inactivation at 56 °C. Finally, the processed serum was stored in a deep freezer at -20 °C for future applications.

### In vitro cultivation of P. falciparum

CQ resistant (K1 strain) and CQ sensitive (3D7 strain) strains of *P. falciparum* were maintained in blood culture medium utilized to test different plant extracts' anti-plasmodial efficacy. These parasite cultures were maintained at Regional Medical Research Centre, Bhubaneswar, Odisha, India, by adopting the methods with minor modification (30). A fresh medium was prepared by taking 10% AB positive serum with 5% hematocrit in RPMI-1640 (Sigma-Aldrich), including 25 mM HEPES buffer, 0.2% sodium bicarbonate, glucose and 40 µg/mL gentamycin sulphate. This culture was then maintained in a CO<sub>2</sub> incubator at a temperature of 37 °C. The culture was routinely propagated by transferring the infected erythrocytes into a newly prepared medium.

### Antiparasite activity evaluation

After a range of incubation periods (0-72 hr at the interval of 8 hr), the contents of the well were collected and stained. The developmental stages of *P. falciparum* were documented by Giemsa staining (10% for 30 min). Parasitemia was observed by adding oil immersion with magnification 100 X and the calculation was made using the formula given in Equation 1 (31).

$$\text{Parasitemia percentage} = \frac{\text{Infected RBCs}}{\text{Total no. of RBCs}} \times 100$$

Eqn. 1

But the growth inhibition percentage is calculated by the formula as given in Eqn. 2.

$$\text{Growth Inhibition} = \frac{\text{Parasitemia in the treated group}}{\text{Parasitemia in negative control}} \times 100$$

Eqn. 2

Meanwhile, 50% growth inhibition was calculated in terms of IC<sub>50</sub> using probit analysis.

### Phytochemical screening by GC-MS analysis

The flower samples are crushed into fine powder by using an electric blender. The relative humidity and temperature should be maintained to ensure the preservation and integrity of the bioactive components. The powdered floral parts of the plant undergo Soxhlet extraction utilizing a variety of solvents and the collected extracts are filtered through a 0.45 µm filter. Using a split ratio 10:1, 2 mL of sample was introduced into GC-MS (Agilent Technologies, USA). Helium gas served as the carrier gas at a 1 mL/g flow rate. Initially, the column temperature was maintained at 60 °C for 2 minutes, then gradually increasing to 160 °C for 5 min with a rate of 5 °C/min. The spectra of the compounds were analyzed by comparing the spectra available in the NIST library.

### Statistical analysis

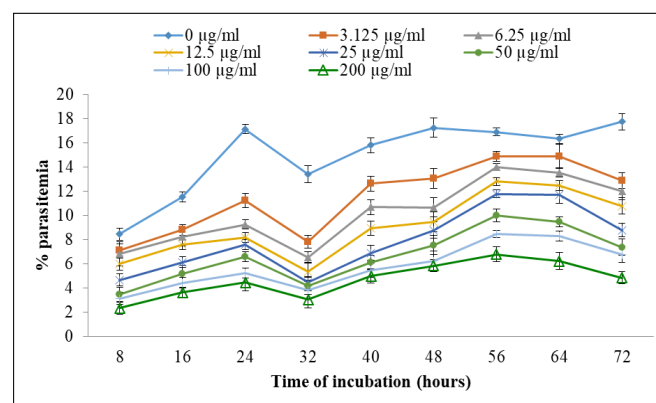
All the results are the mean of three independent experiment replicated (n=6) and the data are reported as mean ± standard error.

## Results and Discussion

### Antiplasmodial activity

The antiplasmodial activity of plant-derived extracts was expressed as a 50% inhibitory concentration (IC<sub>50</sub>) and the results were documented in Tables 1-3. The interpretation of the antimalarial results was categorized as very high activity (IC<sub>50</sub> ≤ 10 µg/mL), moderate activity (IC<sub>50</sub> of 11-50 µg/mL), low activity (IC<sub>50</sub> of 50-100 µg/mL) and inactive (IC<sub>50</sub> of ≥100 µg/mL) as per the effectiveness. The percentage of parasitemia, the rates of growth inhibition and IC<sub>50</sub> values were assessed at 8 hr intervals throughout 72 hr of incubation. The highest inhibition rate was observed in the methanolic flower extracts of *A. nilagirica* against the CQ-resistant K1 (5.76 µg/mL) and the CQ-sensitive 3D7 (6.24 µg/mL) strain after 32 hr of incubation. Whereas in flower extract, moderate anti-plasmodial activity was found in chloroform (7.09

