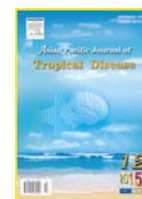




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## *In vitro* antiplasmodial efficacy of mangrove plant, *Ipomoea pes-caprae* against *Plasmodium falciparum* (3D7 strain)

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## ABSTRACT

**Objective:** To evaluate the antiplasmodial activity of mangrove plant, *Ipomoea pes-caprae* (*I. pes-caprae*) (leaves, stems, flowers and roots) against chloroquine-sensitive *Plasmodium falciparum* (3D7 strain) (*P. falciparum*) and cytotoxicity against brine shrimp larvae and THP-1 cell line.

**Methods:** The plants (*I. pes-caprae*) were collected from Machilipatnam mangrove forest (latitude 16°17' N and longitude 81°13' E) of Krishna District, Andhra Pradesh, India. Crude extracts from dried leaves, stems, flowers and roots of *I. pes-caprae* were prepared through Soxhlet extraction using methanol, chloroform, hexane, ethyl acetate and aqueous sequentially. These extracts were tested *in vitro* against *P. falciparum* (3D7 strain) adopted in laboratory. The crude extracts were also tested for their cytotoxicity against brine shrimp larvae and THP-1 cell line. The phytochemical screenings were also conducted with standard methods. As the part of the study, the extracts were checked for any chemical injury to erythrocytes.

**Results:** Out of these extracts, methanolic and aqueous extracts of all plant parts and chloroform extract of stems were active, and the ethyl acetate extracts were weekly active against *P. falciparum*. The extracts of chloroform (except stems) and hexane were inactive. Amongst these extracts, the methanolic extract of root showed excellent antimalarial activity with the IC<sub>50</sub> value of 15.00 µg/mL. Cytotoxic evaluation revealed that methanolic, aqueous and ethyl acetate extracts were slightly cytotoxic whereas chloroform and hexane extracts were more toxic against brine shrimp. All extracts were non-toxic to THP-1 cells. The chemical injury to erythrocytes was also evaluated and it did not show any morphological changes in erythrocytes due to the effect of plant extracts of *I. pes-caprae* after 48 h of incubation. The phytochemical screening of the extracts revealed the presence of alkaloids, triterpenes, flavonoids, tannins, coumarins, carbohydrates, phenols, saponins, phlobatannins and steroids.

**Conclusions:** This is the first report of antimalarial activity of *I. pes-caprae* and it is concluded that the methanolic extract of root is potent for the development of new antimalarial drugs.

### 1. Introduction

Each year, 300 to 500 million new cases of malaria are being diagnosed and nearly 1.5 million people are being subjected death; majority of deaths reported were from Sub-Saharan African countries where most of them were children under 5 years and pregnant women[1]. Malaria has a great impact on child health in malaria endemic countries and contributes to illness, respiratory infection, diarrhoeal disease and malnutrition[2].

AIDS, tuberculosis and malaria are the three most hazardous

infectious diseases of human kind[3]. In spite of all the efforts to eradicate malaria, this disease continues to be one of the greatest health problems facing tropical and sub-tropical regions. Indian sub-continent is known for *Plasmodium vivax*, and *Plasmodium falciparum* (*P. falciparum*) infection and most of the deaths reported were due to *P. falciparum* infection. Malaria dramatically increases in India recently after its near eradication in the early and mid-sixties[4]. The prevalence of malaria increased in 1980s and 1990s as the parasites developed resistance to the most regularly used antimalarial drugs and the vectors became resistance to insecticides[5]. Historically and traditionally plant parts have always been used as an important source in the medicine against malaria. Most effective antimalarial drugs such as chloroquine, quinine and artemisinin are derived from plants. Quinine, the first effective malarial drug was extracted from cinchona tree; based on this

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structure, chloroquine and primaquine were synthesized. The other effective drug, artemisinin was extracted from Chinese herbal tree *Artemisia annua* in 1972[6].

The first effective drug is chloroquine and its resistance was reported in 1957, consequently distributed all over the world and reported from India in 1976. Due to the presence of resistance, the most malaria endemic countries had stopped using chloroquine as the first line treatment for malaria[7]. Recently, artemisinin and its derivatives are being used as the first line treatment according to the World Health Organization proceedings of malaria treatment. Unluckily, artemisinin-resistant strains have been reported from Thai-Cambodia in 2009 and hasten the need for new antimalarials[8]. Hence, there is an urgent need for developing novel, well-tolerated antimalarial drugs for the treatment of malaria due to the difficulty of creating efficient vaccines and also drug resistance of existing antimalarial drugs[9].

Thus, artemisinin and its derivatives are now recommended by World Health Organization worldwide, in combination with other drugs such as lumefantrine, amodiaquine, mefloquine, sulphadoxine-pyrimethamine as the first line treatment of malaria[10]. This fact has encouraged the continuing search for new natural product-derived antimalarial drugs. Several plants are used in traditional medicine for the treatment of malaria and fever in malaria endemic areas[11].

The mangrove plants are specially adopted from woody plants grown interface between land and sea environmental conditions which are tremendously diverse from terrestrial ones[12]. It is surmised that mangrove plants have different characteristics from those of terrestrial plants and therefore they might produce different types of bioactive compound such as alkaloids, tannins, coumarins, phenols *etc.* Many researchers have reported several biological activities of mangrove plants such as antiplasmodial, antibacterial, antifungal, antiviral, antioxidant, antitumor and anticancer activities[13-18].

The plant in the present study is *Ipomoea pes-caprae* (Linn.) (*I. pes-caprae*) which is a mangrove plant commonly known as beach morning glory belonging to Convolvulaceae family. *I. pes-caprae* favorably grows in tropical and sub-tropical mangrove forests[19]. The aim of the present study was to evaluate the *in vitro* antiplasmodial activity and *in vivo* and *in vitro* cytotoxicity of methanol, chloroform, hexane, ethyl acetate and aqueous extracts from leaves, stems, flowers and roots of *I. pes-caprae*.

## 2. Materials and methods

### 2.1. Collection of plant materials

Fresh samples of leaves, stems, roots and flowers from *I. pes-caprae* were collected from Machilipatnam mangrove forest (latitude 16°17' N and longitude 81°13' E) of Krishna District, Andhra Pradesh, India. The mangrove plant (*I. pes-caprae*) was deposited in the Department of Botany, Acharya Nagarjuna University and voucher specimen was also maintained. All the collected plant parts were washed thrice with tap water and twice with distilled water to remove the adhering salts and other associated animals. The authentication of the plant species was done by Prof. K. Khasim, Department of Botany, Acharya Nagarjuna University, Guntur,

Andhra Pradesh, India.

### 2.2. Extract preparation

Shade-dried plant samples were subjected to 90% of different organic solvents (methanol, chloroform, hexane, ethyl acetate and water) at 50–60 °C in a Soxhlet apparatus. After complete extraction, the filtrates were concentrated one by one in rotary vacuum evaporator and then freeze-dried (80 °C) to obtain solid residue. The extraction percentage was deliberated by using the following formula:

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant sample (g)}} \times 100$$

The extracts of plant were screened for the presence of phytochemical constituents by following the methods of Ravikumar *et al.*[20]. The plant extracts were dissolved in dimethyl sulphoxide (DMSO) (50 mg/mL) and in double distilled water (50 mg/mL), and filtered through Millipore sterilized filters (mesh 0.20 µm). The filtrate was used for testing at different concentrations of 200, 100, 50, 25 and 12.5 µg/mL. The standard drug (chloroquine) was dissolved in distilled water.

