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Antimalarial activity of *Carica papaya* (Family: Caricaceae) leaf extract against *Plasmodium falciparum*

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ABSTRACT

Objective: To determine the antimalarial activity of the ethanol leaf extract of *Carica papaya (C. papaya)*, blood stages of CQ-sensitive and CQ resistant strains against *Plasmodium falciparum (P. falciparum)* as target species. **Methods:** *C. papaya* leaf was collected in and around Kalveerampalyam village, Coimbatore, Tamil Nadu, India. *C. papaya* leaf was washed with tap water and shade dried at room temperature. An electrical blender powdered the dried plant materials (leaves). The powder 500g of the leaf was extracted with 1.5 L of organic solvents of ethanol for 8 h using a Soxhlet apparatus. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 2%, 4%, 6%, 8% and 10%, respectively. **Results:** The highest larval mortality in the ethanol leaf extract of *C. papaya* against the 1st to 4th instars larvae and pupae values of LC_{50}= 3.65%, 4.28%, 5.41%, 6.70%, and 7.50%, respectively. The LC_{90} values of 9.61%, 11.75%, 13.53%, 16.36%, and 16.92%, respectively. Plant extracts showed moderate to good antiparasitic effects. These four concentrations (25, 50,100 and 150 μg/mL) of ethanol leaf extracts exhibited promising inhibitory activity against the CQ sensitive strain with (IC_{50}) values 40.75%, 36.54%, 25.30%, and 18.0% and in CQ resistant 50.23%, 32.50%, 21.45%, and 23.12% against *P. falciparum*. **Conclusions:** In conclusion, the results indicate the effective plant extracts have the potential to be used as an ideal eco-friendly approaches for the control of vector mosquitoes.

1. Introduction

Malaria is a vector-borne infectious disease that is widespread in tropical and subtropical regions. The term 'global change' is used to encompass all of the significant drivers of environmental change as experienced by hosts, parasites and parasite managers. The antimalarial potential of compounds derived from plants is proven by examples such as quinine, obtained from Cinchona species, and artemisinin, obtained from Artemisia annua. The selection of plants to be screened for antimalarial activity is done on the basis of traditional reputation of particular plants for efficacy in the treatment of malaria. Here, we studied a single plant used by traditional healers of the Western Ghats to treat "recurrent fever" and evaluated them *in vitro* for antimalarial activity. Scientists therefore have embarked on a mission to survey the flora extensively to discover more and more potential plants have insecticidal properties[1].

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Currently, there is a considerable increase in mortality caused by malaria due to the rapid spread of drug-resistant strains of *P. falciparum*. The asexual erythrocyte cycle of the human malaria parasite, *P. falciparum* causes severe forms of disease[2]. Invasion of an individual parasite into a red blood cell initiates the cycle; approximately 48 h later releases of 16 - 32 daughter parasites terminate the cycle to spread the infection. In South East Asia alone, 100 million malaria cases occur every year and 70% of these are reported from India[3]. The use of chloroquine (CQ) to prevent and treat falciparum malaria has led to the wide-spread appearance of CQ-resistant strains against *P. falciparum* throughout the affected regions. The resistance has at the same time increasingly extended to other available antimalarial drugs[4].

Carica papaya, belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases[5,6]. Originally derived from the southern part of Mexico, *C. papaya* is a perennial plant, and it is presently distributed over the whole tropical area. In particular, *C. papaya* fruit circulates widely, and it is accepted as food or as a quasi drug. Many scientific investigations have

been conducted to evaluate the biological activities of various parts of *C. papaya*, including fruits, shoots, leaves, rinds, seeds, roots or latex. The leaves of papaya have been shown to contain many active components that can increase the total antioxidant power in blood and reduce lipid peroxidation level, such as papain, chymopapain, cystatin, à-tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glucosinolates[7]. Fruit and seed extracts have pronounced bactericidal activities[8]. Leaves have been poulticed into nervous pains, elephantoid growths and it has been smoked for asthma relief amongst tropical tribal communities. The hypoglycemic effect of ethanolic extract of unripe, mature fruits has been reported[9]. Moreover, *C. papaya* leaf juice is consumed for its purported anti-cancer activity by people living on the Gold Coast of Australia, with some anecdotes of successful cases being reported in various publications. *C. papaya* leaf extracts have also been used for a long time as an aboriginal remedy for various disorders, including cancer and infectious diseases.

