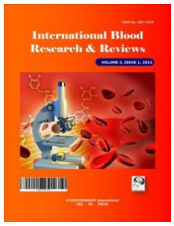

International Blood Research & Reviews
6(2): 1-12, 2016, Article no.IBRR.27960
ISSN: 2321–7219

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Calotropis procera Extract Halts *Plasmodium falciparum* Transgression Through Red Blood Cell (RBC) Membrane

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LBG and HH designed the study, performed the statistical analysis, wrote the protocol. Author KD checked the analytical results. Authors SG and DJ wrote the first draft of the manuscript. Authors SG, DJ and UJ managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2016/27960
Editor(s):
(1) Anamika Dhyani, Laboratory of Biochemistry & Molecular and Cellular Biology Hemocentro-UNICAMP, Brazil.
Reviewers:
(1) Aina Oluwagbemiga Olanrewaju, Nigerian Institute of Medical Research, Yaba, Nigeria.
(2) Abdullahi Maikudi Nuhu, Kaduna Polytechnic, Kaduna, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/15456

Original Research Article

Received 27th June 2016
Accepted 10th July 2016
Published 20th July 2016

ABSTRACT

Aim: To evaluate the potential of *Calotropis procera* extracts in erythrocyte membrane stabilization with special emphasis on *Plasmodium falciparum* entry into RBCs.
Place and Duration of Study: Department of Zoology, BMT and Human Genetics, University School of Sciences, Gujarat University, Gujarat, India, between December-2014 to December-2015.
Methodology: In this study, we evaluated the erythrocyte membrane stabilization properties of *Calotropis procera* leaf extracts. The aqueous and methanolic leaf extracts of *Calotropis procera* were screened for its phytochemical, antioxidant, erythrocyte membrane stabilization and subsequent antiplasmodial activity. The *in vitro* antiplasmodial activity was evaluated on *Plasmodium falciparum* chloroquine-sensitive (MRC2) and chloroquine-resistant (RKL9) strains.
Results: In the present study, phytochemical analysis of aqueous extract has shown only the

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presence of flavonoids, triterpenoids, carbohydrates and phenolic compounds whereas the methanolic extract has shown the presence of all the phyto-components except saponins, oils and fats. Antioxidant activity of the extracts was measured by (DPPH•) radical scavenging assay. The methanolic extract of *Calotropis procera* showed more potent antioxidant activity when compared to aqueous extract. We observed an increased inhibition of entry of the parasites in relation to the concentration of both the extracts. Though enhanced stabilization effects were observed at higher concentrations of the methanolic extract, there was a steady membrane stabilizing property with the aqueous extract. The experimental evidence obtained in our study revealed that the methanolic extract of the leaves of *C. procera*, tends to be more effective in preventing the parasite entry into RBCs.

Conclusion: Our finding confirms the importance of investigating the antimalarial activity of *Calotropis procera* which is used in traditional medicine. Overall, the methanolic extract of *Calotropis procera* appeared to be the best candidate and will be further investigated for their erythrocyte membrane stabilization and antiplasmodial properties of the individual isolated compounds, alone and in combinations.

Keywords: Antioxidant; Calotropis procera; erythrocyte membrane stabilization; anti-plasmodial.

1. INTRODUCTION

Plasmodium falciparum the most widespread etiological agent for human malaria has become increasingly resistant to standard antimalarials *e.g.* chloroquine and antifolates. According to the latest estimates from WHO, there were 214 million new cases of malaria worldwide in 2015 (range 149–303 million). The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%).

In 2015, there were an estimated 438 000 malaria deaths (range 236 000–635 000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%).

Between 2000 and 2015, malaria incidence rates (new malaria cases) fell by 37% globally, and by 42% in Africa. During this same period, malaria mortality rates fell by 60% globally and by 66% in the African Region [1]. The long-term effects of malaria on a child's health and development are often insufficiently recognized and poorly managed. A severe form of the disease, cerebral malaria, kills 10–20% of those children it affects, while an additional 7% are left with permanent neurological problems, including blindness, epilepsy, speech and learning difficulties [2,3].