µg/mL), n-hexane (9.88 µg/mL), petroleum ether (10.24 µg/mL), ethanol (11.37 µg/mL) and aqueous extract (50.15 µg/mL) against the CQ resistant K1 strain (Table 1). Similarly, against the CQ-sensitive 3D7 strain, inhibition rates of 8.01, 10.05, 11.78 and 12.65 µg/mL were observed in chloroform, n-hexane, petroleum ether and ethanol extract respectively (Table 2). Among all the solvent extracts, the root aqueous extract was the least potent, with IC<sub>50</sub> values of 68.83 and 70.02 µg/mL against the K1 and 3D7 strains respectively, after 32 hr of incubation (Table 3). The different concentrations of methanolic flower extracts (0, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL) were applied to analyze the growth inhibition of parasites against *P. falciparum* K1 strain (Fig. 1). The maximum parasitemia percentage was reported in 200 µg/mL concentration of methanolic extract of flower after 32 hr of incubation, followed by 100 µg/mL and 50 µg/mL concentration (Fig. 1). Throughout various phases of the experiment, both parasitized. Uninfected RBCs were examined microscopically, that are illustrated in Fig. 2. Similar results of growth inhibition percentage were also observed in 200 µg/mL concentration of methanolic flower extracts followed by 100, 50, 25, 12.5, 6.25, 3.125 µg/mL of concentrations. However, the maximum



**Fig. 1.** Parasitemia percentage of *P. falciparum* K1 strain in various concentrations of methanolic extracts *A. nilagirica* flowers after incubation of 8 to 72 hr. The data represents mean ± SE of replicates (n=6).

**Table 1.** The IC<sub>50</sub> values of various solvent extracts of flowers of *A. nilagirica* on the growth of *P. falciparum* (K1 strain)

