

In-Vitro Evidence of Effective Anti-Plasmodium Activity by *Plumeria rubra* (L) Extracts

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ABSTRACT

Malaria is known to account for a large segment of death in most tropical countries. In current times the advent of virulent, drug-resistant strains of the Plasmodium parasite has activated the search for innovative, effective, anti-plasmodial agents, with negligible side effects. Hence, plant extracts, loaded with active phytochemicals, are presently proving effective in the battle against malaria and its diverse forms. In the present study therefore, two selected extracts of *Plumeria rubra* were studied to determine the major secondary metabolites and active phyto-components. The anti-malarial efficacy of these extracts were then verified in-vitro on 3D7 and RKL-9 *P. falciparum* strains. The consequence of the test on cultures of infected RBCs for 24 hours were compared with normal RBCs alone, Plasmodia infected untreated RBCs, Plasmodia infected treated RBCs and scored according to the WHO guidelines. The EC 50 values were derived and the study revealed that the dry plumaria rubra leaves and stem methanolic extract proved most potent in containing the parasite proliferation. This was associated with the phytochemical data which showed that these extracts were opulent in active phyto-constituents, and hence attested to be effective anti - plasmodial agents even against the drug resistant strains, such as RKL-9. *Plumeria rubra* is used traditionally for numerous diseases and hence the present study may aid to recognising main molecules in the pursuit of a potent anti-malarial agent.

Keywords: anti-plasmodial agent, *Plumeria rubra*, alcoholic and aqueous extracts, phytochemicals, infected RBCs.

INTRODUCTION

Malaria is a serious and sometimes fatal disease caused by a parasite that commonly infects a certain type of mosquito which feeds on humans. People who get malaria are typically very sick with high fevers, shaking chills, and flu-like illness. Four kinds of malaria parasites infect humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. In addition, *P. knowlesi*, a type of malaria that naturally infects macaques in Southeast Asia, also infects humans, causing malaria that is transmitted from animal to human ("zoonotic" malaria). *P. falciparum* is the type of malaria that is most likely to result in severe infections and if not promptly treated, may lead to death. Although malaria can be a deadly disease, illness and death from malaria can usually be prevented. Globally, the World Health Organization estimates that in 2015, 214 million clinical cases of malaria occurred, and 438,000 people died of malaria, most of them children in Africa. Since malaria leads to often fatal, the disease is a great drain on many national economies¹. Historically, botanicals have been our most fruitful arena in the search for new medicine. Identifying new drugs from traditionally used medicinal plants can, therefore, be the shortest path to success. The leaves of *P. rubra* are used in ulcers, leprosy, inflammations and rubefacient². Flavone glycoside isolated from *Plumeria rubra* shows antioxidant and hypolipidemic activity³. The

extract *P. rubra* flowers found more active than the leaf parts against *Bacillus cereus* with zone of inhibition of 28mm⁴. *Plumeria rubra* containing fulvo plumierin act as inhibitors of human immunodeficiency virus type1 (HIV) reverse transcriptase⁵. The flowers decoction of *P. rubra* was reported to be used in Mexico for control of diabetes mellitus. Its antibacterial activity was confirmed by Baghel *et al.*⁶ Various studies have proved its properties as antitumor, protective against haemotopoietic system, to sooth ititation, as abortifacient and analgesic properties⁷⁻¹⁰. Thus taking into consideration its immense medicinal potential and the fact that it is traditionally used in certain rural areas of our state, this species of the genus *plumaria* has been selected for the present investigation.

MATERIALS AND METHODS

Plant Source and Extraction

The *Plumaria rubra* shoot was collected from Gujarat University campus and in and around Ahmedabad, Gujarat. Samples of plant material were authenticated by the Botany Department, Gujarat University, Ahmedabad, India. The fresh and powdered defatted dry plant material (20 gram) was extracted with 200 ml of solvent for 72 hours; refluxed at a temperature below the solvent boiling point using soxhlet extractor. The crude solvent collected in the flask was concentrated at reduced pressure. The yield collected after drying was kept at 4^oC until further

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Table 1: Preliminary qualitative phytochemical analysis of various extracts of *Plumeria rubra* (L.).