### 2.3. Parasite cultivation

The plant extracts were screened for antiplasmodial activity against chloroquine-sensitive *P. falciparum* (3D7 strain). *P. falciparum* strain was obtained from ongoing cultures in the laboratory. They were cultured according to the method of Trager in candle jar desiccator[21]. Then the *P. falciparum* culture was further cultivated in human O<sup>Rh+</sup> red blood cells using Roswell Park Memorial Institute 1640 medium (Sigma Laboratories Private Limited, Mumbai, India) supplemented with O<sup>Rh+</sup> serum (10%), 5% sodium bicarbonate and 50 µg/mL of gentamycin sulfate. Hematocrits were adjusted at 2% and cultures of parasite were used when they exhibited 2% parasitemia[21].

### 2.4. In vitro antimalarial screening

The *P. falciparum* malaria parasite culture suspension of 3D7 (synchronized with 5% sorbitol to ring stage) was seeded (200 µL/well) in 96-well tissue culture plates. Plant extracts were added to the wells in microlitre to get different concentrations of extract (200, 100, 50, 25 and 12.5 µg/mL). Chloroquine-treated parasites were kept as positive control and DMSO-treated parasites were kept as negative control. The parasites were cultured for 30 h in candle jar desiccator. The cultures were incubated at 37 °C for 48 h in an atmosphere of 2% O<sub>2</sub>, 5% CO<sub>2</sub> and 93% N<sub>2</sub>. For 18 h before the termination of the assay, [<sup>3</sup>H] hypoxanthine (0.5 µCi/well) was added to each well of 96-well plate. The effect of extracts in the cultures was evaluated by the measurement of [<sup>3</sup>H] hypoxanthine incorporation into the parasite nucleic acids[22]. Each treatment had four replicates. At the end of the experiment, one set of the parasite infected red blood cells was collected from the wells and blood smears were prepared. These smears were fixed in methanol and air dried. The smears were stained with acridine orange stain. Stained smears were observed under UV illumination microscope (Carl Zeiss) for the confirmation of [<sup>3</sup>H]

hypoxanthine assay. The experiment was terminated and the cultures were frozen and stored at -20 °C. The parasites were harvested on glass filter papers using Nunc cell harvester and counts per minute (CPM) were recorded in gamma scintillation counter. Control readings were considered as 100% parasite growth and the parasite inhibition was calculated in plant extract treated wells. The parasite inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Average CPM of control} - \text{Average CPM of plant extracts}}{\text{Average CPM of control}} \times 100$$

The IC<sub>50</sub> values were determined by plotting concentration of extract on X-axis and percentage of inhibition on Y-axis with dose response curves using Minitab 11.12. 32. bit software.

### 2.5. Brine shrimp lethality bioassay

In this study, the eggs of *Artemia salina* were hatched for 24 h at room temperature (25–30 °C) in artificial sea water (20 g NaCl and 18 g table salt in 1 L of distilled water) to obtain nauplii (shrimp larvae). Test samples (methanol, chloroform, hexane, ethyl acetate and aqueous extracts of leaf, stem, flower and root of *I. pes-caprae*) were dissolved in DMSO and added to the test tubes. Each test tube contained 4 mL of sea water with sample concentrations of 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 1800 µg/mL in which the concentration of solvent (DMSO) should not be more than 5%, which do not have any destructive effects on the larvae. The same procedure was followed for the standard drug (chloramphenicol) and the final volume for each test tube was made up to 10 mL with artificial sea water with 10 living nauplii in each test tube. The control test tubes contained 10 living nauplii in 10 mL of artificial sea water. After 24 h, the test tubes were observed and the number of survived nauplii in each test tube was counted and the results were noted. The percentage of dead nauplii in the test and standard group was established by comparing with that of control group. The percentage of mortality was plotted against log concentrations and the LC<sub>50</sub> was deliberated by analysis of dose-response logistic curves. The general toxicity activity was considered weak when the LC<sub>50</sub> ranged from 500 to 1000 µg/mL, moderate when the LC<sub>50</sub> ranged from 100 to 500 µg/mL and strong when the LC<sub>50</sub> was ≤ 100 µg/mL[23]. *In vivo* calculating formula of selectivity index (SI) was determined each extract as LC<sub>50</sub> of brine shrimp/LC<sub>50</sub> of *P. falciparum*.

### 2.6. Cytotoxicity of extracts on THP-1 monocyte cells

The assays were carried out using 96-well flat-bottom tissue-culture plates. Cytotoxic properties of active plant extracts were assessed by functional assay using THP-1 cells[24]. The cells were cultured in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum, 0.21% sodium bicarbonate (Sigma) and 100 µg/mL penicillin and 50 µg/mL gentamicin (complete medium). Briefly, cells (0.2 × 10<sup>6</sup> cells/200 µL/well) were seeded into 96-well flat-bottom tissue-culture plates in complete medium. Drug solutions (200, 100, 50, 25 and 12.5 µg/mL) were added after 24 h of seeding and incubated for 48 h in a humidified atmosphere at 37 °C and 5% CO<sub>2</sub>. DMSO as a negative inhibitor and ellipticine as a positive inhibitor were kept as a negative and positive

control. At the end of the experiment, 10 µL of a stock solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 5 µg/mL in phosphate-buffered saline) was added to each well, gently mixed, and incubated for another 4 h. After spinning, the plate was centrifuged at 1500 r/min for 5 min and the supernatant was discarded, subsequently added 100 µL of DMSO (stopping agent). After the formation of formazan, it was read on a micro-titer plate reader (VersaMax tunable multiwell plate reader) at 570 nm and the percentage of cell viability was calculated using the following formula[25]. The SI of *in vitro* toxicity was calculated for each extract as the IC<sub>50</sub> of THP-1 cells/IC<sub>50</sub> of *P. falciparum*.

$$\text{Cell viability (\%)} = \frac{\text{Mean absorbance in test wells}}{\text{Mean absorbance in control wells}} \times 100$$

The IC<sub>50</sub> values were determined by plotting the concentration of extract on X-axis and the percentage of cell viability on Y-axis with dose-response curves using Minitab 11.12. 32. bit software.

### 2.7. Chemical injury to erythrocytes

To evaluate the chemical injury to erythrocytes that were attributed to the extract, 200 µL of erythrocytes were incubated with 200 µg/mL of the extract, a dose equal to the highest dose used in the antiplasmodial assay. The conditions of the experiment were continued as in the case of antiplasmodial assay. After 48 h of incubation, the assay was terminated and thin blood smears were prepared and fixed in methanol and air dried. These smears were stained with Giemsa stain and observed for morphological variations of erythrocytes under high-power light microscope. These morphological findings were compared with the normal erythrocytes of the control group[20].

