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**Antimalarial efficacy of nine medicinal plants traditionally used by the Karens of Andaman and Nicobar Islands, India**

## Antimalarial efficacy of nine medicinal plants traditionally used by the Karens of Andaman and Nicobar Islands, India

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### Abstract

The aim of this study was to assess the antimalarial activity of nine medicinal plants used by Karens of Andaman and Nicobar Islands, against *Plasmodium falciparum* chloroquine-sensitive MRC-2 isolate. The methanol extracts were obtained by cold percolation method and *in vitro* antimalarial activity was assessed using M-III method. The results indicated that out of nine plant species tested, four plants, viz., *Z. spectabilis*, *S. wallichiana*, *C. pulcherrima* and *Amomum* sp. demonstrated significant antimalarial activity (50% inhibitory concentration values were  $5.5 \pm 0.7$ ,  $12.0 \pm 2.5$ ,  $14.6 \pm 1.3$  and  $37.3 \pm 2.5$   $\mu\text{g/mL}$  respectively) with no toxicity effect on erythrocytes.

### Introduction

Andaman and Nicobar Islands, a union territory of India, has been historically known for high malaria transmission. The tropical climate prevailing throughout the year provides an ideal environment for mosquito proliferation and breeding in Nicobar group of islands (Manimunda et al., 2011). Entire land mass in the island has a network of creeks which results in ideal brackish water habitats for the breeding of *Anopheles sundaicus*, the predominant vector of malaria in Andaman and Nicobar Islands (Krishnamoorthy et al., 2005).

Malaria prevalence has increased alarmingly in past few years due to the development of drug resistance by *Plasmodium falciparum* (Parzy et al., 1997). Therefore, new and more effective therapeutic agents are urgently required to overcome the problem of malaria.

The Karen tribe is a lesser-known community in these Islands, settled in Mayabunder, North and Middle Andaman District. Karen derives from the word 'Kavin' as British called them, while they called themselves as

*Pwakanyaw*, the quiet and easy going people, hailing from Henzada, Pathe and Nachaon areas of Pegu district in Western Myanmar (Oberai, 2000). Presently they inhabit three villages depending on agriculture and fishing for livelihood. Most of them depend on traditional medicine, though modern medicine had increased rapidly during recent times (Chander et al., 2015). As a part of the ongoing studies documented their traditional treatment practices and collected plants which were reported to be efficacious in the treatment of traditional medicine system.

In the present study, nine of these plants were subjected to *in vitro* antimalarial activity against *P. falciparum* chloroquine-sensitive (MRC-2) isolate.

### Materials and Methods

#### Plant materials

Nine plants were selected for evaluation on the basis of their ethnomedicinal histories. The names of these



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Table I			
Antimalarial activity of ethnomedicinal plant used by Karens of Andaman and Nicobar Islands			
Scientific name (Voucher No.)	Family	Local Name	Antimalarial activity IC <sub>50</sub> (µg/mL)
<i>Amomum spp.</i> (AN-509)	Zingiberaceae	Thajokha	37.3 ± 2.5
<i>Boesenbergia rotunda</i> (L.) Mansf. (AN-505)	Zingiberaceae	Sue	>100.00
<i>Caesalpinia pulcherrima</i> (L.) Sw. (AN-521)	Caesalpinaceae	Posibo	14.6 ± 1.3
<i>Cinnamomum tamala</i> (Buch.-Ham.) T. Nees & Eberm. (AN-524)	Lauraceae	Thechipogow	>100.0
<i>Moringa oleifera</i> Lam. (AN-512)	Moringaceae	Dandloadaw	>100.0
<i>Senna alata</i> (L.) Roxb. (AN-502)	Caesalpinaceae	Katheepaw	>100.0
<i>Solanum torvum</i> Sw. (AN-522)	Solanaceae	Takotako	>100.0
<i>Strychnos wallichiana</i> Steud. ex DC. (AN-511)	Loganiaceae	Niveka	12.0 ± 2.5
<i>Zingiber spectabilis</i> Griffith (AN-503)	Zingiberaceae	Methalow	5.5 ± 0.7
Chloroquinone			1.3 ± 0.1

IC<sub>50</sub> values are expressed as a mean of three independent values and were recorded in µg/mL

plants in alphabetical order, with their respective scientific name, voucher numbers, family and vernacular name (Karen language) are presented in the Table I. Plant materials were collected in the three villages of Mayabunder, North and Middle Andaman District along with Traditional Knowledge Providers (TKPs) and authenticated by the plant taxonomist at the Botanical Survey of India, Andaman and Nicobar Islands, where voucher specimens were deposited in the Medicinal plant laboratory at the Regional Medical Research Centre (ICMR), Port Blair.

#### Extraction preparation

The plants were washed, shade dried at room temperature and ground into a fine powder. One hundred gram of each plant powder was subjected to percolation by soaking in 95% methanol. After 7 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (45°C). Stock solutions of crude extracts were made in dimethyl sulfoxide (DMSO).

#### Parasite cultivation

The antimalarial activity of plant extracts was assessed against chloroquine-sensitive *P. falciparum* (MRC-2) isolate obtained from the National Institute of Malaria Research (ICMR), New Delhi, India. *P. falciparum* is cultivated in human A Rh<sup>+</sup> red blood cells using RPMI 1640 medium (Sigma, India) supplemented with AB Rh<sup>+</sup> serum (10%), 5% sodium bicarbonate (Sigma, India) and 40 µg/mL of gentamicin sulfate (Sigma, India) (Trager and Jensen, 1976).

