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# Evaluation of *in vitro* antimalarial activity of *Sida acuta* Burm.f. crude extract

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## Abstract

Development of resistant strains malaria parasite to many existing drugs are major concern globally. Plant derived anti-malarials remains a source of bioactive compound in this regard. The crude methanolic extracts of *Sida acuta* Burm.f. was tested *in vitro* for their antimalarial activity against *Plasmodium faciparum* both chloroquine sensitive and resistant strains using SYBR Green I assay. Chemical fingerprints were analyzed by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The methanolic extract of *S. acuta* Burm.f. showed good antiplasmodial activity against both chloroquine resistant (K1) ($IC_{50}$ 25.43 – 35.69 µg $mL^{-1}$) and chloroquine sensitive (3D7) ($IC_{50}$ 24.83 – 27.37 µg $mL^{-1}$). According to the result, the crude methanolic extracts of *S. acuta* Burm.f. branch exhibited the lowest $IC_{50}$ against *P. falciparum* K1 (chloroquine resistance clone) 25.43 ± 4.19 µg $mL^{-1}$ and crude methanolic extracts of *S. acuta* Burm.f. leaves exhibited the lowest $IC_{50}$ against *P. falciparum* 3D7 (chloroquine sensitive clone) 24.83 ± 4.81 µg $mL^{-1}$. The chemical fingerprint of crude methanolic extracts of *S. acuta* Burm.f. using TLC fingerprint exhibited the present of alkaloid when sprayed with dragendorff's reagent and HPLC fingerprint showed the present of quinine. In conclusion, the methanolic extract of *Sida. acuta* Burm.f. exhibited a good *in vitro* antimalarial activity against *P. falciparum* ($IC_{50}$ < 50 µg $mL^{-1}$) and can be developed as a potential antimalarial agents.

**Keywords**: *Sida acuta*; antimalarial property; *Plasmodium falciparum*; medicinal plant



## 1. Introduction

Plants have been served as a major source of medicine since ancient times. World Health Organization report that up to 90% of population in developing countries use plants and its products as traditional medicine for primary health care [1]. Traditional herbal medicine provides a rich source for new drug discovery. Increasing resistance of *Plasmodium falciparum* to commonly use antimalarial drugs has made the need for the development of new agents especially, herbal medicine. A large number of medicinal plants have been identified as potential antimalarial agents [2 – 4]. The constituent alkaloid quinine was identified as an effective anti-malaria drug from the South American plant of the genus *Cinchona* since 1645 and the extracts were used for a long time. However, quinine exhibited relatively high mammalian toxicity and was also not readily available. Artemisinin, Chinese traditional plant and its derivatives isolated from *Artemisia annua,* are currently the drugs of choice for treatment of malaria. Artemisinin has excellent antiplasmodial properties but is more expensive compared to other antimalarial drugs. Due to either limited availability or affordability of conventional medicines in

tropical countries, about 80% of the rural population still relying on the plant extracts to combat the disease [5]. Several studies have been undertaken to evaluate various plant extracts against *P. falciparum*. *Sida* is a medicinal plant belongs to the family Malvaceae comprise of about 200 species. It is native to Central America, but has spread throughout the tropics and sub-tropics in the Pacific, Asia and Africa [6]. *Sida acuta* Burm.f. is one of ethnomedicinally important in this genus. In traditional medicine, *Sida acuta* Burm.f. has been shown to possess a wide spectrum of pharmacological activities such as antipyretic, anti-inflammation, antibacterial, antiulcer as well as antimalaria and several phytochemical screening from this plant showed various compounds. The alkaloid in this plant is the indoloquinolines family and the main alkaloids are cryptolepine and its derivatives [7]. Considering the great potential of Thai herbal medicine, this recent study the antimalarial activity of *Sida acuta* Burm.f. extract was evaluated using *in vitro* SYBR Green I-based assay.