### 2.8. Statistical analysis

The mean and SD of the treated and control group were calculated at 95% confidence interval (CI) levels. The results were analyzed statistically using two-tailed student's *t*-test (Minitab 11.12. 32. bit software) to identify the differences between treated group and control group. The data were considered significant at *P* < 0.05 and *P* < 0.01.

## 3. Results

The weights of leaves, stems, flowers and roots extracts in methanol, chloroform, hexane, ethyl acetate and aqueous respectively were shown as follows: 2.43, 17.54, 11.31, 0.72, 1.63 g; 5.21, 16.14, 10.52, 1.08, 0.97 g; 4.51, 7.66, 15.08, 2.33, 0.54 g and 2.36, 8.00, 6.21, 0.47, 1.22 g. The percentage yields of extracts ranged from 0.94% to 35.08% and were represented in Table 1. It revealed that chloroform extract of leaf (35.08%) showed maximum yield followed by chloroform extract of stems (32.28%). The phytochemical studies revealed that the methanol, chloroform, hexane, ethyl acetate and aqueous extracts of leaves, stems, flowers and roots of *I. pes-caprae* had a variety of phytochemical constituents, namely, alkaloids, triterpenes, flavonoids, tannins, coumarins, carbohydrates, phenols, saponins, phlobatannins and steroids (Table 2).

**Table 1**  
Weight and percentage of yield of different crude extracts from *I. pes-caprae*.

Plant parts	Extracts	Weight of plant part (g)	Weight of extract yield (g)	Yield (%)
Leaves	Methanol	50	2.43	4.86
	Chloroform	50	17.54	35.08
	Hexane	50	11.31	22.64
	Ethyl acetate	50	0.72	1.44
	Aqueous	50	1.63	3.26
Stems	Methanol	50	5.21	10.42
	Chloroform	50	16.14	32.28
	Hexane	50	10.52	21.04
	Ethyl acetate	50	1.08	2.16
	Aqueous	50	0.97	1.94
Flowers	Methanol	50	4.51	9.02
	Chloroform	50	7.66	15.32
	Hexane	50	15.08	30.16
	Ethyl acetate	50	2.33	4.66
	Aqueous	50	0.54	1.08
Roots	Methanol	50	2.36	4.72
	Chloroform	50	8.00	16.00
	Hexane	50	6.21	12.42
	Ethyl acetate	50	0.47	0.94
	Aqueous	50	1.22	2.44

**Table 2**Preliminary phytochemical constituents of *I. pes-caprae* in different extracts of leaf, stem, flower and root.

Tested compounds	Leaves					Stems					Flowers					Roots					
	ME	CH	HE	EA	AQ	ME	CH	HE	EA	AQ	ME	CH	HE	EA	AQ	ME	CH	HE	EA	AQ	
Alkaloids	+	-	-	+	-	+	-	-	-	+	+	-	-	-	+	+	-	-	-	-	-
Coumarins	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Carbohydrates	+	-	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+
Phenols	+	-	-	+	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	+	-
Saponins	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-
Tannins	-	-	-	+	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-	+
Flavanoids	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+
Terpenoids	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Steroids	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-

+: Present; -: Absent. ME: Methanol; CH: Chloroform; HE: Hexane; EA: Ethyl acetate; AQ: Aqueous.

**Table 3**Antiplasmodial activity against *P. falciparum* (3D7 strain) of different crude extracts from *I. pes-caprae*.

Plant parts	Extracts	Percentage of inhibition					IC <sub>50</sub> (µg/mL) (95% CI)
		12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	
Leaves	ME	13.33 ± 1.53 <sup>*</sup>	37.27 ± 0.46 <sup>*</sup>	72.67 ± 2.57 <sup>*</sup>	80.57 ± 2.38 <sup>*</sup>	96.10 ± 1.85 <sup>*</sup>	34.00 ± 1.00 (31.51–36.48)
	CH	4.23 ± 0.92 <sup>*</sup>	11.37 ± 1.40 <sup>*</sup>	29.33 ± 0.58 <sup>*</sup>	44.47 ± 1.27 <sup>*</sup>	67.07 ± 3.10 <sup>*</sup>	125.33 ± 2.52 (119.08–131.58)
	HE	1.17 ± 0.15 <sup>*</sup>	4.23 ± 0.80 <sup>*</sup>	14.30 ± 0.60 <sup>*</sup>	21.00 ± 4.00 <sup>*</sup>	42.57 ± 2.40 <sup>*</sup>	> 200
	EA	7.03 ± 0.30 <sup>*</sup>	17.17 ± 2.02 <sup>*</sup>	42.23 ± 0.40 <sup>*</sup>	62.30 ± 2.52 <sup>*</sup>	77.93 ± 2.57 <sup>*</sup>	69.67 ± 2.08 (64.50–74.84)
	AQ	6.57 ± 0.70 <sup>*</sup>	14.10 ± 1.01 <sup>*</sup>	56.03 ± 0.06 <sup>*</sup>	82.03 ± 3.49 <sup>*</sup>	91.08 ± 3.50 <sup>*</sup>	46.33 ± 1.53 (42.54–50.13)
Stems	ME	34.15 ± 4.00 <sup>*</sup>	64.19 ± 6.03 <sup>*</sup>	85.31 ± 5.29 <sup>*</sup>	90.33 ± 5.51 <sup>*</sup>	98.05 ± 0.08 <sup>*</sup>	18.67 ± 1.53 (14.87–22.46)
	CH	10.28 ± 0.28 <sup>*</sup>	13.11 ± 1.28 <sup>*</sup>	26.35 ± 2.16 <sup>*</sup>	41.13 ± 1.56 <sup>*</sup>	63.45 ± 4.21 <sup>*</sup>	23.00 ± 2.00 (18.03–27.97)
	HE	2.29 ± 0.50 <sup>*</sup>	7.03 ± 1.06 <sup>*</sup>	17.28 ± 2.28 <sup>*</sup>	31.26 ± 1.75 <sup>*</sup>	54.19 ± 0.19 <sup>*</sup>	178.67 ± 1.53 (174.87–182.46)
	EA	15.70 ± 2.23 <sup>*</sup>	27.35 ± 2.65 <sup>*</sup>	46.29 ± 2.30 <sup>*</sup>	78.17 ± 1.26 <sup>*</sup>	84.30 ± 2.70 <sup>*</sup>	48.67 ± 5.13 (35.92–61.41)
	AQ	11.32 ± 1.29 <sup>*</sup>	29.24 ± 3.55 <sup>*</sup>	46.25 ± 2.26 <sup>*</sup>	72.37 ± 1.72 <sup>*</sup>	91.14 ± 1.09 <sup>*</sup>	47.67 ± 3.06 (40.08–55.26)
Flowers	ME	9.36 ± 0.36 <sup>*</sup>	16.00 ± 2.65 <sup>*</sup>	60.47 ± 1.00 <sup>*</sup>	83.12 ± 2.66 <sup>*</sup>	92.16 ± 1.10 <sup>*</sup>	42.00 ± 2.08 (37.16–47.50)
	CH	1.31 ± 0.18 <sup>*</sup>	7.46 ± 2.30 <sup>*</sup>	18.35 ± 0.28 <sup>*</sup>	33.15 ± 2.90 <sup>*</sup>	56.44 ± 2.63 <sup>*</sup>	174.00 ± 7.51 (155.69–192.98)
	HE	3.72 ± 0.55 <sup>*</sup>	9.41 ± 1.03 <sup>*</sup>	24.34 ± 1.91 <sup>*</sup>	31.33 ± 0.80 <sup>*</sup>	43.41 ± 2.14 <sup>*</sup>	> 200
	EA	5.59 ± 0.56 <sup>*</sup>	16.50 ± 2.49 <sup>*</sup>	38.22 ± 2.62 <sup>*</sup>	57.49 ± 4.66 <sup>*</sup>	78.42 ± 1.06 <sup>*</sup>	77.00 ± 7.94 (57.28–96.72)
	AQ	13.05 ± 0.65 <sup>*</sup>	24.31 ± 2.60 <sup>*</sup>	48.06 ± 0.81 <sup>*</sup>	64.25 ± 0.92 <sup>*</sup>	89.17 ± 4.10 <sup>*</sup>	55.67 ± 4.51 (44.47–66.87)
Roots	ME	38.27 ± 3.85 <sup>*</sup>	76.20 ± 4.10 <sup>*</sup>	87.16 ± 3.60 <sup>*</sup>	95.31 ± 0.44 <sup>*</sup>	98.19 ± 0.94 <sup>*</sup>	15.00 ± 1.00 (12.51–17.48)
	CH	2.12 ± 0.22 <sup>*</sup>	7.37 ± 1.47 <sup>*</sup>	16.50 ± 2.10 <sup>*</sup>	23.71 ± 1.49 <sup>*</sup>	37.97 ± 1.29 <sup>*</sup>	> 200
	HE	0.00 ± 0.00	4.60 ± 0.57 <sup>*</sup>	11.71 ± 0.70 <sup>*</sup>	27.25 ± 2.50 <sup>*</sup>	47.45 ± 1.79 <sup>*</sup>	> 200
	EA	7.68 ± 0.61 <sup>*</sup>	15.20 ± 2.59 <sup>*</sup>	38.78 ± 0.50 <sup>*</sup>	59.17 ± 2.57 <sup>*</sup>	67.32 ± 1.44 <sup>*</sup>	78.67 ± 4.16 (68.32–89.01)
	AQ	12.36 ± 2.28 <sup>*</sup>	30.28 ± 0.87 <sup>*</sup>	59.14 ± 4.54 <sup>*</sup>	81.54 ± 1.77 <sup>*</sup>	90.40 ± 2.42 <sup>*</sup>	42.33 ± 3.06 (34.74–49.92)
CQ	-	-	-	-	-	3.680 ± 0.555	