Secondary Metabolites	Alkaloids	Tannins	Flavonoids	Glycosides	Steroids	Saponins	Resins	Terpenoids
FRPLW	-	+	++	+	+	-	+	+
FRPLH	+++	++	+++	++	-	-	-	+++
DRPLH	+	+	+	+	-	-	-	+

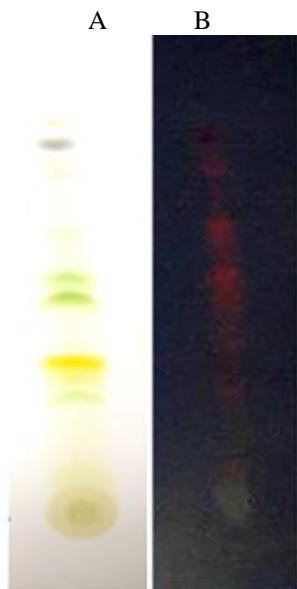


Figure 1: A-B: Showing the Thin Layer Chromatogram of the hydro-alcoholic extracts of the fresh plant under study using the solvent system ethyl acetate: methanol: ammonium (170:20:10 v/v/v). A – *Plumeria rubra* (dried) under white light. B – *Plumeria rubra* (dried) under UV 366 nm.

use.

Phytochemical studies

Qualitative analysis for determining the presence of alkaloids, tannins, flavonoids, terpenoids, steroids, glycosides, saponins, resin and oil in the plant extracts, was carried out using standard methods as described by Harborne¹¹ and Sofowora¹². The dried extracts (0.5 gm) were dissolved in 20 ml distilled water, filtered and used

for various qualitative tests.

High Performance Thin Layer Chromatography (HPTLC)
 Each constituent in the sample was first separated on TLC (Thin Layer Chromatography) plate through selected solvent systems considering the polar, nonpolar or the intermediate nature of the metabolites. This was then followed by scanning, determination of retention factor (R_f) value and λ max profile of metabolites¹³ using a Camag-5 High performance thin layer chromatography (HPTLC) system, complemented by WinCATS evaluation software (Version 1.4.6.8121). The HPTLC technique is supportive to evaluate profiles of the crude plant extracts made by using various solvents for the maximum extraction of the desired metabolites in selected solvents. The comparative analysis with different solvent extracts also serves for value addition of the product¹⁴.

In vitro screening of antiplasmodial property of plant extracts

The *Plasmodium falciparum* strains 3D7 and RKL-9 were obtained from Malaria Parasite Bank of National Institute of Malaria Research Centre (NIMR), New Delhi. The infected RBCs were washed with incomplete media before use. The culture was initiated using fresh RBCs with an initial parasitemia of 1 %. It was seen that the parasites were well acclimatized *in vitro* in RPMI 1640 HEPES medium supplemented with 10-15% O⁺ve treated human plasma and erythrocytes at 37°C in CO₂ incubator. The culture was synchronized using 5% aqueous solution of sorbitol¹⁵ modified by Highland et al. (2015)¹⁶. All other stages except ring stages were found to degenerate. Degenerated stages were removed by centrifuging for 5 minutes at 1500 rpm. The supernatant was discarded, and the pellet was washed thrice with incomplete media. Parasitemia was adjusted to about 1% for assay by

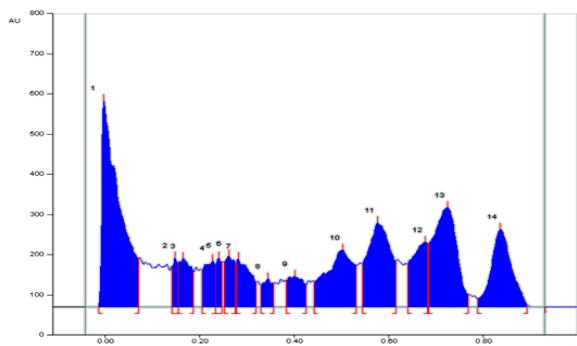


Figure 2A: Showing the HPTLC chromatogram area and peaks of the aqueous extract of *Plumeria rubra* using WinCATS evaluation software (Version 1.4.6.8121).