Consequently, new drugs or drug combinations are urgently needed today for the treatment of malaria. These drugs should have novel modes of action or be chemically different from the drugs in current use. In the present work, we have evaluated the *in vitro* erythrocyte membrane stabilization of *Calotropis procera* for preventing the entry of *P. falciparum* into the erythrocytes. The development of new antimalarial drugs is of paramount importance to combat the rapid spread of multi-drug resistant strains of *Plasmodium*. The growing reality that antimalarial drugs currently available may not be effective any more has led to the investigation of medicinal plants used in traditional medicine for hundreds of years. Medicinal plants for malaria treatment could be a promising source of new antimalarial agents.

Calotropis procera (Ait.) R.Br. (*Asclepiadaceae*) is a plant growing widely throughout the tropical and subtropical regions of Asia and Africa and Middle East [4]. *Calotropis procera* (Ait.) R.Br. is medicinal plant used in several traditional medicines to treat a variety of diseases. The extracts from different parts of the plant have significant therapeutic value. It is found in waste lands and grows as a weed in cultivated areas. It also grows well on rubbish heaps, waste and fallow land, by the roadside and in sand dunes [5].

The plant has attracted much attention due to following biological activities: The previous pharmacological studies include reports of anticancer, antifungal and insecticidal activity of *C. procera* [6]. The flowers of the plant exhibit hepatoprotective activity, anti-inflammatory, antipyretic, analgesic and larvicidal activity. The latex of the plant is reported to possess analgesic and wound healing activity as well as anti-inflammatory and antimicrobial activity while

the roots are reported to have antifertility and anti-ulcer effects [7].

Whole plant was used to treat common diseases such as fever, rheumatism, indigestion, cold, eczema, diarrhoea, for the treatment of boils, to remove thorn from body and for the treatment of jaundice. The root was used for the treatment of eczema, leprosy, elephantiasis, asthma, cough, rheumatism, diarrhoea and dysentery. In case of diarrhoea it changed the faecal matter into a semisolid mass within the first day of treatment. The stem was used for the treatment of skin diseases, enlargements of abdominal viscera, intestinal worms, leprosy and cure leucoderma [8,9].

The plant was recommended in leprosy, hepatic and splenic enlargements, dropsy and worms. The latex is applied to painful joints and swelling, fresh leaves were also use for the same purpose. Oil of the leaves was applied to paralyzed part. The milky juice was used in India as purgative, while flowers were considered as digestive, stomachic, tonic and useful in cough, asthma catarrh and loss of appetite. The root bark was said to promote secretion and to be useful in treating skin disease, enlargement of abdominal viscera, intestinal worms, ascites and anasarca [10].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves of plant *Calotropis procera* were collected from the campus of the School of Sciences, at Gujarat University, Ahmedabad in the month of December, 2014, which was authenticated by Dr. Archana U. Mankad (Vou. No.: GUJBOT/A/S/25/*Calotropis procera*), Head of the Botany Dept., School of Sciences, at Gujarat University, Ahmedabad.

2.2 Processing of Plant Material

The leaves were cleaned thoroughly with single distilled water and dried. The dried leaves were powdered and stored in airtight container. The powder was sieved and defatted with petroleum ether (60ºC) and kept for 24 hours at room temperature with constant stirring. 50 gm of defatted material was extracted at 50ºC with 500 ml two different solvents in the soxhlet apparatus for 8 to 12 hrs till the colour of the solution in the siphon become colourless.

1) Aqueous (100% distilled water)
2) Methanol (30% distilled water +70% methanol)

The extracts were dried at 60ºC in the oven. The yields collected after drying were weighed and kept at 4ºC for further analysis [11,12].

2.3 Qualitative Phytochemical Analysis

The aqueous and methanol extract of the powdered plant material were subjected to various qualitative tests for the detection of various phytochemical constituents groups' present [12].

2.4 High Performance Thin Layer Chromatography (HPTLC)

The aqueous and methanol extracts were further subjected to HPTLC for the conformation of the active constituents. Chromatography was performed on Merck HPTLC pre coated silica gel $60F^{254}$ (20×10 cm) plates (with layer thickness of 0.2 mm). Samples (10 µL) were spotted as 6 mm bands, starting 15 mm from the edge of the plates, by means of a Camag Linomat V sample applicator the nitrogen flow providing a delivery speed of 150 nl/s from the application syringe. These conditions were kept constant throughout the analysis of the samples. The plates were developed to a distance of 80 mm above the position of sample application in a Camag twin-trough chamber previously saturated with mobile phase for 30 minutes. The mobile phase was ethyl acetate: glacial acetic acid: Formic acid: Distilled water (100:11:11:26). Densitometric evaluation of the plates was performed at λ = 366 nm using a Camag Scanner III with tungsten lamp in conjunction with WINCATS III software for quantification [13].