## 2. Materials and Methods

*Plant materials*

*S. acuta* Burm.f. was collected from 3 various locations during January to March in 2015. All plant samples were identified by botanist and voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University.

*Preparation of plant extraction*

All plant materials were washed and dried under shade at about 35 – 40 °C for several days. Dried plant material was then ground to a fine powder using a laboratory scale mill. Ten grams of the dried powder of leaves, branch and stem of each sample from 3 different locations was individually extracted with methanol. The extracts were evaporated to dryness. The percentage yields of the extracts were calculated. The extracts were then stored in a refrigerator until use. Thereafter, the extracts were re-dissolved in methanol at a concentration of 1 mg $mL^{-1}$. The obtained crude extracts were analyzed for their antimalarial activity. Chemical fingerprint was examined using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). All tests were done in triplicate.

*In vitro antimalarial activity*

*In vitro* antimalarial activity of the crude methanolic extracts of *S. acuta* Burm.f. were evaluated against laboratory-adaped *P. falciparum* 3D7 (CQ sensitive clone) and K1 (CQ resistance clone) using SYBR Green I-based assay [8]. Briefly, the parasite was cultured according to the method of Trager and Jensen with some modifications [9] on human erythrocytes. Highly synchronous ring stage parasite by 5% D-sorbital was used in each assay. An aliquot of parasite inoculum (50 µl) with 2% parasitaemia and 1% haematocrit was added into each well of a 96-well microtiter plate. The 96-well drug plates were dosed with the crude methanolic extract of *S. acuta* diluted with complete media to obtain the concentrations range 0 – 100 µg $mL^{-1}$. Dihydroartimisinin (DHA) was used as positive control. The 96-well drug plates were incubated at 37 °C under a gas mixture of 50% $N_2$, 5% $O_2$ and 5% $CO_2$ for 2 days. One hundred microlitre of fluorescent haemolysis reagent (0.01% of fluorescent dye SYBR Green I in lysis buffer) was added to each well and incubated the plates in the dark for 1 hours. Fluorescence intensity was determined at the excitation and emission wave lengths of 485 and 530 nm, respectively. The experiment was done in triplicate and the mean values were calculated. The percent growth inhibition was calculated and the $IC_{50}$ values (concentrations that inhibit the parasite growth by 50) used as indicators of antimalarial activity were determined from a log-dose–response curve plotted using the Calcusyn™ version 1.1 (BioSoft, Cambridge, UK).

*Chemical fingerprint*

*TLC fingerprint*

Three microliters of the crude methanolic extract of *Sida acuta* Burm.f. (1 mg $mL^{-1}$) was spotted onto the 20 × 20 $cm^2$ aluminum sheets silica gel plate (G60 F254, Merck) with 0.25 mm thickness. The plate was developed in the solvent system of butanol: acetic acid: $H_2O$ (6:1:2 v $v^{-1}$) for at least 1 hour. The TLC fingerprint was visualized under UV light at 254 nm and 365 nm and sprayed with dragendorff's reagent. The TLC fingerprint was captured by a digital scanner (Hewlett Packard Deskjet F2280).

*HPLC fingerprint*

The crude methanolic extract of *Sida acuta* Burm.f. was filtrated with 0.45 µm filter membrane before analyzed by HPLC (Thermo Fisher, USA) on Thermo Hypersil gold C18 reversed-phase column, sized 5 µm, 250 × 2.1 $mm^2$. Mobile phase gradient consisted of Acetonitrile: 50 mM $KH_2PO_4$ pH3.0 with the flow rate of 1 mL $min^{-1}$. Injection volume was 5 µl. A UV-PDA detector was set at a maximum absorption wavelength 210 nm for monitoring chromatographic profile. Standard quinine was used as reference for HPLC analysis.

*Data analysis*

The results were presented as mean ± standard deviation (SD).

