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**SHORT COMMUNICATION**

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**STUDIES ON *IN VITRO* ANTIPLASMODIAL ACTIVITY OF *CLEOME RUTIDOSPERMA***ANINDYA BOSE<sup>1\*</sup>, PETER J SMITH<sup>2</sup>, CARMEN A. LATEGAN<sup>2</sup> JAYANTA KUMAR GUPTA<sup>3</sup>  
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Bhubaneswar-751030, Orissa, India.**Keywords:** *Cleome rutidosperma*, aqueous extract, ethanolic extract, fractionation, malaria; *Plasmodium falciparum*, antiplasmodial activity, cytotoxicity

Malaria is a protozoal disease transmitted by the *Anopheles* mosquito, caused by minute parasitic protozoa of the genus *Plasmodium*, which infect human and insect hosts alternatively. It affects over 40% of the world's population, with 120 million cases reported, and about 2 million deaths annually (1). The *P. falciparum* variety of the parasite accounts for 80% of cases and 90% of deaths caused by malaria. The declining efficacy of classical medication in relation to the rapid increase of parasite resistant strains, mainly of *Plasmodium falciparum*, as well as the greater resistance of vectors to insecticides, and the difficulty of creating efficient vaccines have led to an urgent need for new efficient antimalarial drugs (2, 3). Natural molecules may provide innovative strategies towards malarial control, hence active research groups are now working to develop new active compounds as an alternative to chloroquine, especially from artemisinin (4, 5), a plant-based antimalarial drug isolated from the Chinese plant *Artemisia annua* (6). Plants may well, therefore, prove to be the sources of new antimalarial in view of the success with the two important chemotherapeutic agents, quinine and artemisinin, both of which are derived from plants.

*Cleome rutidosperma* (Capparidaceae) is a low-growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves and

small, violet-blue flowers, which turn pink as they age. The elongated capsules display the asymmetrical, dull black seeds. The plant is native to West Africa, although it has become naturalized in various parts of tropical America as well as Southeast Asia (7, 8). The diuretic, laxative, anthelmintic, antimicrobial, analgesic, anti-inflammatory, antipyretic, antioxidant and free radical scavenging activities of *Cleome rutidosperma* were reported earlier by the authors (9-13). The plant is used as anti-malarial by the traditional healers in Cameroon and mild antiplasmodial activity of chloroform/methanol (1:1) extract of leaves of *Cleome rutidosperma* against chloroquine-sensitive (F32) laboratory strain of *P. falciparum* was reported earlier in Cameroon (14). The present study investigates the *in vitro* antiplasmodial activity of ethanolic extract and its fractions of aerial parts of *Cleome rutidosperma* against the chloroquine sensitive (CQS) D10 strain of the parasite, as well as their toxicity against a mammalian cell lines.

**MATERIALS AND METHODS****Plant materials**

The plant material (whole plant) was collected from North 24-Pargana district of West Bengal, India during September 2004 and was authenticated at

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Botanical Survey of India, Shibpur, Howrah, West Bengal, India. A voucher specimen (C.R.-1) has been kept in our research laboratory for future reference. The fresh aerial parts were washed under running tap water to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder.

#### Preparation of extracts

The aerial parts were extracted with 90% ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure, which gave a greenish-black colored sticky residue (yield 11.6% w/w on dried material basis). A portion of dried ethanolic extract was suspended in water and fractionated successively with petroleum ether (40-60°C), diethyl ether, ethyl acetate and n-butanol. All the fractions were dried by distillation under reduced pressure and kept in a desiccator until used. For preparation of aqueous extract, 50 g of powdered aerial parts were stirred in 450 mL of boiling distilled water. Boiling was allowed to continue for 5 min. The mixture was then kept aside for 30 min to allow it to infuse. It was then filtered initially with cloth filter then with filter paper, evaporated and dried under reduced pressure to yield solid residue, which was kept in a desiccator until used.

#### *In vitro* antiplasmodial activity

Continuous *in vitro* culture of asexual erythrocyte stages of *Plasmodium falciparum* were maintained using a modified method of Trager and Jensen (15). Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase (pLDH) assay using a modified method described by Makler (16). The pLDH assay compares well with other available assays (17). The fractions were tested in duplicate against the chloroquine sensitive (CQS) D10 strain of the parasite. The fractions were dissolved in 10% methanol or 10% DMSO and stored at -20°C until use. The fractions were tested as a suspension if not properly dissolved. The highest concentration of solvent to which the parasites were exposed had no measurable effect on the parasite viability. Chloroquine (CQ) was used as the reference drug in all experiments. The starting concentration of the full dose-response experiment was 100 µg/mL, which were serially diluted 2-fold in complete medium to give 10 concentrations, with the lowest concentration being 0.195 µg/mL. CQ was tested at an initial concentration of 100 ng/mL. The same dilution technique was used for the other samples. The 50% inhibitory concentration (IC<sub>50</sub>) values were

obtained using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4.0 software.