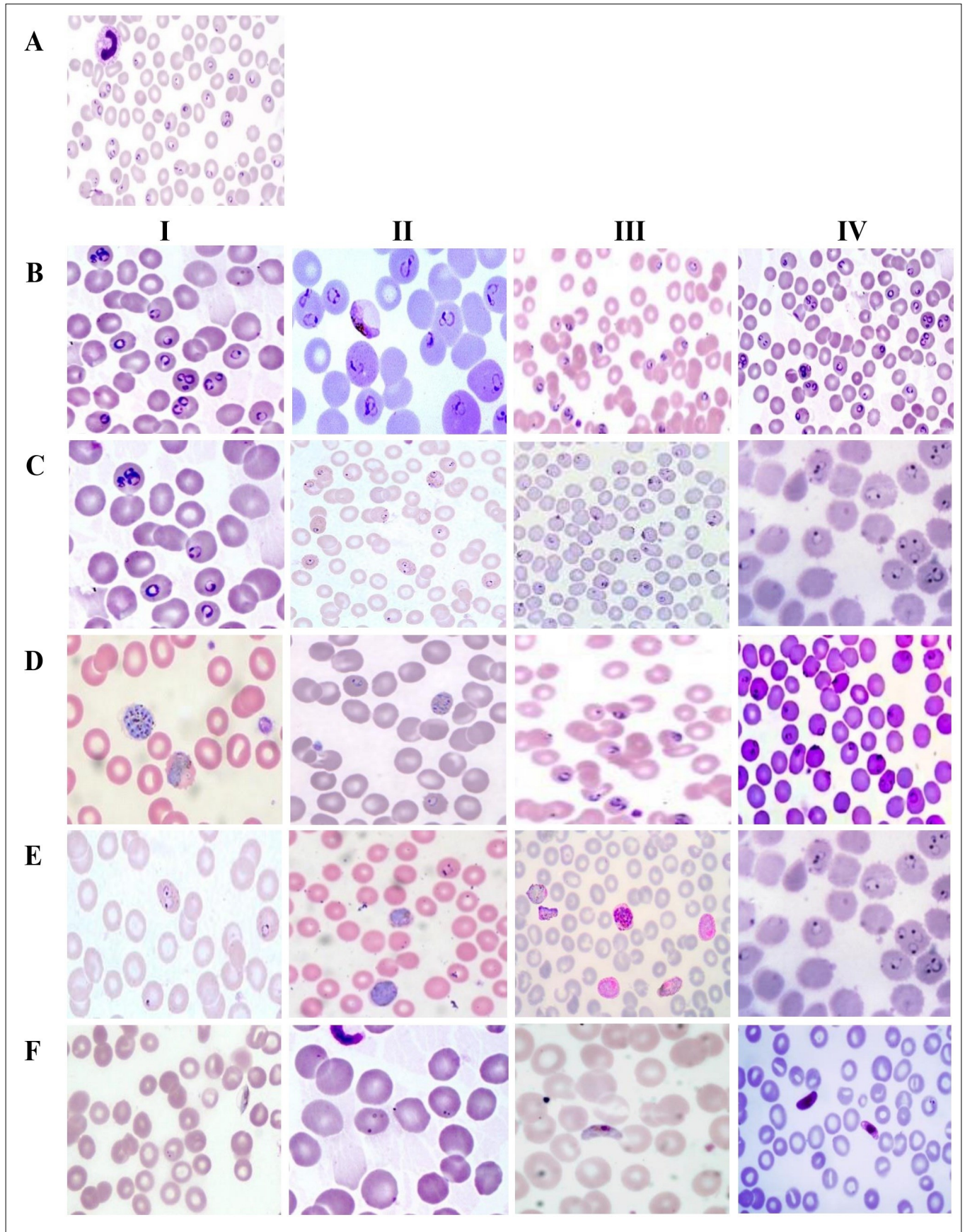
Observation (hr)	IC <sub>50</sub> (µg/mL)					
	Methanol	Chloroform	n-Hexane	Petroleum Ether	Ethanol	Aqueous
8	21.93 ± 3.16	22.74 ± 73.49	24.51 ± 3.14	25.92 ± 3.04	27.09 ± 3.46	62.21 ± 7.49
16	34.19 ± 3.88	37.34 ± 3.75	39.34 ± 4.26	41.2 ± 4.50	43.81 ± 5.03	75.92 ± 10.59
24	10.24 ± 1.43	13.44 ± 1.54	17.86 ± 1.77	18.43 ± 2.08	20.54 ± 2.59	55.25 ± 7.03
32	5.76 ± 0.82	7.09 ± 1.09	9.88 ± 1.13	10.24 ± 1.52	11.37 ± 1.77	50.15 ± 6.16
40	16.35 ± 2.01	16.04 ± 1.65	20.25 ± 3.01	21.17 ± 3.34	21.78 ± 2.91	58.46 ± 5.21
48	26.66 ± 3.74	31.18 ± 3.83	35.12 ± 4.05	37.56 ± 4.11	39.71 ± 5.76	73.83 ± 9.11
56	96.18 ± 13.22	106.37 ± 17.48	112.26 ± 15.33	104.09 ± 20.10	110.82 ± 18.16	143.59 ± 27.51
64	93.96 ± 11.06	97.84 ± 12.26	98.48 ± 12.67	99.31 ± 12.89	100.73 ± 11.12	128.04 ± 23.09
72	23.98 ± 2.66	24.62 ± 3.22	25.16 ± 2.82	27.34 ± 3.34	28.55 ± 3.46	65.06 ± 8.01

The data represents the mean ± SE of replicates (n=6).

**Table 2.** The IC<sub>50</sub> values of various solvent extracts of flowers of *A. nilagirica* on the growth of *P. falciparum* (3D7 strain)

Observation (hr)	IC <sub>50</sub> (µg/mL)					
	Methanol	Chloroform	n-Hexane	Petroleum Ether	Ethanol	Aqueous
8	23.03 ± 1.30	24.14 ± 4.32	26.11 ± 0.23	26.55 ± 5.01	28.0 ± 3.40	63.11 ± 7.01
16	35.01 ± 2.01	38.04 ± 3.12	40.04 ± 0.56	42.02 ± 1.20	45.01 ± 5.13	76.02 ± 1.03
24	11.33 ± 1.77	15.40 ± 1.50	18.06 ± 1.56	19.13 ± 2.77	21.14 ± 2.79	56.15 ± 3.12
32	6.24 ± 0.21	8.01 ± 0.71	10.05 ± 0.63	12.65 ± 0.48	12.65 ± 0.48	52.60 ± 0.09
40	17.05 ± 2.75	17.14 ± 1.02	21.05 ± 3.55	22.07 ± 2.21	22.50 ± 2.01	59.46 ± 1.12
48	27.44 ± 1.45	32.45 ± 4.02	36.22 ± 1.45	38.16 ± 3.14	40.11 ± 1.06	75.03 ± 2.14
56	97.98 ± 1.36	108.07 ± 1.80	114.09 ± 1.33	106.0 ± 2.15	111.02 ± 1.16	144.09 ± 2.11
64	90.76 ± 1.25	98.90 ± 1.11	99.08 ± 1.09	100.11 ± 1.90	102.03 ± 11.2	129.14 ± 2.09
72	25.08 ± 2.60	26.12 ± 5.23	26.06 ± 2.01	29.04 ± 3.11	29.05 ± 3.06	66.16 ± 4.11