## 3. Results and Discussion

Ten grams of the dried powder of leaves, branch and stem of each sample from 3 different locations were individually extracted with methanol. The extracts were evaporated to dryness. The percentage yields of the extracts were shown in Table 1.

**Table 1** The characteristic and percentage yields of the crude methanolic extract of *S. Acuta*

| Plant | (parts used) | Color | Yield (% w $w^{-1}$) |
|---|---|---|---|
| *S. acuta* Burm.f. | leaves | Brown-green sticky compound | 15.25 |
| | branch | Dark-brown sticky compound | 9.81 |
| | stem | Dark-brown sticky compound | 5.87 |

*In vitro antimalarial activity*

*In vitro* antimalarial activity of the crude methanolic extract of *S. acuta* Burm.f. was investigated using SYBR Green I assay. The $IC_{50}$ values used as indicators of antimalarial activity were determined from a log-dose–response curve plotted using the Calcusyn™ version 1.1 (BioSoft, Cambridge, UK) and the result was shown in Table 2. Artesunate and quinine were used as positive control. The methanolic extract of *S. acuta* Burm.f. showed antiplasmodial activity against both chloroquine resistant (K1) ($IC_{50}$ 25.43 – 35.69 µg $mL^{-1}$) and chloroquine sensitive (3D7) ($IC_{50}$ 24.83 – 27.37 µg $mL^{-1}$).

**Table 2** The $IC_{50}$ values of *in vitro* antimalarial activity of the crude methanolic extract of *S. acuta* Burm.f. against *P. falciparum* 3D7 and K1. Data presented as mean ± standard derivation

| The crude methanolic extract of *S. acuta* Burm.f. | *In vitro* antimalarial activity $IC_{50}$ (µg $mL^{-1}$) | |
|---|---|---|
| | K1 (CQ Resistant clone) | 3D7(CQ Sensitive clone) |
| leaves | 27.87 ± 0.63 | 24.83 ± 4.81 |
| branch | 25.43 ± 4.19 | 26.38 ± 1.09 |
| stem | 35.69 ± 3.86 | 27.37 ± 1.06 |
| Artesuante (AS) | 0.00067 ± 0.0007 | 0.00052 ± 0.0003 |
| Quinine (QN) | 0.12 ± 0.003 | 0.017 ± 0.003 |

*Chemical fingerprint*

*TLC fingerprint*

The TLC fingerprint of the crude methanolic extract of *S. acuta* Burm.f. from different parts used was visualized under UV light at 254 nm and 365 nm and sprayed with dragendorff's reagent. The TLC fingerprint of methanolic extract of *S. acuta* Burm.f. leaves was shown in Fig. 1.