In-vitro Experimental test series:

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

CAQ : *C. procera* leaves aqueous extract.
CHA : *C. procera* leaves methanol extract.

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

RBCs : Normal RBCs in culture.
iRBCs: RBC infected with *Plasmodium falciparum*.

RBCs + CAQ: RBCs pre-treated with 7.81-250 µg/ml *Calotropis* aqueous extract.

RBCs + CHA: RBCs pre-treated with 7.81-250 µg/ml *Calotropis* methanol extract.

2.5 Antioxidant Assay by DPPH

The free radical scavenging activity of the extract was measured *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay [14]. Three milliliters of 0.1 mM DPPH solution prepared in methanol was added to 1 mL of the test extracts (1–10 mg/mL) dissolved in distilled water. The content was mixed and allowed to stand at room temperature for 30 min in the dark. The reduction of DPPH free radical was measured by recording the absorbance at 517 nm. Ascorbic acid was used for comparison. The percentage scavenging activities (% inhibition) at different concentrations of the extracts fractions were calculated using the following formula. The principle behind this assay in the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant.

$$I\ (\%) = [(AC - AS)/AC] \times 100$$

Where I is inhibition, absorbance values of the control (AC) and absorbance values of the sample (AS). Three replicates were made for each sample and results were expressed as mean ± SD.

2.6 Lipid Peroxidation Assay

The method is based on the reaction of Thiobarbituric acid (TBA) with malonyl dialdehyde (MDA) and other breakdown products of peroxidised lipids collectively called as thiobarbituric acid reactive species (TBARS). Thiobarbituric acid reactive species (TBARS) level in normal RBCs infected RBCs (iRBCs) and RBCs pre-treated with plant extracts determined by the method of Ohkawa, et al. [15].

2.7 *In vitro* Study of Erythrocyte Membrane Stabilization

The membrane stabilizing activity of both extracts of leaves of *C. procera* assessed by using haemolysis assay by Jansen, et al. [16].

Preparation of plant extracts:

Aqueous extract (1 mg/ml) of *C. procera* leaves was dissolved in 5% DMSO in RPMI 1640 media and used for this assay.

Methanol extract (1 mg/ml) of *C. procera* leaves was dissolved in 5% DMSO in RPMI 1640 media and used for this assay.

2.8 *In vitro* Percent Inhibition of Entry of Parasites

In vitro percent inhibition of entry of parasites determined by Jonvile, et al. [17] with some modifications. Normal RBCs were pretreated with the plant extracts for 24 hours and then exposed to iRBCs with 2% parasitemia for next 24 hours. Normal RBCs, without treatment with plant extracts were considered as control. The plates were incubated under the incubator with 5 percent CO_2. The numbers of schizonts of the control and treated samples were counted for 200 cells each to assess the consequence of the aqueous extract and methanol extract. All these values are expressed as (%) inhibition of entry of parasites.

3. RESULTS AND DISCUSSION

3.1 Qualitative Phytochemical Analysis

Preliminary phytochemical analysis was performed and the results are given in Table 1. From the result it has been observed that the methanol extract of the leaves of *C. procera* has shown greater number and quantity of phytochemicals as compared to the aqueous extract. The aqueous extract has shown only the presence of flavonoids, triterpenoids, carbohydrates and phenolic compounds whereas the methanolic extract has shown the presence of all the phytocomoponents except saponins, oils and fats.

The powdered leaves of *Calotropis* are used for the fast healing of wound, as a purgative and to treat digestion. They are used to promote sexual health including penis dysfunction and are reported to be an aphrodisiac. The leaves of *C. procera* are used by various tribes of Central India as curtive agent for jaundice [18]. The leaves are used to treat joint pain and reduce swelling [19]. It is also used by traditional medicine practitioners in Gwari communities for the treatment of ringworms [20]. It has been proved that plants are one of the major sources of drug discovery and development. Plants are reported to have anticancer, antimicrobial, antidiabetic, anti inflammation, antioxidant properties [21]. The bioactive plant extract is a new concept and has been recently reported [22].