#### In vitro test for antimalarial activity

The *in vitro* activity of *P. falciparum* intraerythrocytic stage on crude plant extracts was evaluated by Schizont maturation Inhibition M-III method (WHO, 2001). Briefly, crude extracts were dissolved in DMSO and serially diluted with RPMI 1640 medium to reach 1 mg/mL before use. Serial double dilutions were made in 96-well microtiter plates (in triplicate) with a concentration range of 1.56-100 µg/mL. The cultures, before testing, were synchronized by treatment with 5% D-sorbitol with a parasitemia of 0.6-0.8%. Each well received 10 µL of parasite-infected erythrocytes, 5% hematocrit and 90 µL of different drug dilutions. Chloroquine and solvent controls contained similar concentrations of solvent, as that of test wells. The plates were incubated at 37°C for 24 hours. After confirmation of the presence of 10% mature schizonts in control wells (without drug), the blood from each well was harvested, and a thick film was prepared on a glass slide. The blood films were stained for 40 min with Giemsa stain at a dilution of 10% in double distilled water. Three independent optical-microscopy readings of the number of schizonts with three or more nuclei were carried out in 200 parasitized red blood cells for each dilution and duplicate. Growth inhibition was expressed as the percentage of schizonts in each concentration, compared with controls.

#### Antimalarial activity calculation and analysis

The number of schizonts observed per well was directly entered into the nonlinear regression software, HN-

NonLin V 1.1, which was specific for the analysis of *in vitro* drug sensitivity assay for malaria. Individual dose response curves were generated and their IC<sub>50</sub> values were determined.

#### Chemical injury to erythrocytes

In order to determine the possibility of chemical injury to the erythrocytes due to the extract, 100 µL of 5% erythrocytes were incubated with 100 µL/mL of the extract at a dose equal to the highest used in the antimalarial assay. During the experiment, the conditions were maintained as in the case of antimalarial assay. After 24 hours of incubation, thin blood smears were stained with Giemsa and observed microscopically for any morphological changes. The morphological findings were compared with those of erythrocytes which are not exposed to the extract.

### Results

The leaf extract of *Z. spectabilis* (IC<sub>50</sub> 5.5 ± 0.7 µg/mL) showed excellent antimalarial activity followed by extracts of *S. wallichiana* (IC<sub>50</sub> 12.0 ± 2.5 µg/mL), *C. pulcherrima* (IC<sub>50</sub> 14.6 ± 1.3 µg/mL) and *Amomum* sp. (IC<sub>50</sub> 37.3 ± 2.5 µg/mL). Moreover, the leaf extract of *C. tamala*, *S. toroum*, *M. oleifera*, *S. alata* and *B. rotunda* were relatively inactive with IC<sub>50</sub> values higher than 100 µg/mL (Table I).

The microscopic observation of uninfected erythrocytes incubated with the methanol extracts and uninfected erythrocytes from the blank column of the 96-well plate showed no morphological differences after 48 hours of incubation.

### Discussion

In the early studies, we reported the list of plants used by the Karens for various illnesses (Chander et al., 2015) and we selected nine plants routinely used by the Karens. Methanol extracts of *Z. spectabilis*, *S. wallichiana* and *C. pulcherrima* showed the antimalarial activity and no previous reports available on the antimalarial properties of these medicinal plants in the literature. A previous phytochemical investigation on non-volatile constituents of the rhizomes of *Z. spectabilis* afforded several flavonoids, terpenoids and also demonstrated the antibacterial activity (Sivasothy et al., 2012). In the present study, the leaf extract of *Z. spectabilis* showed high antimalarial activity in crude form.

According to Rasaonaivo et al. (1992), an extract is very active if IC<sub>50</sub> < 5 µg/mL, active 5 µg/mL < IC<sub>50</sub> < 50 µg/mL, weakly active 50 µg/mL < IC<sub>50</sub> < 100 µg/mL and inactive IC<sub>50</sub> > 100 µg/mL.

*S. wallichiana* is specifically used for malaria fever in Karen community and it showed good antimalarial activity. In

previous reports, the presence of various phytochemical and antimicrobial activities of *S. wallichiana* was demonstrated (Mallikharjuna et al., 2010). Vivek et al. (2013) reported the antimicrobial and anti-oxidant properties of leaf and flower extracts of *C. pulcherrima*. In the present study, leaf extract showed the active antimalarial property.

The *in vitro* antimalarial activity might be due to the presence of alkaloids, flavonoids, triterpenes, sterols, tannins and saponins in the methanol extracts of tested plants. The mechanism of action might be due to the inhibition of hemozoin biocrystallization, inhibition of protein synthesis, decreased mitochondrial membrane potential and DNA fragmentation (Dubar et al., 2011; Lopez et al., 2010).

### Conclusion

The methanol leaf extracts of *Z. spectabilis*, *S. wallichiana* and *C. pulcherrima* possess significant suppressive effects on *in vitro* cultures of chloroquine-sensitive *P. falciparum*. These plants could serve as useful sources for new antimicrobial agents.

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### Conflict of Interest

Authors declare no conflict of interest

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