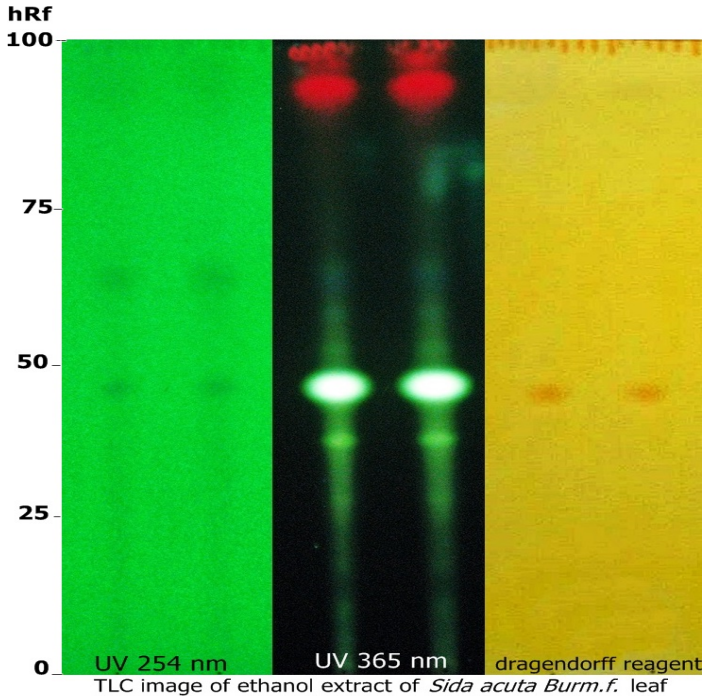


**Fig. 1** TLC fingerprint of methanolic extract of *S. Acuta* Burm.f. leaves using solvent system of butanol: acetic acid: $H_2O$ (6:1:2 v $v^{-1}$) under UV light at 254 nm and 365 nm and sprayed with dragendorff's reagent.

*HPLC fingerprint*

The HPLC separations were carried out on a Phenomenex Luna $C_{18}$ reversed-phase column with gradient of acetonitrile: 50 mM $KH_2PO_4$ pH 3.00. Detection wavelength was set at 210 nm. HPLC fingerprint of methanolic extract of *S. acuta* Burm.f. from all parts used presents peak at same retention time of standard quinine (19.73 min ). Fig. 2. Showed the HPLC chromatogram of *S. acuta* Burm.f. leaves.