Table 1. Qualitative analysis showing the presence or absence of various phytochemicals in the leaves of *C. procera*

No.	Compounds	Aqueous extract	Methanol extract
1	Alkaloids	-	+
2	Glycosides	-	++
3	Tannins	-	+
4	Saponins	-	-
5	Flavonoids	+	++
6	Carbohydrates	+	++
7	Steroids	-	++
8	Triterpenoids	+	++
9	Phenolic compounds	++	+++
10	Oils and fats	-	-

(+) present (-) absent

Flavonoids have already been studied as antioxidants and demonstrated to be very active [23]. Phenolics and flavonoids have been reported to exert anti-inflammatory, antiviral, antibacterial, antiallergic, antitumour, lipid peroxidation and platelet aggregation inhibitor activities [24,25]. They exert these effects as antioxidants and free radical scavengers [26,27].

3.2 High Performance Thin Layer Chromatography (HPTLC)

HPTLC technique was used to separate the phytochemicals and WIN CATS evaluation software (Version 1.4.6.8121) was used to analyse the data. The results of HPTLC analysis are shown in Figs. 1-3.

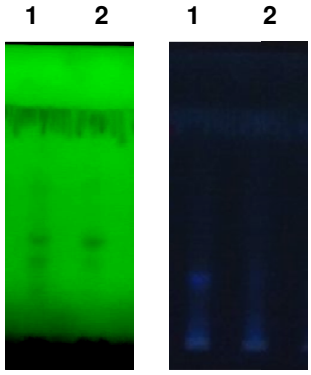

Fig. 1. Showing the chromatogram under UV 254 nm and 366 nm
Lane 1- Methanolic extract and
Lane 2- Aqueous extract of *C. procera leaves*

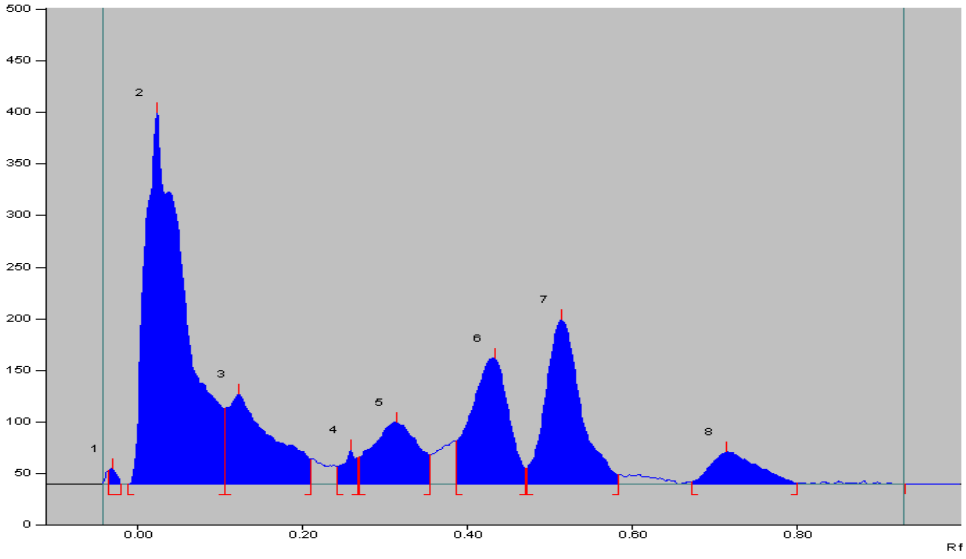

Fig. 2. Showing the HPTLC chromatogram area and peaks of the methanolic extract of *C. procera* using Win CATS evaluation software (Version 1.4.6.8121) at 366 nm