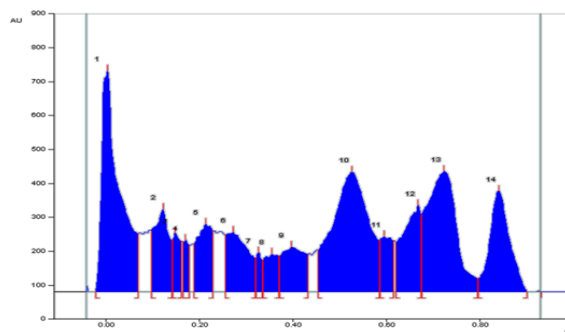


Figure 2B: Showing the HPTLC chromatogram area and peaks of the hydro-alcoholic extract of *Plumeria rubra* using WinCATS evaluation software (Version 1.4.6.8121).

diluting with fresh washed RBCs. The plant crude extracts were dissolved in 10% DMSO with RPMI media having a final concentration of 1mg/ml. The tests were performed using a 96-well micro plate (flat-bottomed) with 2 fold serial dilutions. All tests were run in triplicate. Synchronized parasite culture was incubated in test well plates with each extract for 24 hours.

In-vitro Experimental test series

The following plant extracts were evaluated *in-vitro*:

- 1.FRPLW: fresh *Plumaria rubra* shoot Aqueous extract
- 2.FRPLH: fresh *Plumaria rubra* shoot hydro-alcoholic extract
- 3.DRPLH: dry *Plumaria rubra* shoot hydro-alcoholic extract.

The *In-vitro* experimental tests carried out included:

RBC : Normal cultured RBCs

iRBC : RBCs infected with *Plasmodium falciparum*

iRBC + FRPLW :: Infected RBCs treated with fresh *Plumaria rubra* shoot Aqueous extract

iRBC + FRPLH:: Infected RBCs treated with fresh *Plumaria rubra* shoot hydro- alcoholic extract

iRBC +DRPLH:: Infected RBCs treated with dry *Plumaria rubra* shoot hydro-alcoholic extract

In-vitro Efficacy Evaluation

After 24 hours of each culture, Normal RBCs alone, Plasmodia Infected RBCs, Synchronized infected RBCs and Cultures of infected RBCs with test extract; the slides were prepared as a thin smear, stained and scored according to the WHO guidelines¹⁷. The extracts were verified at concentrations of 1.95, 3.91, 7.81, 15.63, 31.25, 62.50 and 125.0µg/ml. The values were compared between control and test wells.

Oxidative stress parameters

Lipid Peroxidation (LPO) -Thiobarbutiric acid reactive species assay (TBARS)

The Thiobarbutiric acid reactive species (TBARS) levels in normal RBCs, infected RBCs (iRBCs) and RBCs treated with the plant extract were detected by the method of Okhawa *et al.*¹⁸.

Superoxide Dismutase (SOD)

The activity of superoxide dismutase (SOD) in normal RBCs, infected RBCs (iRBCs) and treated RBCs were assayed by the method of Kakkar *et al.*¹⁹.

Total Protein Estimation

Total Protein content was estimated in normal RBCs, infected control and samples treated with the plant extracts, by the method of Lowry *et al.*²⁰ using bovine serum albumin as standard. The blue colour that developed is quantitatively proportional to the total protein present and was measured at 540 nm. The protein content was expressed as mg/100 mg tissue weight.

Hemoglobin (Hb) Determination

Hemoglobin determination was carried out using a Sahli's hemoglobinometer with standard colour comparator and the final value was recorded as g/dl²¹.

Molecular docking and bioavailability studies of selected phytochemicals against Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1)

Preparation of Protein Target Structure and Ligands

The X-ray crystal structure of *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) was recovered from the Protein Data Bank (PDB ID- 2YK0)²² were subjected to energy minimization using GROMOS96 utility (without reaction field) executed in Swiss-Pdb Viewer 4.0.1.²³.

Active site prediction

Structures with unbound ligands were computationally analyzed for active site using Q-Site Finder²⁴. It differentiates pockets on the protein surface through calculation of van der Waals interaction energies using a methyl probe and probes with favourable interaction energies were gathered and ranked.