The data represents the mean ± SE of replicates (n=6).

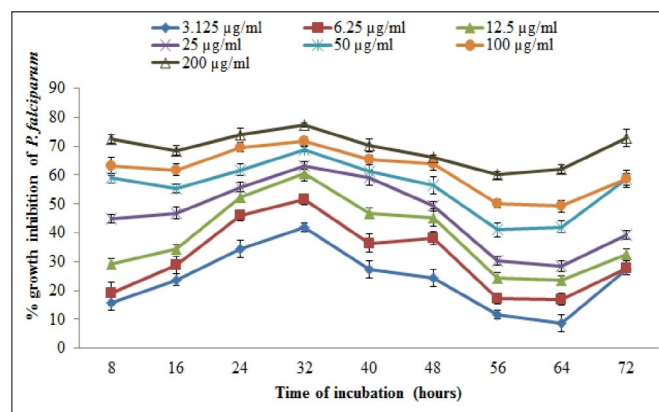


**Fig. 2.** Degree of parasitemia observed under microscope from methanolic extract of flower, leaf, stem and root of *A. nilagirica* on growth of *P. falciparum* K1 strain. Column- A: Control (without plant extract); B: 8 hr of incubation; C: 24 hr of incubation; D: 40 hr of incubation; E: 56 hr of incubation; F: 72 hr of incubation. Row- I: flower methanol extract; II: leaf methanol extract; III: stem methanol extract; IV: root methanol extract.

reduction in growth was noted after 32 hr of incubation across all tested concentrations (Fig. 3). There was a significant difference in  $IC_{50}$  between aqueous and methanolic extracts of *A. nilagirica* tested against K1 and 3D7 strains of *Plasmodium*. All organic solvent extracts exhibited higher activity than aqueous extracts, with flower extracts showing the highest activity. The high percentage yields (1.90%) were recorded against aqueous extracts of leaf of *A. nilagirica*, followed by aqueous stem (1.78%), ethanolic leaf (1.67%), methanolic leaf (1.63%), ethanolic root (1.50%), ethanolic stem (1.46%), methanolic stem (1.45%), aqueous root (1.43%) and flower (1.30%). However, the minimum yield of 0.10% was recorded in flower petroleum ether extract (Table 3).

Over many years, the exploration of plant-based medicine has steadily risen, serving as a key method in discovering beneficial herbs and new chemical entities to combat illnesses such as malaria. Traditional medicine remains one of the most favoured forms of healthcare globally (32). The main issue of the recent malarial control program is the establishment of resistance to the first line of antimalarial medications (33). The scientific community ensures using plant-based products, which are inexpensive and efficient antimalarial medicines (34). The antimalarial activity of ethanolic extract of *Avicennia africana* was represented with  $IC_{50} = 49.30 \mu\text{g/mL}$  against parasite *P. berghei* and the same extract was tested against mice at 1500 mg/kg with 100% parasitic inhibition with  $LD_{50} > 5000 \text{ mg/kg}$  in mice (35). Similarly, the ethanolic extracts of the stem and bark of *Terminalia macroptera* were tested for antimalarial and antioxidant activities in mice infected with *P. berghei* and found to have inhibitory action against the malaria parasite (36). It was also reported anti-plasmodium activities of three leaf extracts (n-hexane, ethyl acetate and ethanol) of *Sonchus aevensis* against *P.*