Values are represented as mean ± SD of three replicates at 95% CI. \*: P &lt; 0.001, \*\*: P &lt; 0.05 compared with DMSO control. CQ: Chloroquine; ME: Methanol; CH: Chloroform; HE: Hexane; EA: Ethyl acetate; AQ: Aqueous.

In the present study, crude extracts of methanol, hexane, chloroform, ethyl acetate and aqueous from leaves, stems, flowers and roots of *I. pes-caprae* were evaluated for their antimalarial potencies. The IC<sub>50</sub> values of the tested plant extracts against *P. falciparum* were listed in Table 3. The *in vitro* antiplasmodial activity of biological active substances was categorized into four groups based on IC<sub>50</sub> value *i.e.*, < 5 µg/mL (very active), 5–50 µg/mL (active), 50–100 µg/mL (weakly active) and > 100 µg/mL (inactive)[20].

Based on above categorization, the IC<sub>50</sub> value of the methanol, aqueous, ethyl acetate extracts of leaves, stems, flowers and roots along with chloroform extract of stems of *I. pes-caprae* showed a range (IC<sub>50</sub> = 15.00–78.67 µg/mL) of inhibitory concentrations against chloroquine sensitive *P. falciparum* strain. The methanolic extracts of leaves, stems, flowers and roots, aqueous extracts of leaves, stems and roots, ethyl acetate extracts of stems and chloroform extracts of stems showed IC<sub>50</sub> value of < 50 µg/mL. Among these, methanolic extracts of root showed excellent antiplasmodial activity (IC<sub>50</sub> = 15.00 µg/mL) followed by methanolic extract of stems (IC<sub>50</sub> = 18.67 µg/mL). The microscopic observation of inhibition of *P. falciparum* to methanol extract treatment (200 µg/mL) was shown in Figure 1.

The ethyl acetate extracts of leaves, flowers and roots, and aqueous extracts of flowers showed low antiplasmodial activity (IC<sub>50</sub> = 50–100 µg/mL). Moreover, the chloroform extracts of leaves, flowers and roots, and hexane extracts of leaves, stems, flowers and roots showed IC<sub>50</sub> value > 100 µg/mL (Table 3). The CPM were represented in Figure 2 during the treatment of all the extracts at the highest concentration (200 µg/mL).

The cytotoxic evaluation of different extracts of *I. pes-caprae* against brine shrimp (*Artemia salina*) showed LC<sub>50</sub> values ranging from 253.33 to 1437.00 µg/mL. The general toxicity activity was considered weak when the LC<sub>50</sub> was between 500 and 1000 µg/mL, moderate when LC<sub>50</sub> was between 100 and 500 µg/mL and strong when the LC<sub>50</sub> was ≤ 100 µg/mL [23]. Based on the above classification, out of the 20 tested extracts, 3 extracts exhibited

moderate toxicity, while 17 extracts displayed weak toxicity. Amongst the tested extracts, none showed LC<sub>50</sub> value of < 100 µg/mL. The SI was also calculated and most of the extracts showed SI value > 10 indicating that the extracts were safer for further studies (Table 4). The cytotoxicity studies of twenty different extracts against THP-1 cell line showed IC<sub>50</sub> values > 200 µg/mL. An extract was classified as non-toxic when the IC<sub>50</sub> value was > 20 µg/mL. Based on the above, the plant extracts were not harmful for further studies. The SI was also calculated and listed in Table 5.

The microscopic observation of uninfected erythrocytes incubated with the extracts of *I. pes-caprae* and uninfected erythrocytes from the blank column of the 96-well plate showed no morphological differences after 48 h of incubation (Figures 3 and 4).