Virtual screening

Ligand dataset selected for this study were virtually screened (docked) into the binding site of the target protein *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) using Argus Lab 4.0.1 from Planaria Software LLC. To allow fast sampling, the binding site was constructed which consist of all remnants that have at least one atom within 3.5 Å from any atom in the co-crystallized ligand. This method of constructing binding site was helpful for protein structures with co-crystallized ligand. A varied approach was performed for proteins with unbound ligand whose active site was predicted using Q-Site Finder. Best 3 scored pockets were computationally examined for each protein target using Jmol Java plugin executed in Q-Site Finder and the amino acids entrenched in the predicted cavity volume were utilized as active site residues. These two methods gave a good representation of the important residues in the binding pocket for a protein target. A grid box of size 22 X 15 X 16 with atom scaling of 0.40 Å was produced and a high precision Argus Dock engine with A Score as scoring function were chosen. After grid generation, the ligands were flexibly docked with the protein and 1000 poses were created, among which best 10 poses of low-energy, which were collected in rank 1, were examined. Argus Dock engine makes use of ligand torsionality as a hierarchical tree in which the root's node (group of bonded atoms that do not have rotatable bonds) is placed in a search point inside a grid containing residues of the active site. A set of varied and energetically favourable translations are generated and poses that survive in torsional search through an estimated thorough search are retained and finally clustered.

RESULTS AND DISCUSSION

The results obtained in the study provided evidence and confirmation of the potent anti-oxidant and anti-plasmodial activity of the tested plant extracts. Preliminary phytochemical analysis revealed in fresh *Plumaria rubra* bark and leaves maximum yield was obtained in methanol extract (22%) while minimum yield was obtained in chloroform (7.3%). In dry *Plumaria rubra* bark and leaves Petroleum ether extract gave 2.1% while methanol gave 3.1%. Dry *Plumaria rubra* flower's methanolic extract gave 1.5% yield revealed an extraction yield of 5.3% and 9.5% for aqueous and hydro-alcoholic

Table 2: *In vitro* anti-plasmodial activity of the *Plumaria rubra*(shoot) extracts on 3D7 strain.

Malaria <i>in vitro</i> drug sensitivity (3D7)								
Plate	Result							
Conc.: µg/ml	Control	1.95	3.91	7.81	15.63	31.25	62.5	125
FRPLW	121.641	74.524	82.641	46.992	60.331	22.631	3.634	3.419
FRPLH	107.144	79.953	54.288	42.779	36.495	53.981	52.658	19.574
DRPLH	84.996	80.307	49.650	63.310	13.276	11.816	7.559	2.1753

Regression Parameters	Unit: µg/ml					
	Polynome	EC ₅₀	EC ₉₀	EC ₉₅	EC ₉₉	R ²
FRPLW	3	6.050	57.696	77.044	95.866	0.9033
FRPLH	3	2.678	111.709	120.782	127.891	0.9513
DRPLH	3	7.619	28.279	35.706	45.448	0.9112

Table 3: *In vitro* anti-plasmodial activity of the *Plumaria rubra* (shoot) extracts on RKL9 strain.

Malaria <i>in vitro</i> drug sensitivity (RKL-9)								
Plate	Result							
Conc.: µg/ml	Control	1.95	3.91	7.81	15.63	31.25	62.5	125
FRPLW	123.586	110.452	65.819	73.138	43.315	58.994	22.631	3.9148
FRPLH	121.019	79.454	55.515	46.600	26.637	54.887	54.188	21.179
DRPLH	84.996	83.971	70.013	49.324	62.643	10.969	9.966	9.252

Regression Parameters	Unit: µg/ml					
	Polynome	EC ₅₀	EC ₉₀	EC ₉₅	EC ₉₉	R ²
FRPLW	3	7.573	94.638	107.398	117.733	0.9371
FRPLH	3	2.389	109.722	120.134	128.203	0.9467
DRPLH	3	12.447	41.879	51.802	64.436	0.9046

Table 4: Table showing Superoxide dismutase activity in unparasitized, parasitized and treated RBCs.

	SOD (units/mg protein)
RBC	0.0871±0.003
iRBC	0.070±0.002 **
iRBC+FRPLW	0.079±0.002
iRBC+FRPLH	0.080±0.008
iRBC+DRPLH	0.075±0.005

Values are Mean ± S.E. ** P<0.001

Table 5: Table showing Lipid Peroxidation level in unparasitized, parasitized and treated RBC.