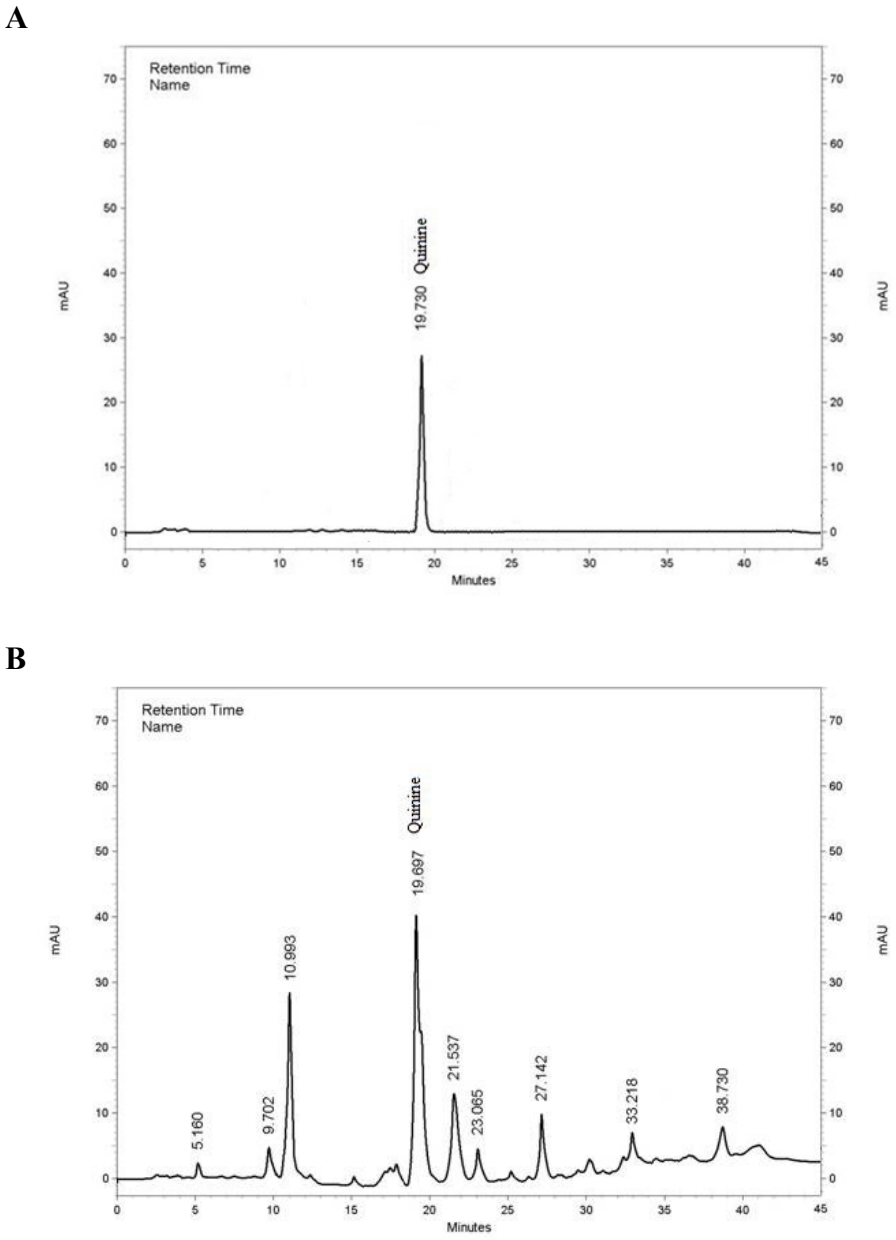


**Fig. 2** The HPLC chromatogram of (A) standard quinine and (B) the HPLC fingerprint of methanolic extract of *S. acuta* Burm.f. leaves. The peak of quinine present at retention time 19.73

A large number of herbal medicines have been traditionally used to treat malaria or fever for thousands of years including quinine and artemisinin, two main groups of modern antimalarial drugs derived from plants. Recently, increasing of drug resistance malaria parasites are major problem in malaria elimination. Malaria is the most commonly found in Africa and Southern Asia especially, in poor areas of being able to afford and access effective antimalarial drugs, traditional medicines could be an important and sustainable source of treatment [10]. *Sida acuta* Burm.f., a traditional medicine for the treatment of a wild range of human diseases, has been previously reported for its biological activities [11 – 14]. The present study showed that methanolic extract of *S. acuta* was tested *in vitro* for their antimalarial activity against laboratory *Plasmodium falciparum* both chloroquine sensitive and resistant clones. The result indicated the good antimalarial activity ($IC_{50}$ < 50 µg $mL^{-1}$). Previous study of ethanolic extract of *S. acuta* Burm.f. tested on fresh clinical isolates of *P. falciparum* using *in vitro*

semi-microtest showed the good antimalarial activity ($IC_{50}$ = 4.37 μg $mL^{-1}$) and suggested that alkaloids of the plant may responsible for antimalarial activity. Their result which is consistent with the report of Banzouzi *et al.*, (2004) in which ethanolic extract of *S. acuta* Burm.f. was shown to be active against *P. falciparum* using flow cytometry with incorporation of [$^3$H] hypoxanthine and the activity was related to its alkaloid contents [15]. Several Thai traditional medicines have been reported, ethanolic extracted of *E. longifolia* Jack showed active antimalarial activity both 3D7 and K1 with the $IC_{50}$ of 2.16 μg $mL^{-1}$ and 1.79 μg $mL^{-1}$ respectively, suggesting that this Thai medicinal plant has a potential to be an antimalarial agent when compare to artesunate, a standard antimalarial drug [16]. According to the TLC fingerprint sprayed with dragendorff's reagent indicated the present of alkaloid in methanolic extract of *S. acuta* Burm.f.. However, the mode of action of alkaloids with antiprotozoal activities is unknown and it is possible that some of these may act on biochemical targets unique to protozoa [17]. It is often argued by protagonists of herbal medicine that the total plant extract contains a mixture of substances which act synergistically and hence it is better for a patient to take the whole plant or an extract rather than a single isolated active ingredient. HPLC is the primary analytical tool for quantifying chemical compounds in plant materials. HPLC fingerprint indicate the present of quinine in methanolic extract of *S. acuta* Burm.f..

## 4. Conclusion

The methanolic extract of *S. acuta* Burm.f. exhibited a good *in vitro* antimalarial activity against *Plasmodium falciparum* ($IC_{50}$ < 50 μg $mL^{-1}$) due to their alkaloids and quinine contents in the extract which can be developed as potential antimalarial agents from Thai herbal plants.

## 5. Acknowledgement

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