**Table 4**  
Cytotoxicity against brine shrimp larvae of different crude extracts from *I. pes-caprae*.

Plant parts	Extracts	Percentage of mortality								LC <sub>50</sub> (µg/mL) (95% CI)	SI	
		100 µg/mL	200 µg/mL	400 µg/mL	600 µg/mL	800 µg/mL	1000 µg/mL	1200 µg/mL	1400 µg/mL			1600 µg/mL
Leaves	ME	4.87 ± 1.82*	10.75 ± 1.40*	17.23 ± 1.12*	37.44 ± 1.10*	73.52 ± 2.67*	95.27 ± 2.57*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	651.67 ± 10.41 (625.81–677.52)	19.00
	CH	18.48 ± 1.28*	38.42 ± 2.41*	81.53 ± 6.86*	90.40 ± 2.21*	98.08 ± 0.94*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	253.33 ± 11.02 (226.97–281.70)	2.02
	HE	11.50 ± 0.50*	26.59 ± 2.92*	58.29 ± 1.17*	89.20 ± 2.61*	99.53 ± 0.38*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	348.33 ± 7.64 (329.36–367.31)	1.74
	EA	6.49 ± 1.14*	11.12 ± 1.23*	24.46 ± 2.38*	51.57 ± 4.96*	81.29 ± 1.21*	99.33 ± 0.46*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	599.33 ± 11.02 (571.97–626.78)	8.60
	AQ	0.00 ± 0.00	2.20 ± 0.31*	7.24 ± 1.31*	17.58 ± 1.14*	40.41 ± 2.01*	78.30 ± 7.84*	99.23 ± 0.71*	100.00 ± 0.00*	100.00 ± 0.00*	851.00 ± 17.7 (807.10–894.90)	18.36
Stems	ME	5.45 ± 0.68*	13.36 ± 2.85*	27.17 ± 1.76*	54.42 ± 2.02*	86.25 ± 2.03*	98.42 ± 1.37*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	578.30 ± 13.6 (519.60–637.00)	30.97
	CH	0.00 ± 0.00	4.64 ± 1.57*	9.50 ± 2.02*	19.46 ± 2.79*	33.43 ± 1.15*	64.33 ± 0.90*	93.36 ± 1.87*	99.38 ± 0.18*	100.00 ± 0.00*	914.67 ± 5.51 (900.99–928.35)	39.76
	HE	3.60 ± 1.26*	8.28 ± 2.77*	15.46 ± 3.02*	31.30 ± 0.91*	69.56 ± 4.48*	99.21 ± 0.30*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	699.00 ± 6.56 (682.71–715.29)	3.91
	EA	5.48 ± 0.80*	9.17 ± 2.60*	21.44 ± 0.50*	26.43 ± 1.39*	61.19 ± 5.92*	99.05 ± 0.09*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	737.70 ± 19.70 (688.80–786.50)	15.15
	AQ	0.00 ± 0.00	0.00 ± 0.00	1.27 ± 0.48*	6.77 ± 0.67*	14.89 ± 1.48*	26.26 ± 3.01*	47.23 ± 0.98*	84.48 ± 2.92*	99.10 ± 0.89*	1216.00 ± 5.29 (1202.86–1229.14)	25.50
Flowers	ME	2.29 ± 0.29*	5.32 ± 1.20*	12.24 ± 2.02*	23.27 ± 3.02*	45.23 ± 1.91*	79.18 ± 0.95*	99.17 ± 0.20*	100.00 ± 0.00*	100.00 ± 0.00*	825.67 ± 6.66 (809.13–842.21)	19.65
	CH	3.66 ± 1.31*	7.44 ± 1.36*	12.29 ± 4.19*	23.34 ± 2.74*	58.40 ± 2.50*	89.28 ± 3.50*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	742.00 ± 4.16 (724.09–759.91)	4.26
	HE	11.91 ± 1.86*	23.23 ± 3.14*	44.20 ± 4.06*	82.25 ± 1.89*	99.14 ± 0.16*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	427.67 ± 15.70 (388.68–466.66)	2.13
	EA	5.21 ± 1.03*	8.38 ± 1.14*	18.36 ± 2.63*	33.45 ± 1.94*	71.61 ± 3.82*	99.32 ± 0.60*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	685.33 ± 7.02 (667.89–702.78)	8.90
	AQ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.25 ± 0.32*	8.29 ± 1.66*	20.38 ± 3.09*	42.31 ± 4.26*	88.32 ± 2.19*	99.37 ± 0.54*	1230.67 ± 10.07 (1205.66–1255.67)	22.10
Roots	ME	0.00 ± 0.00	0.00 ± 0.00	3.42 ± 0.63*	7.76 ± 1.50*	13.32 ± 2.05*	27.29 ± 3.16*	59.38 ± 1.14*	87.50 ± 4.83*	99.40 ± 1.12*	1139.33 ± 12.50 (1108.27–1170.39)	75.95
	CH	0.00 ± 0.00	9.30 ± 0.25*	17.52 ± 1.25*	29.45 ± 4.07*	66.18 ± 1.99*	99.31 ± 0.44*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	715.00 ± 15.00 (677.74–752.26)	3.57
	HE	0.00 ± 0.00	0.00 ± 0.00	3.27 ± 0.29*	8.68 ± 1.26*	15.36 ± 0.83*	28.26 ± 0.79*	60.19 ± 2.93*	90.31 ± 2.17*	100.00 ± 0.00*	1135.67 ± 7.09 (1118.04–1153.29)	5.67
	EA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.53 ± 1.44*	10.24 ± 0.88*	19.19 ± 1.00*	42.56 ± 1.68*	86.26 ± 3.68*	1437.00 ± 8.54 (1415.78–1458.22)	18.26
	AQ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.37 ± 2.15*	8.36 ± 1.27*	17.23 ± 1.94*	26.21 ± 2.00*	61.62 ± 1.59*	99.10 ± 0.10*	1335.00 ± 3.00 (1327.55–1342.45)	16.96
CP	-	-	-	-	-	-	-	-	-	19.45 ± 1.23		

Values are represented as mean ± SD of three replicates at 95% CI. \*: P < 0.001, \*\*: P < 0.05 compared with DMSO control. SI was determined as LC<sub>50</sub> brine shrimp larvae/IC<sub>50</sub> *P. falciparum* (3D7 strain). CP: Chloraphenicol; ME: Methanol; CH: Chloroform; HE: Hexane; EA: Ethyl acetate; AQ: Aqueous.