	LPO (×10 ⁴ n moles of MDA formed/100mg of tissue wt/60 mins
RBC	0.21±0.04
iRBC	1.36±0.06**
iRBC+FRPLW	0.62±0.07*
iRBC+FRPLH	0.83±0.03*
iRBC+DRPLH	0.52±0.04*

Values are Mean ± S.E. ** p<0.001; * p<0.001

extracts respectively. The analysis revealed that alkaloids, flavonoids and terpenoids were much higher in the alcoholic extract as compared to aqueous extract. The

presence of tannins and glycosides were detected in all the three extracts, the FRPLW, FRPLH and DRPLH extracts. Certain phytochemicals such as saponins etc were absent in the extract (Table 1). Figure 1 demonstrates the varied phytochemical constituents in the FRPLW and FRPLH extracts as obtained by thin layer chromatography. In the HPTLC fingerprinting of hydro-alcoholic and aqueous extracts of fresh *P.rubra* shoot revealed several peaks as recorded in PLATE 1.HPTLC profile under UV 254 nm was recorded and the corresponding HPTLC chromatograms are presented in Figure 2A and 2B. 14 peaks were obtained in both the aqueous and hydro-alcoholic extract.

In vitro antiplasmodial activity of *P. rubra* against 3D7 and RKL-9 strains

In vitro anti-plasmodial study revealed that the dry *Plumaria rubra* shoot hydro-alcoholic extract was most active, when compared with the other extracts against the chloroquine sensitive 3D7 and chloroquine resistant RKL9 strains of Plasmodium falciparum, with 50% inhibitory concentration of parasite growth observed at 2.678µg/ml and 2.389µg/ml respectively when compared with that of the other extracts; fresh *Plumaria rubra* shoot Aqueous extract (3D7: 6.050µg/ml & RKL: 7.573µg/ml) and fresh *Plumaria rubra* shoot hydro-alcoholic extract (3D7: 7.619µg/ml & RKL: 12.447µg/ml). The plate readings and the EC₅₀, EC₉₀, EC₉₅, EC₉₉ and R² values

Table 6: Table showing the total protein content in 3D7 and RKL-9 control, infected and treated strains.

Samples	Protein (mg/100mg)	
	3D7	RKL-9
RBC	11.53 ± 0.32	11.25 ± 0.37
iRBC	18.63 ± 0.75**	18.40 ± 0.214**
iRBC + DRPLH	15.26 ± .216**	-----
iRBC + FRPLW	13.43 ± 0.517*	14.35 ± 0.24*
iRBC + FRPLH	15.52 ± 0.323**	14.71 ± 0.13*

Values are Mean ± S.E. ** P<0.001; * P<0.001

Table 7: Table showing Haemoglobin content (g/dl) in 3D7 and RKL-9 control, infected and treated strains.

	Haemoglobin (g/dl)	
	3D7	RKL-9
RBC	18.61 ± 0.013	18.7 ± 0.015
iRBC	14.81 ± 0.014**	14.3 ± 0.016**
iRBC + DRPLH	15.57 ± 0.072*	15.34 ± 0.035*
iRBC + FRPLW	15.36 ± 0.037**	15.65 ± 0.021*
iRBC + FRPLH	15.71 ± 0.014*	15.63 ± 0.036*

Values are Mean ± S.E. * p<0.01; ** p<0.001

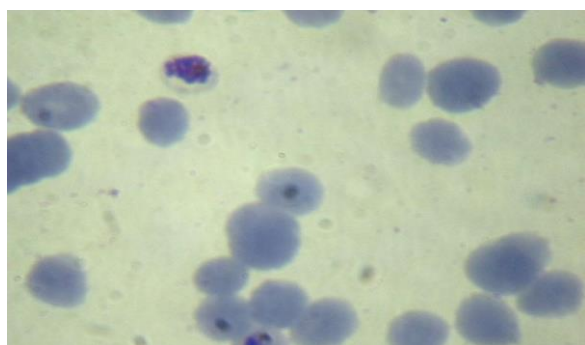


Figure 3: Showing a sample of cultured RBC as a thin smear of controlled sample 100 X.