C. papaya contains two important biologically active compounds viz., chymopapain and papain which are widely used for digestive disorders[10]. It showed that papaya derived papain, caricain, chymopain, and glycerin endopeptidase can improve acidic pH conditions and pepsin degradation. Other active compounds of *C. papaya* are lipase, or CPL, a hydrolase, which is tightly bonded to the water-insoluble fraction of crude papain and is thus considered as a "naturally immobilized" biocatalyst[11]. According to the folk medicine, papaya latex can cure dyspepsia and also applicable for external burns and scalds. Seeds and fruits are excellent antihelminthic and anti-amoebic[12]. Dried and pulverized leaves are sold for making tea; also the leaf decoction is administered as a purgative for horses and used for the treatment of genetic-urinary system. Unripe and semi-ripe papaya fruits are ingested or applied on the uterus to cause absorption. However, the consumption of unripe and semi-ripe papaya fruits could be unsafe during pregnancy causes no risk[13]. The larvicidal properties of crude extract of three plants, viz., *C. papaya*, Murraya paniculata and Cleistanthus collinus against *C. quinquefasciatus* as target species. The relative efficacy of the plant extracts in vector control was as follows: *C. papaya* seed extract > *M. paniculata* fruit extract > *M. paniculata* leaf extract > *C. collinus* leaf extract. To examine the potential role of *C. papaya* as anti-cancer therapy, we analyzed in this report the anti-tumor activity of the aqueous extract of the leaves of *C. papaya* against various cancer cell lines, as well as its potential immunomodulatory effects, and attempted to identify the active components[14].

In the present study, an attempt has been made to screen and evaluate the larvicidal, pupicidal activity of *C. papaya* against malaria vector, *A. stephensi* and Invitro antiplasmodial activity of CQ-sensitive and CQ-resistant strains against malaria parasite, *P. falciparum*. These extract were tested for their antimalarial potential against malaria parasite, *P. falciparum*.

2. Materials and methods

2.1. Collection of eggs and maintenance of larvae

The eggs of *A. stephensi* were collected from National Centre for Disease Control (NCDC) field station of Mettupalayam, Tamil Nadu, India, using an "O" type brush. These eggs were brought to the laboratory and transferred to 18 cm×13 cm×4 cm enamel trays containing 500 mL of water for hatching. The mosquito larvae were fed with pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage. The pupae were collected from the culture trays and transferred to plastic containers (12 cm×12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in a 90 cm×90 cm×90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at (27+2) ℃, 75%-85% RH under a photoperiod of 14 L: 10D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

2.2. Blood feeding of adult A. stephensi

The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

2.3. Plant materials and preparation of plant extracts

C. papaya leaf was collected in and around Kalveerampalyam village, Coimbatore, Tamil Nadu, India. *C. papaya* leaf was washed with tap water and shade dried at room temperature. An electrical blender powdered the dried plant materials (leaves). The powder 500 g of the leaf was extracted with 1.5 L of organic solvents of ethanol for 8 h using a Soxhlet apparatus[15]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The crude plant extracts were evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations were prepared ranging from 2, 4, 6, 8 and 10%, respectively.

2.4. Larval/Pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty-five numbers of first to fourth instars larvae and puape were introduce into 500 mL glass beaker containing 249 mL of de-chlorinated water and 1 mL of desired concentrations of ethanolic leaf extract were added. Larval food was given for the test larvae. At each tested concentration two to five trials were made and each trial consisted of five replicates. The control was setup by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae and pupae were exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbott's formula[16]. The LC_{50} and LC_{90} were calculated from toxicity data by using probit analysis[17].

2.5. Parasite sample collection

Malaria positive blood samples were collected from K.M.C.H hospital, Coimbatore, Tamil Nadu, India. The samples are collected in EDTA tubes and stored at 4 ℃.

2.6. Staining and visualizing of parasites

The simple, direct microscopic observation of blood specimens to observe the malaria parasite is still the gold standard for malaria diagnosis. Microscopic diagnosis of malaria is performed by staining thick and thin blood films on a glass slide to visualize the malaria Parasite Parasites are stained using Leishman stain (0.15%). On light microscopic examination of the blood film the number, species, and morphological stage of the parasites can be reported.