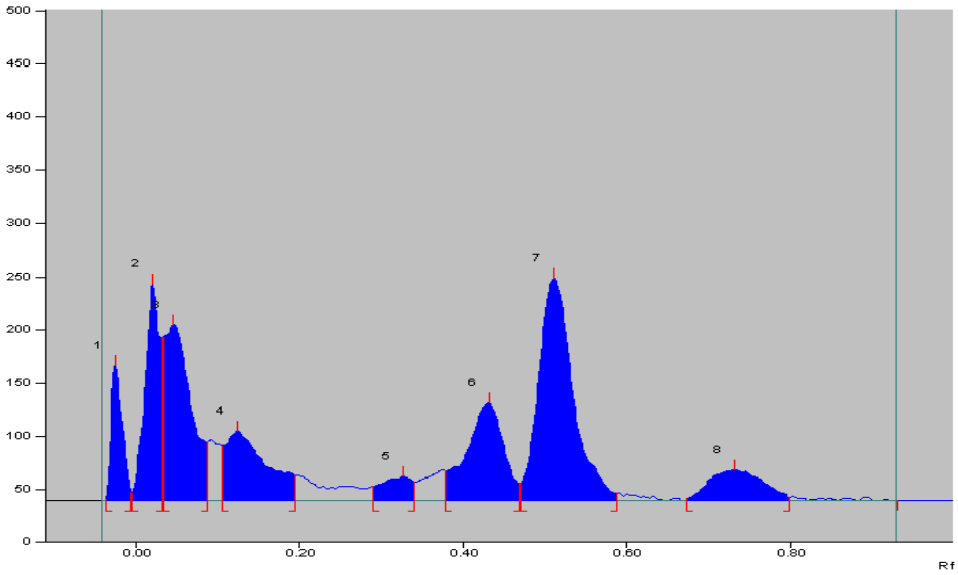

Fig. 3. Showing the HPTLC chromatogram area and peaks of the aqueous extract of *C. procera* using win CATS evaluation software (Version 1.4.6.8121)

HPTLC with both the extracts of leaves of *C. procera* revealed the presence of various flavonoids, triterpenoids and phenolic compounds. It is observed that both the extracts showed similar peak distribution with slight difference in its area covering under peak graph. Thus HPTLC studies also showed similar qualitative profile with minor quantitative variations in both the extracts. HPTLC analysis was also performed for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug. From the present study, it can be concluded that the different therapeutic properties of *C. procera* are due to the various phytochemicals and secondary metabolites present in the plant. However, further research is needed in the characterization and mode of action of these phytochemicals on the various systems.

3.3 Antioxidant (DPPH) Activity

Free radical scavenging potentials of *C. procera* aqueous and methanol extract at different concentrations (7.81–250 µg/mL) were measured by the DPPH radical scavenging assay and the results are shown in Table 2.

Considerable scavenging potential (50% with aqueous extract and 60% with methanolic extract) was found at lower concentrations (up to 7.81 µg/mL) of the extract. Further increase in extract concentration produced little enhancement in activity (about 70%). DPPH radical is a commonly used substrate for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay [28]. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds [29]. Phenolic compounds which were identified in significant concentration in the extracts are known to be powerful chain breaking antioxidants. Pietta [30] have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. Flavonoids are capable of effectively scavenging the reactive O2 species because of their phenolic hydroxyl groups and hence work as potent antioxidants [31]. The antioxidant capacities of many flavonoids are much stronger than those of vitamins C and E [32]. Flavonoids are widespread plant secondary metabolites, including flavones, flavanols and condensed tannins. Epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases associated with oxidative stress. *In vitro*, flavonoids from several plants sources have shown free-radical scavenging activity and protection against oxidative stress [33]. Natural phenolics exert their beneficial health effects mainly through their antioxidant activity [34]. These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing 1st-chain initiation by scavenging initial radicals such as

hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non radical species and breaking chains to prevent continued hydrogen abstraction from substances [35].

Tannins are produced through the process known as condensation of simple phenolics and have a variety of molecular structures [36]. Generally, they are divided into hydrolysable and condensed proantho- cyanidins (polymers of flavan-3-ols). Tannins are biologically active compounds and may have beneficial or adverse nutritional effects [37]. The TLC and HPTLC data therefore confirmed that these extracts were rich sources of flavonoids, tannins, phenolic compounds which explain the potent antioxidant properties of *C. procera* extracts.