**Table 5**  
Cytotoxicity against THP-1 cell line of different crude extracts from *I. pes-caprae*.

Plant parts	Extracts	Percentage of inhibition (P values)					IC <sub>50</sub> (µg/mL)	SI
		12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL		
Leaves	ME	0.00 ± 0.00	0.00 ± 0.00	4.27 ± 0.84*	10.42 ± 1.05*	22.38 ± 2.25*	> 200	> 5.88
	CH	0.00 ± 0.00	0.00 ± 0.00	1.29 ± 0.10*	1.44 ± 0.35**	4.13 ± 0.12**	ND	ND
	HE	3.45 ± 0.58*	7.50 ± 0.87*	16.35 ± 1.86*	21.57 ± 1.65*	27.57 ± 1.54*	> 200	0
	EA	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.42 ± 1.97*	17.39 ± 2.96*	> 200	> 2.87
	AQ	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.47 ± 0.33	1.52 ± 0.36	ND	ND
Stems	ME	4.44 ± 0.90*	9.34 ± 1.54*	14.49 ± 1.80*	17.25 ± 0.88*	25.45 ± 3.75*	> 200	> 10.71
	CH	0.00 ± 0.00	2.54 ± 0.57*	8.70 ± 2.14*	12.78 ± 2.26*	15.38 ± 1.05*	> 200	> 8.69
	HE	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.47 ± 0.30*	2.89 ± 0.82**	ND	ND
	EA	0.00 ± 0.00	0.00 ± 0.00	2.61 ± 0.80*	2.77 ± 0.61**	4.29 ± 0.80	ND	ND
	AQ	0.00 ± 0.00	3.38 ± 1.12*	7.33 ± 2.06*	13.54 ± 2.83*	17.30 ± 1.10*	> 200	> 4.19
Flowers	ME	4.75 ± 1.33	0.00 ± 0.00	5.70 ± 2.03*	2.84 ± 1.07	10.29 ± 3.44**	ND	ND
	CH	1.58 ± 0.79**	4.70 ± 1.13*	10.35 ± 2.43*	16.50 ± 2.29*	24.50 ± 4.47*	> 200	> 13.33
	HE	0.00 ± 0.00	1.74 ± 0.33*	3.89 ± 0.79*	3.45 ± 1.03**	3.82 ± 0.81	ND	ND
	EA	4.44 ± 1.22*	9.27 ± 2.12*	17.79 ± 3.03*	22.39 ± 1.90*	25.43 ± 4.26*	> 200	0
	AQ	0.00 ± 0.00	1.48 ± 0.60**	2.86 ± 1.00**	2.28 ± 0.78	3.87 ± 0.70	ND	ND
Roots	ME	4.79 ± 1.31*	2.59 ± 1.07**	8.79 ± 3.61**	7.43 ± 1.86*	10.65 ± 1.05*	ND	ND
	CH	0.00 ± 0.00	0.00 ± 0.00	9.23 ± 0.88*	15.54 ± 3.86*	27.54 ± 2.11*	> 200	> 4.76
	HE	0.00 ± 0.00	0.00 ± 0.00	2.63 ± 0.58*	2.45 ± 0.48*	4.65 ± 1.02	ND	ND
	EA	0.00 ± 0.00	0.00 ± 0.00	7.22 ± 1.76*	14.36 ± 2.04*	22.60 ± 3.31*	> 200	> 2.59
	AQ	0.00 ± 0.00	0.00 ± 0.00	1.78 ± 0.68**	1.68 ± 1.06	3.64 ± 1.34	> 200	> 3.59
Ellipticine	-	-	-	-	-	0.59 ± 0.25		

Values were represented as mean ± SD of three replicates at 95% CI. \*: P < 0.001, \*\*: P < 0.05 compared with DMSO control. SI were determined as IC<sub>50</sub> THP-1 cell line/IC<sub>50</sub> *P. falciparum* (3D7 strain). ND: Not determined; ME: Methanol; CH: Chloroform; HE: Hexane; EA: Ethyl acetate; AQ: Aqueous.

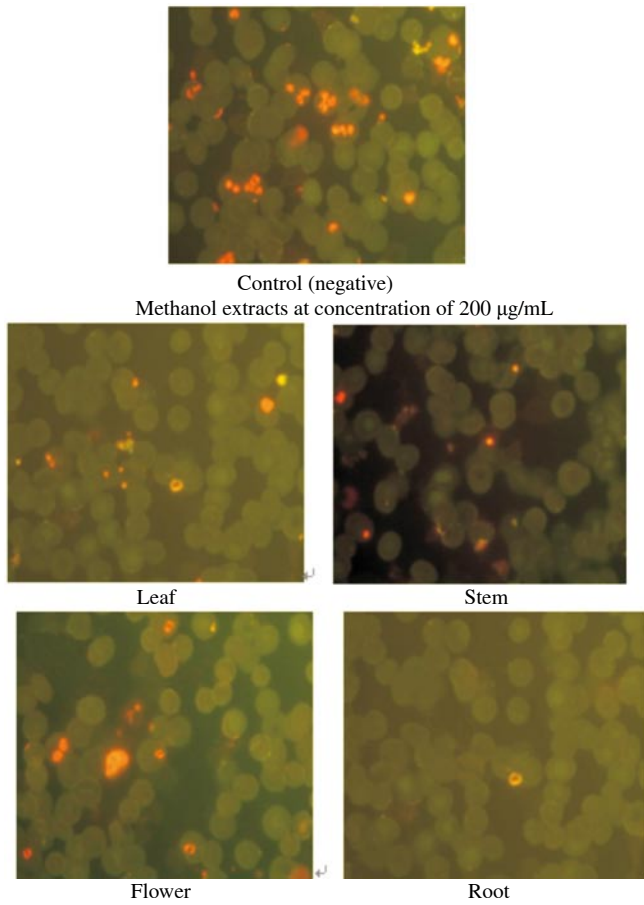


Figure 1. Microscopic observations after methanol extract treatment against *P. falciparum* (3D7 strain) at the highest concentration (200 µg/mL).

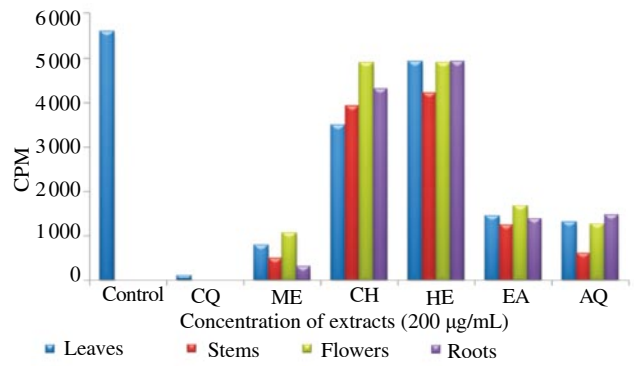


Figure 2. CPM of untreated (control) group and treated groups against *P. falciparum* (3D7 strain).

Methanol extracts showed low CPM similar to chloroquine. CQ: Chloroquine; ME: Methanol; CH: Chloroform; HE: Hexane; EA: Ethyl acetate; AQ: Aqueous.

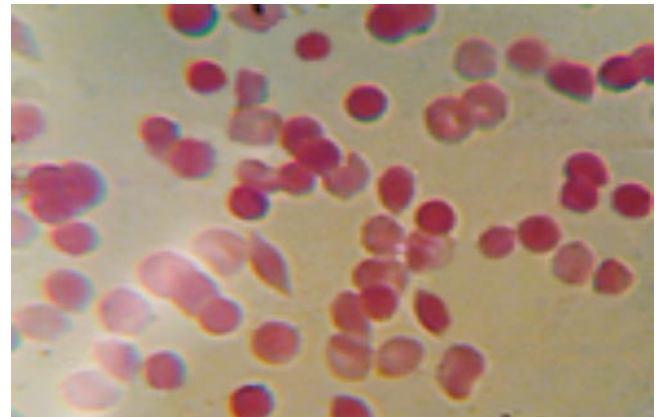


Figure 3. The normal erythrocytes of the control group in chemical injury to erythrocytes assay.