Figure 4: Showing a sample of cultured RBC thin smear of treated sample 100 X.

found for these extracts against 3D7 and for RKL-9 strains by using Hn-NonLin software are shown in Table 2 and 3. According to Tran *et al.*²⁵ activity is effective if EC₅₀ value ≤10 µg/ml, which is conformation to the data, obtained in this study.

Superoxide Dismutase (SOD)

A highly significant (p<0.001) decline was observed in the activity of Superoxide dismutase (SOD) of the cultured infected RBCs (iRBC) however the treatment with the extracts showed an insignificant increase in SOD activity (Table -4).

Lipid Peroxidation (LPO)

The LPO levels of the RBCs were significantly increased after parasite infection; here also the treatment with both extracts was able to bring about a significant decline in lipid peroxidation. (Table 5).

Estimation of Protein content

In the present investigation the protein content increased significantly (p<0.001) in the infected RBCs, but it was observed that there was a sharp decrease in the protein content after 24 hrs in the treated iRBCs (Table-6) of both strains, bringing about a recovery.

Haemoglobin Content

A significant decline was recorded in the haemoglobin content (p<0.001) of the infected RBCs of both strains studied. Treatment with both aqueous and hydroalcoholic extracts of *Plumeria rubra* brought about significant (p<0.001) recovery in the haemoglobin content towards the normal, control values (Table 7).

Molecular docking and bioavailability studies of selected phytochemicals against Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP1)

The ligand dataset was virtually screened with the protein targets using Argus lab (2004) software 4.0.1 and the binding energy values were analyzed for each docked conformation. Conformations having low energy and exhibited favourable hydrogen bonding with the amino acids side chain and its amide nitrogen was considered (Table 8). Binding energies of the protein ligand interactions are important to describe how fit the ligand binds to the target macromolecule. Molecular docking through *in-silico* analysis and bioavailability studies of selected phytochemicals against *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), it was concluded that all the docked compounds of *Plumeria rubra* (Table 8) could be considered as a probable

Table 8: Showing the docking results of selected phytochemicals as ligand against PfEMP1 as target.

Compound Name	Compound ID(PUBchem)	Moldock Score	H-bond Interaction amino acids
Plumericin	CID_5281545	-113.484	[Arg518][Tyr516]
Isoplumericin	CID_5281543	-105.141	[Arg578]
Fulvoplumericin	CID_5281541	-102.439	[Arg578][Tyr651]

inhibitor of *Plasmodium* development and life cycle of erythrocytic stages. The emergence and spread of *Plasmodium falciparum* drug resistance have triggered an urgent need to investigate new sources of anti-malarial drugs. The most potential sources being investigated are traditional herbal drugs. Herbal extracts from medicinal plants have immense therapeutic potential, are cost effective and have less side effects as well as they are the richest source of primary and secondary metabolites having potent biological activities²⁶. The saponin extract was used for testing anti-inflammatory and antihelmintic activity of *P. rubra* leaves. Several phytochemicals were isolated from the stem bark of *Plumeria rubra* Linn. These compounds exhibited antifungal, antialgal, antitumor and antibacterial activities. The chloroform and ethanolic extracts of *P. rubra* leaves show antiulcer activity in albino rats. Ethanol extract of leaves of *Plumeria rubra* possessed protective action on the haemopoietic system²⁷. Since the phyto-therapeutic potential of *P. rubra* is well recognized, the present study was carried out to demonstrate its anti-plasmodial activity, if any, on cultured infected Red Blood Cells. Alcoholic and aqueous extracts of fresh *Plumaria rubra* shoot showed significant anti-plasmodial activity against the drug sensitive strain Pf3D7 (EC₅₀: 7.619 & 6.050 µg/ml respectively) while dry alcoholic extract also showed significant anti-plasmodial activity (EC₅₀: 2.678 µg/ml). In addition, these extracts also manifested potent anti-plasmodial activity against PfRKL-9, which is a known drug resistant strain. Preliminary TLC analysis revealed the presence of potent phytochemicals in both extracts. HPTLC analysis confirmed the presence of Tannins, Glycosides, flavanoids, terpenoids in greater quanta in the hydro-alcoholic extract, as compared to aqueous extract. These phytochemicals are known to possess a wide range of activities to help in protection against chronic diseases²⁸. Certain constituent phytochemicals such as flavonoids and terpenoids are known to anti-inflammatory effects while the phenolic compounds have been reported to act as free radical scavengers, having dynamic antioxidant activity. The presence of antioxidants in the extracts is therefore accountable for the effective free radical scavenging activity. The antioxidant potential of *Plumeria rubra* extracts has been emphasized by Rehman et al.²⁹, which substantiates our findings. Keeping these facts in mind, an attempt was therefore made using an *in-vitro* approach, to determine the effect of *Plumaria rubra* extracts in controlling the propagation of *Plasmodium falciparum* within the RBC and thus manifest an anti-plasmodial effect through the interaction of the phyto-constituents. The study revealed that while the infected RBCs in culture manifested a high level of lipid