*falciparum* 3D7 strain with  $IC_{50}$  values  $5.119 \pm 3.27 \mu\text{g/mL}$ ,  $2.916 \pm 2.34 \mu\text{g/mL}$  and  $8.026 \pm 1.23 \mu\text{g/mL}$ , respectively (37). These results align with our study, which found that n-hexane and ethanol flower extracts had  $IC_{50}$  values of 9.88 and 11.37  $\mu\text{g/mL}$  respectively. The antimalarial activity of *Annona muricata* ethanol extract was studied under an *in silico* approach against the *P. berghei* NK-65 strain and confirmed the hypolipidemic effect of plant extracts that completely deprives the essential lipid molecule for parasite (38). It was reported the n-hexane and ethanolic leaf and bark extract of *P. berghei* had antiplasmodial activity, but the maximum activity reported in ethanol bark (72.16% activity) extract that may be due to the various chemical interactions with active compounds of plants (39). It was discovered the antimalarial properties of ethanolic flower extracts of Asteraceae and Rubiaceae family against CQ-resistant *P. falciparum* with  $IC_{50}$  of *M. erythrophylla* 3.73  $\mu\text{g/mL}$ , *B. balsamifera* with  $IC_{50} = 5.94$  and 9.66  $\mu\text{g/mL}$  (40). Secondary



**Fig. 3.** Growth inhibition percentage of *P. falciparum* K1 strain in various concentrations of methanolic flower extracts of *A. nilagirica* after incubation of 8 to 72 hr.

**Table 3.** Extraction yield and antiplasmodial activity of various extracts and fractions from *A. nilagirica* ( $IC_{50}$  values) after 32 hr of cultivation.

Plant Extract/ Fraction	Plant parts	Extraction Yield(% w/w)	$IC_{50}$ ( $\mu\text{g/mL} \pm \text{SD}$ ) against <i>P. falciparum</i> K1 strain after 32 hr of incubation	$IC_{50}$ ( $\mu\text{g/mL} \pm \text{SD}$ ) against <i>P. falciparum</i> 3D7 strain after 32 hr of incubation
Methanolic	Root	1.20	$10.73 \pm 0.71$	$11.09 \pm 0.41$
	Stem	1.45	$8.36 \pm 0.89$	$9.07 \pm 0.50$
	Leaf	1.63	$7.33 \pm 0.02$	$8.11 \pm 0.01$
	Flower	1.09	$5.76 \pm 0.82$	$6.24 \pm 0.21$
Chloroform	Root	0.52	$12.0 \pm 0.90$	$13.12 \pm 0.40$
	Stem	0.60	$10.01 \pm 0.08$	$10.88 \pm 0.84$
	Leaf	0.68	$9.24 \pm 1.01$	$9.67 \pm 0.20$
n- Hexane	Flower	0.32	$7.09 \pm 1.09$	$8.01 \pm 0.71$
	Root	0.15	$15.99 \pm 0.45$	$16.68 \pm 0.90$
	Stem	0.20	$12.08 \pm 0.81$	$12.98 \pm 0.55$
Petroleum ether	Leaf	0.24	$10.18 \pm 0.23$	$11.77 \pm 0.80$
	Flower	0.11	$9.88 \pm 1.13$	$10.05 \pm 0.63$
	Root	0.23	$17.16 \pm 1.16$	$18.83 \pm 0.71$
Ethanolic	Stem	0.30	$13.73 \pm 0.33$	$14.27 \pm 0.22$
	Leaf	0.35	$11.03 \pm 0.88$	$12.75 \pm 0.14$
	Flower	0.10	$10.24 \pm 1.52$	$11.78 \pm 0.71$
Aqueous	Root	1.50	$20.90 \pm 0.03$	$21.87 \pm 0.05$
	Stem	1.46	$14.57 \pm 0.08$	$15.44 \pm 0.17$
	Leaf	1.67	$12.03 \pm 1.23$	$13.0 \pm 0.63$
	Flower	1.28	$11.37 \pm 1.17$	$12.65 \pm 0.48$
	Root	1.43	$68.83 \pm 0.65$	$70.02 \pm 0.10$
	Stem	1.78	$64.71 \pm 0.76$	$65.10 \pm 0.44$
Aqueous	Leaf	1.90	$52.43 \pm 0.81$	$53.71 \pm 0.20$
	Flower	1.30	$50.15 \pm 6.16$	$52.60 \pm 0.09$

The data represents the mean  $\pm$  SE of replicates (n= 6).

metabolites like flavonoids, tannins, anthraquinones, terpenoids, phenols and glycosides, among others, have demonstrated antimalarial properties in various plant extracts through different mechanisms. However, due to their antioxidant capabilities, flavonoids may contribute to this antimalarial effect (41). Antioxidant substances can obstruct hemozoin formation, which is crucial because free heme is highly harmful to the malaria parasite (42). Furthermore, metabolites like glycosides are proven to have direct antiplasmodial impacts (43). Consequently, the antimalarial efficacy of plants may result from the individual or combined effects of these compounds.