In addition to providing a diagnosis of malaria the blood

smear can also provide useful prognostic information; the parasite count, number of circulating pigment-containing phagocytes and the presence of late asexual stages of the parasite are all positively correlated with a fatal outcome.

2.7. Culture of parasites

Parasites are grown in human erythrocytes in a settled layer of cells in RP-C: RPMI-Complete. Incomplete medium (RP-I) is prepared by dissolving 16.2 g of powdered RPMI 1640 (supplemented with L-glutamine and 25 mM HEPES buffer but without sodium bicarbonate. Complete medium (RP-C) is obtained by adding 4.2 mL of 5% sodium bicarbonate solution and 5 mL of 8% Albumax stock solution per 100 mL of RP-I. Parasites from cultures are added to the freshly washed erythrocytes to give a starting parasitemia between 0.1-1.0%. The cultures are provided appropriate atmosphere using the candle-jar method with 1% O_2, 5% CO_2, and 94% N_2 with 24-h medium changes, requiring subculture by addition of fresh erythrocytes every 4-5 days[18].

2.8. In vitro antiplasmodial assay

The antiplasmodial activity of the extract and test compounds was performed in 96-well tissue culture plates as described[19] with modifications reported[20]. Twofold serial dilutions of test samples dissolved in sterile methanol were placed in microtiter plates (Figure 1) and diluted with culture medium (RPMI 1640 plus 10% human serum). A suspension of parasitized erythrocytes (0.5-1% parasitaemia, 2.5% haematocrit) containing mainly trophozoites was added to the wells to give a final volume of 100 mL. Chloroquine was used as positive control and uninfected and infected erythrocytes were included as negative controls. The plates were incubated at 37 ℃ and after 24 and 48 h the culture medium was replaced with fresh medium with or without test samples.

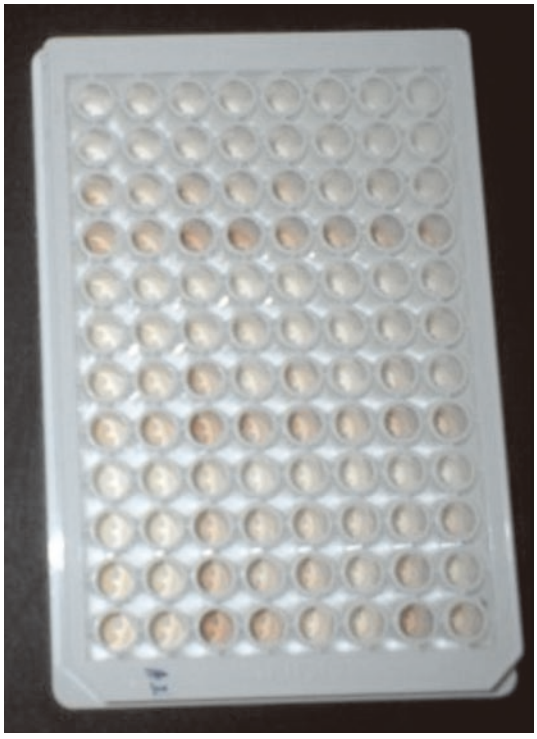

Figure 1. Round-bottom 96-well plates, with culture malarial parasite, *P. falciparum*.

After incubation for 24 h, Giemsa-stained thick blood films were prepared for each well, and the percentage of inhibition of parasite growth was determined (Figure 2) under microscope by comparison of the number of schizonts with three or more nuclei out of a total of 200 parasites with that of control wells. The percent inhibition at each concentration was determined and the mean of the least three IC_{50} values of parasite viability was calculated using mathematical log-concentration-response probit analysis.