peroxidation, the infected RBC cultures treated with the hydro-methanolic extracts showed a significant decline in lipid peroxidation. Balanheru and Nagarajan (1992)³⁰ showed significant decline in lipid peroxidation in heart microsome in vitro by the flavones, glycosides, Ursolic acid which is abundantly present in *Plumeria rubra*. Becker et al.³¹ have shown an increase in the lipid peroxidation of plasmodium infected RBCs. Moreover Erel et al.³² have demonstrated that plasmodia succeed in accumulating free radical scavenging enzymes within their own cells but deplete them in red blood cells of the host. Polyphenols (flavonoids) have been known to effectively curb free radical induced peroxidation of lipid. According to Verstrachten et al.³³ in addition to their protein binding and direct scavenging activity, these potent antioxidants interact with membrane lipids and prevent the access of deleterious molecules across the cell membrane. It was also found that related to an increase in lipid peroxidation in the infected RBCs, a decline was noted in SOD, a free radical scavenging enzyme. The antioxidant potency of the *Plumaria rubra* extracts however appeared to overcome this deficiency. Kirk³⁴ and Lew et al.³⁵ have mentioned that at the same time the premature haemolysis of the highly permeabilized iRBC are prevented by the excessive ingestion, digestion and detoxification of the host cell haemoglobin (Hb) and its discharge through the NPP, thereby preserving the osmotic stability of the iRBCs. Diez-Silva et al.³⁶ had reported that a protein pf155/Ring infected erythrocyte surface antigen (RESA) is expressed enormously during the first 24 hrs of erythrocytic stage and this protein single handedly alters the mechanical properties of RBC membranes and impedes the microcirculation of iRBCs. Therefore anaemia is detected as common haematological changes in malaria *P. falciparum* infection. According to Agravat and Dhruva³⁷ 93% cases of anaemia were reported during *P. falciparum* infection. Anaemia correlates with the severity of the malaria infection^{38,39}. However, during treatment anaemia increases due to effect of the anti-malarial drug used, a finding reported by Sowunmi et al.⁴⁰. These researchers have specified that artemisinin drugs (anti-malaria drug) are reported to cause falls in haematocrit during treatment, resulting in anaemia. In the present study, extracts of *Plumaria rubra* brought a significant recovery in Hb levels after the treatment. Docking simulations carried out to confirm the *in vitro* finding revealed conformations of low energy and favourable binding with selected reactive groups of certain phytochemicals present in the extracts. Plumericin, isoplumericin and fulvoplumericin showed good binding compatibility against *pfemp1* protein target with the most favorable binding energies. Hence from the

molecular docking through *in-silico* analysis and bioavailability studies of selected phytochemicals against *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1), it was concluded that the docked compounds of *Plumeria rubra* could be considered as potential inhibitors of *Plasmodium* development and life cycle of erythrocytic stages. This study therefore emphasises, the importance of identifying novel molecular sites and molecules showing selective binding interaction of the specific target protein PfEMP1 of the *Plasmodium falciparum* and erythrocytic membrane. This innovative *in silico* approach lends support to the *in vitro* findings in the present study, confirming the efficacy of the extract.

CONCLUSION

In conclusion, the experimental work was focused towards to determining the anti-malarial activities of the crude extracts of selected medicinal plants, and the study did yield evidence of effective components which could have potent anti-plasmodial activity. The present study has explicit significance in a tropical country such as ours, to yield semi-purified herbal extracts that could control the *Plasmodium* growth and multiplication, at the erythrocytic stages.

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