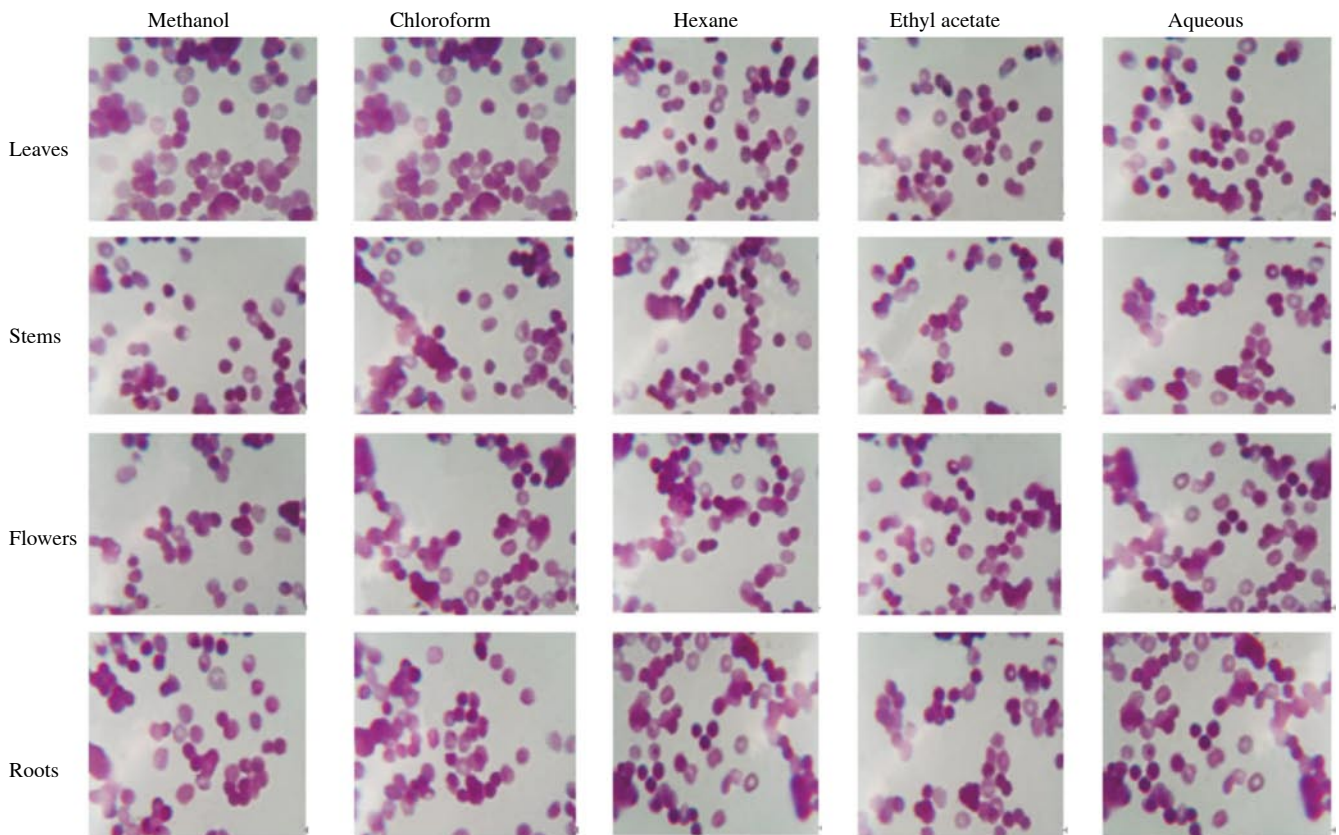


Figure 4. Screening for chemical injury to erythrocytes after the treatment of different crude extracts from *I. pes-caprae* (at the highest concentration of 200 µg/mL).

#### 4. Discussion

Malaria is still the most hazardous parasitic infectious disease which causes two million deaths every year. It is a great burden to developing nations, a number that could rise due to increasing multidrug resistance to all antimalarial drugs currently available[7,8]. There are several genetic polymorphisms identified in *P. falciparum* and *Plasmodium vivax* that can provide reliable data about the prevalence of drug resistance. Among all, the *pfprt*, *pfmdr1*, *pfdhfr* and *pfdhps* associated with drug sensitivity that have great role in drug resistance mechanisms in parasites are directly connected to treatment failure[26]. From the past 20 years, many strains of *P. falciparum* have developed resistance to chloroquine and other antimalarial drugs. The growth and spread of drug resistant strains of *P. falciparum* has limited effectiveness to the currently used malarial drugs. In view of this fact, the emergence and expand of parasites resistant to antimalarial drugs has caused an urgent need for novel effective alternative antimalarial drug compounds which are discovered and developed with minimal side effects[27].

Plants have proved to be a good source of chemotherapeutic agents over the years. Today, many of the drugs have been derived from plants resources such as quinine, chloroquine and artemisinin. Historically, medicinal plants have provided a source of encouragement for novel therapeutic drugs, as plant-derived medicines have made large contributions. According to the World Health Organization, at the present time, 80% of the world's population depends on plants for their primary health care[28]. Plants can produce secondary metabolites for their defense, which play an important role of physiological activities in human body[29]. The medicinal values of plants are due to the substances that it contains, which generate a physiological action on the human body. Some examples of these plant substances are alkaloids, resins, tannins, essential oils and many others[30]. India has remarkable biodiversity and rich cultural traditions of plant use. Interestingly, today, many of the pharmaceutical companies are utilizing the plant-based drug formulations in therapeutic treatment of various diseases and disorders worldwide[31].

The present plant (*I. pes-caprae*) is a mangrove plant commonly known as beach morning glory belonging to Convolvulacea family. The plant (*I. pes-caprae*) favorably grows in tropical and sub-tropical mangrove forests[21]. Mangroves are wide-ranging in tropical and sub-tropical regions, growing in the seashore of saline intertidal zones of sheltered coast lines and contain biologically active antibacterial, antiviral and antifungal compounds[32]. Mangrove plant extracts traditionally have been used for centuries as popular method for treating several disorders related to health. Plant-derived materials have recently

became of great interest owing to their versatile applications. Hence, the current studies were undertaken to evaluate the *in vitro* antiplasmodial activity of mangrove plant (*I. pes-caprae*) along with cytotoxicity assay.

Out of the 20 tested extracts, 9 extracts showed good activity ( $IC_{50}$  ranged from 15.00  $\mu\text{g/mL}$  to 48.67  $\mu\text{g/mL}$ ), 4 extracts exhibited low activity ( $IC_{50}$  ranged from 55.32  $\mu\text{g/mL}$  to 78.60  $\mu\text{g/mL}$ ), while 7 extracts were inactive ( $IC_{50} > 100 \mu\text{g/mL}$ ) to *P. falciparum*. The present study revealed that the methanolic extract of root exhibited excellent antiplasmodial activity with  $IC_{50}$  value of 15.00  $\mu\text{g/mL}$  followed by methanolic extract of stems with  $IC_{50}$  value of 18.51  $\mu\text{g/mL}$ . Ethyl acetate extracts have displayed moderate activity whereas the chloroform extracts of leaves, flowers and roots, and hexane extracts of leaves, stems, flowers and roots of *I. pes-caprae* have not shown antiplasmodial activity. The  $IC_{50}$  value of the methanol root extract of *I. pes-caprae* was quite higher than the positive control of chloroquine and showed active antimalarial activity. Thus, the results of our study are also consistent with the outcomes of many researchers who reported the antiplasmodial activities of several plants including polyherbal extracts[33-38].