### GC-MS analysis

After confirmation of antiplasmodial properties, GC-MS analysis was conducted by taking the methanolic flower extract of *A. nilagirica*. This process revealed a variety of bioactive compounds, each exhibiting distinct phytochemical activities. The chromatogram obtained is depicted in Fig. 4, while the discovered phytocomponents are presented in Table 4 with details of their retention times, molecular weights, peak areas and biological functions. Notably, phytocompounds with mosquitocidal and/or larvicidal properties, such as N-acetyl norephedrine, 1,6-cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl), naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) and alpha-caryophyllene, were identified. Further compounds like bicyclo(2.2.1)heptan-2-one,1,7,7-trimethyl (Dihydro-artemisinin), bicyclo(3.1.1)

heptane,2,4,6 -trimethyl, 1,3-propanediamine, N-(2- aminoethyl) and 1,6-cyclodecadiene,1-methyl-5-methylene-8-(1-methylethyl) are recognized that are responsible for antimalarial activity. These findings further support the antimalarial capabilities of the *A. nilagirica* flower extracts.

GC-MS qualitative analysis of *A. nilagirica* flower methanol extracts has revealed a range of active compounds implicated in mosquitocidal, larvicidal and antimalarial activities. Predominantly, these compounds are classified as sesquiterpenes, sesquiterpenoids and alkaloids, which are recognized for their strong antimalarial properties (40, 44). Sesquiterpene lactones are gaining attention as promising leads

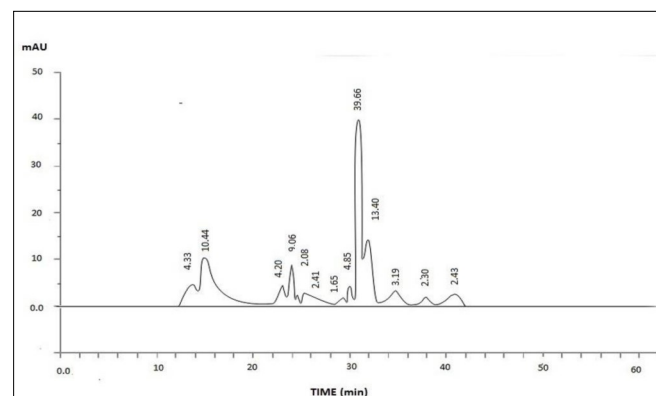


Fig. 4. Chromatogram of methanol flower extract of *A. nilagirica* by GC-MS analysis

Table 4. Identified phytocomponents from the methanol flower extract of *A. nilagirica* by GC-MS analysis