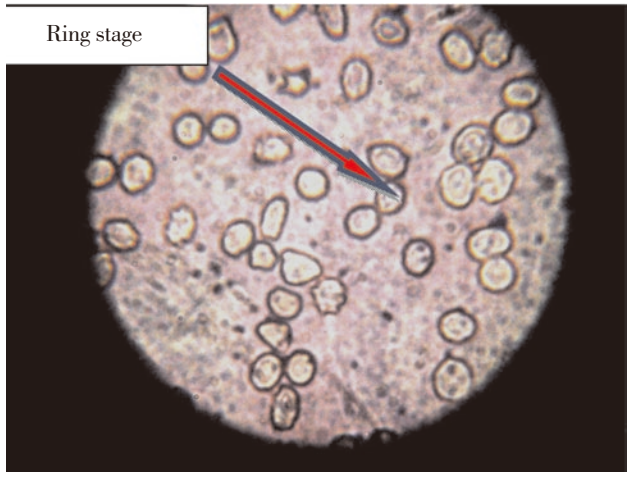

Figure 2. Ring stages of malarial parasite, *P. falciparum*.

2.9. Statistical analysis

All data were subjected to analysis of variance; the means were separated using Duncan's multiple range tests[21]. SPSS (Statistical software package) 9.0 version was used. Results with $P<0.05$ were considered to be statistically significant.

3. Results

Larvicidal and pupicidal activity of ethanol extract of *C. papaya* leaf extract (CPLE) at various concentrations against malarial vector, *A. stephensi* is given in the Table 1. Considerable mortality was evident after the treatment of CPLE for all larval instars and pupae. Mortality was increased as the concentration increased, for example, 41% mortality was noted in I instar larvae by the treatment of CPLE at 2% whereas it has been increased to 96% at 10% of CPLE treatment. Similar trend has been noted for all the larval instars and pupae of *A. stephensi* at different concentrations of CPLE treatment. The LC_{50} and LC_{90} values were represented as follows: LC_{50} value of I instar was 3.65%, II instar was 4.28%, III instar was 5.41%, IV instar was 6.70%, and pupa was 7.50%, respectively. The LC90 values of I instar was 9.61%, II instar was 11.75%, III instar was 13.53%, IV instar was 16.36%, and pupa was 16.92%, respectively.

Table 2 shows the antimalarial activity of ethanolic leaf extract of *C. papaya* against *P. falciparum*. These four concentration of ethanolic leaf extracts exhibited promising IC_{50} (μg/mL) of 25, 50, 100 and 150, respectively also against the CQ sensitive and control 40.75, 36.54, 25.30, 18.0 and 1.0 CQ resistant and control 50.23, 32.50, 21.45, 23.12 and 5.0 against malaria parasite, *P. falciparum*.

Table 1
Larval and pupal toxicity effect of *C. papaya* leaf extract against malarial vector, *A. stephensi*.

Mosquito larval instars and pupae	Percentage of larval and pupal mortality Concentration of *C. papaya* (%)					LC_{50} (LC_{90})	95% confidence limit		x^2
	2	4	6	8	10		LFL	UFL	
							LC_{50} (LC_{90})	LC_{50} (LC_{90})	
I	41.6±1.24^{de}	51.0±0.81^{de}	62.6±1.69^{de}	80.3±1.24^{e}	96.0±1.63^{e}	3.65 (9.61)	1.00 (7.76)	5.02(14.63)	6.61*
II	38.0±0.81^{d}	47.0±1.41^{d}	58.3±1.24^{d}	70.6±1.69^{d}	87.6±0.94^{d}	4.28(11.75)	3.39 (10.40)	4.98 (13.91)	2.63*
III	31.3±1.69^{bc}	41.0±1.63^{c}	50.6±0.94^{bc}	65.0±0.81^{c}	78.6±1.24^{cd}	5.41 13.53)	4.61 (11.82)	6.14 (16.37)	0.80*
IV	28.0±1.63^{b}	33.3±1.24^{b}	47.0±0.81^{b}	57.6±1.69^{b}	66.6±0.47^{b}	6.70 16.36)	5.85 (13.84)	7.69 (21.02)	0.46*
Pupa	21.6±1.24^{a}	32.3±1.69^{b}	43.0±0.81^{a}	53.0±1.63^{a}	62.3±0.94^{a}	7.50 (16.92)	6.66 (14.32)	8.62 (21.67)	0.18*

Table 2
In vitro antiplasmodial activity of the ethanol leaf extract of *C. papaya* against *P. falciparum*.