Ravikumar *et al.* also reported the antiplasmodial activity of several mangrove plants from southeast coast of India which supports the present study where *I. pes-caprae* is also a mangrove plant[33]. The root extract of *I. pes-caprae* has shown the potent antiplasmodial activity supported by the work of Prakash *et al.*, in which *Brucea mollis* root extracts have shown *in vitro* and *in vivo* antiplasmodial activity[39]. Previous studies on *I. pes-caprae* plant parts and their major chemical classes show various level of biological activities such as antioxidant, antianalagic, antiinflammatory, antispasmodic, antinociceptive, antiarthritic, antihistaminic, antidiabetic, antibacterial, antifungal and immunostimulatory activities[40-48].

Bagavan *et al.*, also did a similar work and they have reported the antimalarial activity of methanol, hexane, chloroform, acetone and ethyl acetate extracts of *Citrus sinensis* (peel), *Leucas aspera*, *Phyllanthus acidus* (leaf), *Ocimum sanctum* and *Terminalia chebula* (seed) against chloroquine-sensitive (3D7) strain of *P. falciparum* and their cytotoxicity on human laryngeal cancer cell line (HEp-2) and normal cell line (Vero) were tested. Out of the 25 test extracts, they have concluded that methanol and ethyl acetate extracts of leaf of *Lepiota aspera*; ethyl acetate, methanol, and acetone extracts of leaf of *Phyllanthus acidus* and acetone extract of seed of *Terminalia chebula* exhibited good antiplasmodial activity ( $IC_{50}$  ranged from 4.76 to 22.76  $\mu\text{g/mL}$ ) with SI ranged from 2.04 to 9.97 for HEp-2 and 2 to 12 for Vero cells respectively[49].

Chenniappan and Kadarkarai tested the antimalarial activity of 50 traditionally used Western Ghats plants alone and in combination with chloroquine against chloroquine-resistant

*P. falciparum* strains from India[37]. Out of 200 extracts, 29 extracts showed significantly high *in vitro* antiplasmodial activity with IC<sub>50</sub> values ranging from 3.96 to 4.85 µg/mL, 53 extracts showed significantly good *in vitro* antiplasmodial activity with IC<sub>50</sub> values ranging from 5.02 to 9.87 µg/mL and 28 extracts showed significantly moderate *in vitro* antiplasmodial activity with IC<sub>50</sub> values ranging from 10.87 to 14 µg/mL respectively. In combination with chloroquine, 103 extracts showed significant synergistic *in vitro* antiplasmodial activities with synergistic factor values ranging from 1.03 to 1.92 µg/mL and these activities were up to a fold higher with chloroquine, suggesting synergistic interactions of the two drugs[37].

Kaushik *et al.*, screened the ethyl acetate extracts and methanol extracts of 17 Eastern Ghats plants from South India and Buchpora and North India for antiplasmodial activity against chloroquine-sensitive (3D7) and chloroquine-resistant (Indo) strains *P. falciparum* and their toxicity against HeLa cell line[50]. They have reported the ethyl acetate and methanol promising antiplasmodial activity (IC<sub>50</sub> *P. falciparum* 3D7: ≤ 20 µg/mL) from *Aerva lanata*, *Anisomeles malabarica*, *Anogeissus latifolia*, *Cassia alata*, *Glycyrrhiza glabra*, *Juglans regia*, *Psidium guajava* and *Solanum xanthocarpum*. Ethyl acetate extracts from leaves of *Couroupita guianensis*, *Euphorbia hirta*, *Pergularia daemia*, *Tinospora cordifolia* and *Tridax procumbens* as also methanol extracts from *Ricinus communis* displayed excellent antiplasmodial activity (*P. falciparum* 3D7 IC<sub>50</sub>: 21–40 µg/mL). Moderate activity (*P. falciparum* 3D7 IC<sub>50</sub>: 40–60 µg/mL) was reported from ethyl acetate extracts of leaves of *Cardiospermum halicacabum*, *Indigofera tinctoria* and *Ricinus communis* while the other extracts showed marginal activities (*P. falciparum* 3D7 IC<sub>50</sub>: 60 to 100 µg/mL). The promising extracts illustrated good resistance indices (0.41–1.4 µg/mL) against the chloroquine-resistant (Indo) strain of *P. falciparum* and good selective indices (3 to 22.2) from tested HeLa cell line[50].

In the present investigation, the plant extracts of methanol, ethyl acetate and aqueous have shown good antiplasmodial activity and *in vivo* cytotoxicity LC<sub>50</sub> values from 500 to 1000 µg/mL (weak toxicity) which is considered safer for therapies. And the *in vitro* cytotoxicity IC<sub>50</sub> values were > 200 µg/mL (non-toxic) for all the test extracts of the present study and are not harmful for further therapeutic studies[34]. Majority of the tested extracts shown SI > 10 during both *in vivo* and *in vitro* cytotoxicity studies indicates the safer therapy[38]. Thus, our results are in corroboration with the findings of Musila *et al.*, who reported the cytotoxicity of stem bark of *Launaea cornuta* on brine shrimp larvae (weakly toxic)[51]. However, the low toxicity of *I. pes-caprae* extracts suggests that it is potential for inhibiting the growth of *P. falciparum* without the inherent toxicity.

Cytotoxicity is also endorsed to the occurrence of various secondary metabolites found in plant extracts. Not only their existence but also the quantity of the phytochemical components in a given plant extract will determine the scope of its bioactivity. In addition, the occurrence of more than one class of secondary metabolites in a certain plant extract determines the nature and scope of its biological activity[51]. The current *in vitro* antiplasmodial activity of the test extracts may be due to the presence of major chemical classes such as alkaloids and phenols. The alkaloids are strong antiplasmodial compounds. Except the alkaloids, the major chemical classes such as coumarins, phenols, polysaccharides and flavanoids are also concerned with strong antiplasmodial activities[52].

The present results are supported by Ouattara *et al.*, who reported that polysaccharides and polysaccharide derivatives (prumycin) had potential antiplasmodial activity. Alkaloids, flavonoids and sesquiterpenes were reported to be potent plant secondary metabolites with extensive range of bioactivities[53]. Alkaloids, major classes of compounds, have power of antimalarial activity and quinines, one of the most important and oldest antimalarial drugs, belongs to alkaloids. Biological activity is attributed to the presence of various secondary metabolites in plants[51]. In view of this, it envisages that any one of the classes of compounds may be responsible for the activity.

In addition, none of the test extracts of *I. pes-caprae* have shown any of the chemical injury to the erythrocytic membrane throughout the experimentation. Commonly, the erythrocytic membrane is a fragile structure that can be significantly altered by drug interactions. The mechanical stability of the erythrocytic membrane is an excellent indicator of *in vitro* studies for cytotoxicity screening, because of its structural dynamics favoring interactions with drugs and this signifies the possible use of these extracts as antiplasmodial drug in future. The mechanism of action might be due to the inhibition of hemozoin biocrystallization by the alkaloids and inhibition of protein synthesis by triterpenoids[54].

So, it can be concluded from the present study that methanolic extract of root, stem, leaf and flower of *I. pes-caprae* displayed *in vitro* antimalarial activity and warranted further investigation of those plants as potential sources of antiplasmodial agents. Additional *in vitro* and *in vivo* work aimed at understanding the mechanisms of action of the active plant extracts, isolating and characterizing the bioactive constituents is underway in our laboratories and will be reported in due course of time.

#### Conflict of interest statement

We declare that we have no conflict of interest.



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