Name of the compound	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)	Biological activity
Myo-inositol	C <sub>6</sub> H <sub>12</sub> O <sub>6</sub>	180.16	31.943	39.66	Anti-cancer, anti-convulsant and anti-HIV activity
1,4-dioxin-2-yl-alpha-methylfuranoside	C <sub>9</sub> H <sub>18</sub> O <sub>6</sub>	222.237	31.943	39.66	Carbohydrate derivatives, gap junction forming compounds
N, N-Dimethylvaleramide	C <sub>7</sub> H <sub>15</sub> NO	129.203	32.029	13.40	Analgesic activity
Thiophene, tetrahydro-2-methyl	C <sub>5</sub> H <sub>10</sub> S	102.198	32.029	13.40	Antiinflammatory activity, Insecticidal activity
L-Asparagine	C <sub>4</sub> H <sub>8</sub> N <sub>2</sub> O <sub>3</sub>	132.119	32.029	13.40	Osmoprotectant
Aminopyrazine	C <sub>3</sub> H <sub>5</sub> N <sub>3</sub>	95.105	15.539	10.44	Antimicrobial and anti-tubercular agents
Isoborneol	C <sub>10</sub> H <sub>18</sub> O	154.253	15.539	10.44	Antibacterial activity
Cyclopentene,1,2,3 trimethyl	C <sub>8</sub> H <sub>14</sub>	110.2	15.539	10.44	Anti-fungal activity
2H-1-Benzopyran-2-one	C <sub>9</sub> H <sub>6</sub> O <sub>2</sub>	146.145	24.503	9.06	Component of anticoagulants, antimicrobial, antiinflammatory and antioxidant activity
N- Acetylnorephedrine	C <sub>11</sub> H <sub>15</sub> NO <sub>2</sub>	193.242	30.911	4.85	Insecticidal and mosquitocidal activity
1,3-propane-diamine, N-(2- aminoethyl)	C <sub>5</sub> H <sub>15</sub> N <sub>3</sub>	117.196	30.911	4.85	Insecticidal and antimalarial activity
L- Alanine, N- (1-oxopentyl)-methyl-1-ester	C <sub>7</sub> H <sub>15</sub> NO <sub>5</sub>	193.197	30.911	4.85	Anti-cancer activity
Bicyclo(2.2.1)heptane-2-one,1,7,7-trimethyl (Dihydro-artemisinin)	C <sub>10</sub> H <sub>16</sub> O	152.233	14.731	4.33	Insect repellent and insecticidal activity, Antimalarial activity
Caryophyllene	C <sub>15</sub> H <sub>24</sub>	204.357	23.84	4.20	Insecticidal activity
Bicyclo(3.1.1) heptane,2,4,6 -trimethyl	C <sub>10</sub> H <sub>18</sub>	138.249	35.04	3.19	Anti-cancer and antioxidant compounds
3,7,11,15-Tetramethyl-1-2-hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O	296.531	35.04	3.19	Antimalarial activity
3-Eicosyne	C <sub>20</sub> H <sub>38</sub>	278.524	41.139	2.43	Antioxidant activity, Insecticidal activity
Citronellylisobutyrate	C <sub>14</sub> H <sub>26</sub> O <sub>2</sub>	226.355	41.139	2.43	Palmitic acid
1,6-Cyclodecadiene,1-methyl-5-methylene-8-(1-methyl ethyl)	C <sub>15</sub> H <sub>24</sub>	204.351	25.672	2.41	Insect repellent activity
Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methyl ethyl)	C <sub>14</sub> H <sub>24</sub>	201.357	25.672	2.41	Mosquito larvicidal and Antimalarial activity
n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	985	38.292	2.30	Mosquito repellent
Alpha-caryophyllene	C <sub>15</sub> H <sub>24</sub> O	220.356	24.855	2.08	Lipid derivative
Tetraacetyl -d-xylonic nitrile	C <sub>14</sub> H <sub>17</sub> NO <sub>9</sub>	343.288	29.526	1.65	Insecticidal and mosquitocidal activity
3,3 -Iminosprolamine	C <sub>10</sub> H <sub>25</sub> N <sub>3</sub>	187.33	29.526	1.65	Antiasthematic and antiinflammatory activity
3-methyl-3,5-(cyanoethyl) tetrahydro-4-thiopyranone	C <sub>12</sub> H <sub>16</sub> N <sub>2</sub> OS	236.333	29.526	1.65	Antioxidant activity
					Insect repellent

for creating drugs that block disease transmission, showing considerable potential. Additionally, artemisinin and its derivatives stand out as the most extensively studied compounds in this category, common in tested plants (45). Several groups investigated the GC-MS analysis of *A. nilagirica*. However, the studies are restricted to leaf, root and stem (46, 47).

## Conclusion

The current research reveals the methanolic extract from *A. nilagirica* flowers exhibits significant antimalarial properties against CQ-sensitive 3D7 and CQ-resistant K1 strains of *P. falciparum*. The study confirms a substantial decrease in plasmodial count within infected blood at higher extract concentrations, highlighting its potential as a malarial treatment. Additionally, an assessment of antiplasmodial activity of the flower extracts suggests that *A. nilagirica* could be a source for new antimalarial medications. The finding supports the antiplasmodial nature of the extracts, lending credence to their traditional use in combating malaria. Employing tissue culture techniques could facilitate further research into this plants' pharmacological and phytochemical properties, potentially leading to the discovery of new active compounds and aiding in its conservation. Further research into these extracts is recommended to develop a stable galenic formulation, which may enhance traditional medicine and aid in progressing from malaria control to pre-elimination. Moreover, additional studies are necessary to identify and characterize the active constituents using HPLC-MS and NMR spectroscopy techniques.

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## Authors' contributions

SP performed the experimental work and wrote the initial draft, SLS and MR conceptualized and supervised the work, JRR done data analysis and extensively edited the whole manuscript which was then reviewed and approved by all authors.

## Compliance with ethical standards

**Conflict of interest:** The authors declared that they have no competing interests.

**Ethical issues:** None

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