3.4 Lipid Peroxidation (LPO) - Thiobarbutiric Acid Reactive Species Assay (TBARS)

The levels of alteration in LPO are depicted in Table 3. There are significant alterations in both the extracts of *C. procera.* A significantly decreased level of LPO has been observed in the infected RBCs, when treated with methanolic extract of *C. procera* as compared to the aqueous extract.

Atamna et al. [38] observed that erythrocytes infected with *P. falciparum* produced OH• radicals and H2O2 about twice as much compared to normal erythrocytes. *Plasmodium* parasites are subjected to high levels of oxidative stress during development in host cells. A potential source of free radical production in this disease is the host's hemoglobin molecule, since the parasite uses this molecule as a source of amino acids for its own nutrition during the erythrocytic stage of the disease, resulting in the liberation of large amounts of circulating heme. By having Fe^{2+}-associated groups, these heme groups are able to induce intravascular oxidative stress, causing changes in erythrocytes and endothelial cells and facilitating the internalization of the parasite in tissues such as the liver and brain [39]. Lipid peroxidation causes damage to unsaturated fatty acids and thereby decreases membrane fluidity which ultimately leads to many pathological events in the body [40].

The study revealed that while the infected RBCs in culture manifested a high level of lipid peroxidation, the infected RBC cultures treated with the aqueous and methanolic extracts showed a significant decline in lipid peroxidation. This may be due to a decrease in the number of parasites entering the pre-treated RBCs. Antioxidants also protect membranes from peroxidative damage through their metal ion chelating and radical scavenging capabilities [41]. Plant-derived antioxidants such as lignans, tannins, stilbenes, quinones, coumarins, xanthones, phenolic acids, flavones, flavonols, catechins, anthocyanins, and proanthocyanidins have the potential to delay or provide protection to living organisms from the damage caused by uncontrolled production of ROS and the concomitant lipid peroxidation, protein damage, and DNA strand breaking because of their redox properties, which allow them to act as hydrogen donors, reducing agents and free radical scavengers [42-44].

3.5 Erythrocytes Membrane Stabilization Assay

Result of percent erythrocyte membrane stabilization assay is given in Table 4. We observed a decline in the entry of the parasites into the RBC, in relation to the concentration of both the extracts. Constant membrane stabilization effects are observed in the higher concentrations of both the extracts.

Table 2. Table showing the percent antioxidant activity of the both extracts of leaves of *C. procera*

	Concentration of the extract					
	250 µg/ml	**125 µg/ml**	**62.5 µg/ml**	**31.25 µg/ml**	**15.63 µg/ml**	**7.81 µg/ml**
Ascorbic acid	93.25±1.78	91.70±1.71	89.31±1.95	78.33±2.31	75.90±1.55	66.46±1.98
Aqueous	71.35±0.58	70.98±0.56	69.88±0.22	64.06±0.27	60.33±0.48	48.45±0.32
Methanol	74.22±0.48	73.89±0.96	73.33±0.45	72.27±0.44	69.89±0.63	56.59±0.49

Values are mean ± S.E

Table 3. Table showing lipid peroxidation in control RBCs, iRBCs and treated with both the extracts of the leaves of *C. procera*

RBCs	LPO (×10^4n moles of MDA formed/100 mg of tissue wt/60 mins) (MRC-2)	LPO (×10^4n moles of MDA formed/100 mg of tissue wt/60 mins) (RKL-9)
Control RBCs	0.158±0.02	0.158±0.02
iRBCs	0.949±0.01**	0.949±0.01**
RBCs+ CAQ (250 µg/ml)	0.500±0.03**	0.340±0.02**
RBCs+ CHA (250 µg/ml)	0.580±0.02**	0.520±0.02**

*Values are mean ± S.E. *p<0.01 **p<0.001*

Table 4. Showing percent of erythrocyte membrane stabilization

Extract	Concentration of the extract					
	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.63 µg/ml	7.81 µg/ml
Aqueous	89.15±0.16	89.48±0.21	87.48±0.31	79.55±0.15	83.25±0.10	82.99±0.14
Methanol	92.69±0.14	92.11±0.11	90.66±0.13	90.40±0.10	70.34±0.79	65.24±0.20

Values are mean ± S.E. (Control untreated RBCs - 34.08±0.18%)