Con. (μg/mL)	IC_{50} values (%)	
	Sensitive	Resistant
25	40.8±3.6	50.2±1.0
50	36.5±3.6	32.5±2.0
100	25.3±1.0	21.4±2.6
150	18.0±1.7	23.1±2.6
Control	1.0±0.3	5.0±0.8

4. Discussion

Malaria is still the most important parasitic disease in the world and caused by the protozoans belonging to the genus Plasmodium. The *P. falciparum* causes severe malaria, and the protozoan comes in contact with humans by the bites of female Anopheles mosquitoes. Nearly 1 million deaths, mostly of children under the age of 5 years were caused by malaria. There are currently 109 malarias countries and territories, of which 45 are within the World Health Organization African region[22]. *C. papaya* is a tree-like herbaceous plant, a member of the small family Caricaceae and widely cultivated for its edible fruits and the leaves of the plant is used for the analysis. Natural products isolated from plants used in traditional medicine which have potent *in vitro* antiplasmodial action represent potential sources of new antimalarial drugs[23,24].

The biological activity of plant extract might be due to the various compounds including the important compounds are chymopapain, papain, *etc.*, exist in *C. papaya* and these compounds may jointly or independently contribute to produce larvicidal activity against *A. stephensi*. Many researchers have reported on the effectiveness of plant extract against mosquito larvae and the plant extract essential oils against vector mosquito larvae and the recent reports[25-32]. The hexane, chloroform, ethyl acetate, acetone and methanol extracts of the bark of *A. squamosa* have been reported as very active agents against the IV instars larvae of the malaria vector, *A. stephensi* and the lymphatic filariasis vector, *C. quinquefasciatus*[33]. The ethanol extract of whole plant Leucas aspera against the first to fourth instar larvae and pupae values of LC_{50} = 9.695, 10.272, 10.823, 11.303 and 12.732%, respectively[34,35]. The larvicidal activities of *L. aspera* whole plant of the different solvents of hexane, chloroform, ethyl acetate, and methanol extracts against *C. quinquefasciatus* (LC_{50} =126.91, 131.56, 129.63, and 96.87 mg/L; LC_{90} = 692.71, 638.92, 635.02, and 411.13 mg/L), respectively. The plant *S. xanthocarpum* leaf extract treatment at 24 h exposure; the LC_{50} values of first to fourth instars and pupae were 155.29, 198.32, 271.12, 377.44, and 448.41 mg/L, respectively and LC_{90} value of 687.14, 913.10, 1011.89, 1058.85 and 1,141.65 mg/L, respectively[36].

The present study reports the toxic effects of the selected plant against malarial vector, *A. stephensi*. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plant products produce a large variety of compounds that increase their resistance to insect attack[37,38]. Differential susceptibilities of larva of three mosquito species to petroleum ether extract of *Acorus calamaus* L. Citrus medica[39]. The crude extract of the fruit pods from Swartzia madagascariensis Desvaux produced higher mortality inlarva of Anopheles gambiae (Giles) than larva of *A. aegypti* but was ineffective against larvae of C. quinquefasciatus[40]. Larvicidal activity of Pseudocalymma alliaceum and Allium sativum against *C. quinquefasciatus*[41]. Ethanol extracts of Allium sativum (Garlic bulb) against the filarial vector, *C. quinquefasciatus*[42]. In the present results, the highest larval mortality of the ethanol leaf extract of *C. papaya against* the I to IV instars larvae and pupae values of LC_{50}= I instar was 3.65%, II instar was 4.28%, III instar was 5.41%, IV instar was 6.70%, and pupa was 7.50%, respectively. The LC_{90} values of I instar was 9.61%, II instar was 11.75%, III instar was 13.53%, IV instar was 16.36%, and pupa was 16.92%, respectively.

The methanol extract of A. squamosa leaves showed high antiplasmodial activity with IC_{50} values of 2 and 30 μg/mL against CQ-sensitive strain 3D7 and CQ-resistant strain Dd2 of *P. falciparum*, respectively[43]. Antiplasmodial activity of Solanum nudum steroids against blood stages of *P. falciparum* was recorded[44]. Previous studies for antiplasmodial properties in Z. zanthoxyloides have been conducted, but were focused only on the roots which are not recommended for specie preservation, and the parasite strains used were the sensitive *P. falciparum* line, 3D7 and IC_{50} reported was 4.9 μg/mL for crude extracts[45]. The leaves and twigs extracts of *R. mucronata* were showed antiplasmodial activity against *P. falciparum* strain D10, respectively[46].