#### Cytotoxicity test on mammalian cells

The most potent extract fraction (diethyl ether fraction) was evaluated for *in vitro* cytotoxicity against a mammalian cell-line, Chinese Hamster Ovarian (CHO) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-assay. The MTT-assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays (18, 19). The tetrazolium salt MTT was used to measure both growth and chemosensitivity. The sample was dissolved in 10% methanol and tested as a suspension. The initial concentration of stock solutions was 2 mg/mL for all samples. Samples were stored at -20°C until use. The highest concentration of solvent to which the cells were exposed had no measurable effect on the cell viability. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 µg/mL, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 µg/mL. The same dilution technique was applied to the test compounds with an initial concentration of 100 µg/mL, to give 5 concentrations, with the lowest concentration of 0.01 µg/mL. All samples were tested in triplicate on a single occasion. The 50% inhibitory concentration (IC<sub>50</sub>) values for these samples were obtained from dose-response curves, using a non-linear dose-response curve fitting analyses via GraphPad Prism v.4 software.

## RESULTS AND DISCUSSION

The IC<sub>50</sub> values obtained from the tests on the CQS D10 strain of *Plasmodium falciparum* are shown in Table 1. The selectivity index (SI), defined as the ratio of cytotoxicity to antiplasmodial activity and determined by dividing the IC<sub>50</sub> values for the CHO cells by the IC<sub>50</sub> value for *Plasmodium falciparum* is also shown for the most active fraction (Table 2). High selectivity (high SI values) indicates potentially safer therapy of the drugs against malaria parasite.

Evaluation of *in vitro* antiplasmodial activity of ethanolic extract of *Cleome rutidosperma* showed moderate activity against *P. falciparum* CQS D10 strain (IC<sub>50</sub> value of 34.4 µg/mL), although the aqueous extract was found to be inactive (IC<sub>50</sub> > 100 µg/mL). Fractionation process enhanced the activity of the initial crude ethanolic extract, whereas petroleum ether and diethyl ether fractions were found to

Table 1. *In vitro* antiplasmodial activity of *Cleome rutidosperma* against *P. falciparum* CQS D10 strain.

Treatment	IC <sub>50</sub>
Ethanol extract (E)*	34.4 µg/mL
Petroleum ether fraction of E*	20.0 µg/mL
Diethyl ether fraction of E*	8.1 µg/mL
Ethyl acetate fraction of E*	54.3 µg/mL
n-Butanol fraction of E*	76.4 µg/mL
Water extract	> 100 µg/mL
Chloroquine	11.1 ng/mL (n = 3)

\*Tested as a suspension. Values are the average of three replicates. n = number of data averaged.

Table 2. IC<sub>50</sub> values of diethyl fraction and emetine against CHO cell line

Treatment	IC <sub>50</sub> (µg/mL)	Selectivity index <sup>a</sup>
Diethyl ether fraction	> 100	> 12.3
Emetine	0.05	

Values are the average of three replicates.

<sup>a</sup>Selectivity index (SI) = cytotoxicity IC<sub>50</sub> / antiplasmodial IC<sub>50</sub>

be more active than the main ethanolic extract. As the ethanolic extract and its fractions were fairly insoluble, even in DMSO and were tested as a suspension, this might have affected the accuracy of the results and the actual IC<sub>50</sub> values might be even lower. The diethyl ether fraction was found to be the most potent fraction and showed good antiplasmodial activity with IC<sub>50</sub> value of 8.1 µg/mL. The fraction did not show any cytotoxicity against the CHO cell-line at the concentrations tested. This indicated the activity selectivity for the tested parasite.

The presence of phytoconstituents like terpenoids or flavonoids have been previously found to be responsible for antimalarial activities in plants (20-28). The presence of these constituents in ethanolic extract and its potent fractions of *Cleome rutidosperma*, as reported earlier (13), may be responsible for the observed activities. Investigations are in progress to identify the active antimalarial compounds of *Cleome rutidosperma* by bioassay-guided fractionation.

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