Haemolytic assays have long been used to measure free radical damage and counteraction by antioxidants. It is useful for screening for oxidizing or antioxidizing molecules. Many primary or secondary plant metabolites have been found to protect cells from oxidative damage. These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation. Human erythrocytes are the key target for the free radicals or reactive oxygen species because of polyunsaturated fatty acid membrane and also their oxygen transport linked redox activity. H_2O_2 is a potent oxidizing molecule which causes cell membrane depolarization and subsequent release of hemoglobin from the cells [45]. Anti-haemolytic activity is a characteristic of antioxidant molecules to safeguard the cells against these reactions. Medicinal plants are efficient in boosting the immune system of resistance against infections, healing the allergies, raising and renewing the body's vitality [46]. The synergistic characters of these phytochemicals have made them more unique, as they can mitigate many diseases like atherogenesis, thrombosis, carcinogenesis, hepatotoxicity and variety of disease via inhibiting lipid peroxidation [47].

Oyedapo et al. [48] have shown that herbal drugs are capable of stabilizing the red blood cell membrane. The mode of action of the extracts could be connected with binding of certain extract components to the erythrocyte membranes with subsequent alteration of the surface charges of the cells. This might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the hemolysis of red blood cells. It has been reported that certain flavonoids exert profound stabilizing effect on lysosomal membrane both *in vivo* and *in vitro*, while tannins possess ability to bind cations, thereby stabilizing the erythrocyte membrane and other biological macro molecules [49,50]. Such molecular phenomena could have a major impact in halting the transgression of the parasite through the RBC membrane.

3.6 *In vitro* Percent (%) Inhibition of Entry of Parasites

The experimental evidence obtained in our study revealed that the hydroalcoholic extract of the leaves of *C. procera*, tends to be more effective in preventing the parasite entry into RBCs. The result has been tabulated in Table 5.

The malaria parasite passes through numerous stages of development and these stages have their individual unique shapes and structures and protein complements. According to Silvie, et al. [51] the merozoites released from the liver recognize, attach and enter the RBCs by multiple receptor-ligand interactions in as little as 60 seconds. Silvie et al. [51] have explained that varieties of duffy binding like (DBL) homologous proteins of *P. falciparum* recognize different RBC receptors other than the duffy blood group or the reticulocyte receptor.

Table 5. Percent (%) inhibition of entry of parasites into the RBCs

Drug conc. (µg/ml)	Control RBCs (untreated)		iRBCs (No. of parasites) (treated)		Percent inhibition	
	MRC-2	RKL-9	MRC-2	RKL-9	MRC-2	RKL-9
0.00	200	200	-	-	-	-
3.91	-	-	113	128	43.50±0.25	36.00±0.24
7.81	-	-	98	99	51.00±.0.07	50.50±0.13
15.63	-	-	89	91	55.50±0.07	54.50±0.37
31.2	-	-	69	88	65.50±0.25	56.00±0.22
62.5	-	-	51	80	74.50±0.12	60.00±0.12
125	-	-	32	67	84.00±0.04	66.50±0.23
250	-	-	21	52	89.50±0.21	74.00±0.12

Values are mean ± S.E

The malarial parasites secrete hundreds of diverse proteins to seize control of RBCs [52]. Proteins located on the surface of merozoites are divided into proteins anchored to the merozoite plasma membrane and others associated by interaction with surface proteins [53].

Diez-Silva, et al. [54] 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. In our study it was observed that there was a sharp decrease in the protein content after 48 hrs in the treated iRBCs which could probably be due to the effect of certain phytochemicals in blocking the synthesis of this protein (RESA) and thus explaining the inhibition of the parasite growth.

4. CONCLUSIONS

The present investigation clearly reveals the antimalarial nature of the aqueous and methanolic extract of *Calotropis procera* by stabilizing the red blood cell membrane and suggests that this plant could be used in the management of malaria caused by *P. falciparum* in human. On the basis of the results obtained in the study, it can be inferred that the leaves of *C. procera* are rich sources of secondary metabolites/phytocomoponents which contribute to its efficacy in RBC membrane stabilization and restriction of entry of parasites into the cell.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the grant of a major project under the FAP-2012 Programme of Gujarat State Biotechnology Mission (GSBTM).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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