The extracts from Desmopsis panamensis, Pseudomalmea boyacana, Rollinia exsucca, and Rollinia pittieri (Annonaceae) showed good antiplasmodial activity (IC_{50}<10 μg/mL), respectively, against CQ-sensitive (F32) and CQ-resistant (W2) *P. falciparum*[47]. The compounds (+)-3- acetylaltholactone, goniotriol, (+)-altholactone, and an aporphine alkaloid; (-)-nordicentrine were isolated from flowers of Goniothalamus laoticus exhibited antiplasmodial activity against *P. falciparum* (IC_{50} 2.6, 7.9, 2.6, and 0.3 μg/mL), respectively[48]. The leaf ethyl acetate extracts of A. squamosa displayed a moderate activity against *P. falciparum* (IC_{50} 3D7, 33 μg/mL)[49] and the emodin and lupeol were isolated from the ethyl acetate fraction of stem bark of Cassia siamea found to be the active principles responsible for the antiplasmodial property with IC_{50} values of 5 μg/mL, respectively, against the multi-drug-resistant strain of *P. falciparum*[50]. The lowest parasitemia inhibition rate (20.0%) was observed at 25 μg/mL concentrations of the silver nanoparticles from Catharanthus roseus. The parasitemia inhibitory concentration values for silver nanoparticles from *C. roseus* were 20.0, 41.7, 60.0 and

75.0% for 25, 50, 75 and 100 μg/mL, respectively[51].

In our experience, culture of the infected cell line was possible for up to 48 h, this was enough to obtain trophozoites on hepatocytes, and thus, with this methodology, it is possible to study the effect of natural compounds during this period. However, to evaluate the activity of the compounds on schizonts or mature stages, it is essential to improve the culture conditions in order to extend the observation period up to 120 h (5 days), as previously described[52,53]. The petroleum ether extracts of the rind of *C. papaya* and *Citrus sinensis* also showed antimalarial activity against strain *P. falciparum* FCK 2 *in vitro*[54]. The isolation of active antiplasmodial components from reputed traditional antimalarial medicinal plant species such as Creptolepis sanguinoleta, Dichroa febrifuga, Neurolaena lobata, Vernonia brachycalyx, Khaya grandifolia, and Nardostachys chinensis has shown promising *in vitro* as well as *in vivo* antiplasmodial activity[55-57].

In earlier, *A. paniculata* was used to control different parasites[58,59]. *In vitro* antiplasmodial activity of medicinal plant extracts against malaria parasite, *P. falciparum*[60]. The isolated 20 quinones from Cassia alata, Cassia occidentalis, Garcinia kola and Ocimum basilicum and investigated the *in vitro* antiplasmodial activity against *P. falciparum*[67]. Six of the 20 isolated quinines were found to be active with an IC_{50} value of below 1 μg/mL; the other quinines were bearing a moderate activity with IC_{50} values between 5 and 20 μg/mL, respectively. In the present results, the ethanol leaf extract of *C. papaya* showed a high antiplasmodial activity promising of IC_{50} (μg/mL) of 25, 50, 100 and 150, respectively also against the CQ sensitive and control 40.75, 36.54, 25.30, 18.0 and 1.0 CQ resistant and control 50.23, 32.50, 21.45, 23.12 and 5.0 against malaria parasite, *P. falciparum*.

In conclusion the overall, increasing the global spread of multi-drug resistant malaria parasite showed that there is a need for new chemotherapeutic agents to combat malaria. In the present study, to aiming the search of new anti-malarial drugs, *in vitro* anti-plasmodial activities of the plants *C. papaya*. Development of new active and safe drugs for the community is therefore an urgent need. Towards this goal, research into new antimalarial drugs from natural products, traditional healers use parts of many plants for the treatment of several pathologies, including malaria, and have done so for centuries. The use of plants can take into account their preservation because inadequate use of these plants could lead in terms to ecological disruption. These medicinal plants and their survival could be directly linked to our own behavior. These results confirm that extracts from leaves of these plants which displayed good activities against *P. falciparum* are suitable for their traditional use in the treatment of malaria fever.

Conflict of interest statement

We declare that we have no conflict of interest.

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