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In-vivo antimalarial activity of aqueous root extract of *Barringtonia acutangula* in mice

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ABSTRACT

This study scientifically investigated the antimalarial activity of an aqueous root extract (ARE) of *Barringtonia acutangula* (L.) Gaertn. (Lecythidaceae), claimed to be used in malaria therapy in indigenous medicine in Sri Lanka. Three doses of the ARE (400, 800 and 1200 mg/kg), chloroquine or distilled water (DW) was orally administered for 3 consecutive days and the schizonticidal activity was examined in the *Plasmodium yoelii* murine model. The ARE exhibited dose dependent schizonticidal activity, the highest being recorded at 800 mg/kg dose. Oral administration of ARE at 800 mg/kg dosage significantly ($p< 0.05$) suppressed the parasitaemia by 60.88 % compared with the control. However, the ARE was 40 % less potent than chloroquine in the *P. yoelii* murine model. The ARE was well tolerated by mice over a period of 30 days showing no overt signs of toxicity, stress and hepatic (in terms of serum SGOT, SGPT levels), renal (in terms of serum creatinine, urea, Na^+, K^+ levels) and haematological toxicity. Slightly but a significant ($p< 0.05$) reduction in food and water intake was observed during 2nd and 3rd week of the experiment. In conclusion, the ARE of *B. acutangula* is orally active, relatively non-toxic and possesses antimalarial activity justifying the claims of indigenous medicine of Sri Lanka.

KEY WORDS: Sri Lanka; *Barringtonia acutangula;* antimalarial activity; *in vivo*; *Plasmodium yoelii*

INTRODUCTION

Malaria is a life threatening disease responsible for 1-2 million deaths each year (1). Despite much effort, the incidence of malaria continues to increase at a significant rate (2). This situation has been compounded by the emergence and spread of drug resistant strains of *P. falciparum* and *P. vivax* in much of the malarious parts of the world (3). The declining efficacy of classical medication in relation to the rapid extension of drug resistant strains of malaria parasites has led to the need for novel efficient anti malaria agents (4). A principal approach to chemotherapeutic research against malaria consists of investigating the traditional plant based antimalarials (5).

The plant kingdom represents a virtually untapped reservoir of novel chemical compounds, many of them extraordinary biodynamic. Eighty percent of the world's population relies on herbal medications to manage disease (6). In Sri Lanka, about 35% of the population relies on indigenous systems of health care (7).

Barringtonia acutangula (L.) Gaertn. (Lecythideae) (English: Indian Oak, Sinhala: Diyamidella, Tamil: Adambu) is a small evergreen plant (2.7- 3.6 m in height). This plant occurs throughout India, Sri Lanka, and North Australia (8).

It has a brownish grey bark with glabrous young parts. The leaves are simple, alternate and stipulate, and are 7.5 -12.5 cm long. Leaves are oval shaped, bright green in colour with tapering bases and contain a reticulate venation pattern. Their petioles are 0.6-1.2 cm long. Flowers are regular, bisexual and about 2.5 cm in diameter (9). Petals are cream coloured and stamens are dark bright crimson. Tacemes are 22.5- 30 cm long sepals and fused into a calyx tube adnate to the ovary. Ovaries are inferior to the flowers in long pendulous racemes, fragrant, with bright red stamens. Fruits are bluntly quadrangular and 2.5- 4.0 cm long (9).

Phytochemically, the bark and the root of *B. acutangula* contain saponins, tannins and triterpenes, and phenolic compounds (9). The juice of the leaves is used for diarrheoa (9). The water extract of the bark provides a scorpion venom antidote (10) and is also used on wounds (9). The powdered seeds are inhaled as snuff for relief in headache and externally applied for insect stings (9). The water extracts of roots and barks are employed as a febrifuge for malaria as these supposedly possess similar properties to the cinchona bark (9).

However, the claimed activities of this plant have not been scientifically investigated so far. Therefore, this study was undertaken to validate the claim of

antimalarial activity of *B. acutangula* aqueous root extract.

MATERIALS AND METHODS

Collection of plant material

Roots of *B. acutangula* were collected from 5 trees located at Bulathkopitiya in the Kegalle district, Sri Lanka in January, 2006. The specimen was identified and authenticated by Dr H Kathriarachchi of the Department of Plant Sciences of the University of Colombo, Sri Lanka. A voucher specimen (wdr/cdj 1003) was deposited in the museum of the Department of Zoology of the University of Colombo, Sri Lanka.

Preparation of the extract

Roots of *B. acutangula* were cleaned and air dried under the shade for 14 days and powdered by using a mechanical grinder (Sumeet Master 864, Sumeet Machines Ltd, Nasik, India). Sixty grams of root powder were mixed with 1920 mL of distilled water and boiled for 3 hours in a round bottom flask until the final volume reached 240 mL. The resultant brown coloured solution was freeze-dried (yield 1.6%) and the final product was stored at 4^0 C. Freeze-dried powder was dissolved in DW to obtain the required dosages in 1 mL of solution. The animals were orally treated with the ARE so that they received 400, 800 and 1200 mg/kg of body weight. Taking in to consideration the metabolic rate of mice, the lower dose is approximately equal to the usually prescribed dose by the traditional practitioners (11) which is within the accepted range for the murine model (12).

Experimental animals

Healthy, adult female C57BL/6JNcl mice weighing 20-25 g, purchased from the Medical Research Institute, Colombo, Sri Lanka were used in this study. All animals were housed in plastic cages in the animal house, Department of Zoology, University of Colombo under standard conditions (temperature 28-31^0C; photoperiod, approximately 12 hours natural light per day, relative humidity 50- 55%). The animals were fed with pelleted food (Master Feed Ltd. Colombo, Sri Lanka) and clear drinking water *ad libitum*. Except at the time of experimental procedure the animals were handled only during cage cleaning. All the experiments were conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines and rules of the Faculty of Science, University of Colombo, Sri Lanka, for animal experimentations.

Phytochemical screening

Phytochemical screening of the ARE was carried out according to Farnsworth (13). The ARE was screened for alkaloids, flavanoids, triterpenes, polyphenolic compounds, saponins, sterols and leucocanthocynins.

In vivo antimalarial drug susceptibility testing

Parasite isolates

Chloroquine sensitive, 17XL (lethal strain) *Plasmodium yoelii* was used to assess the *in vivo* antimalarial activity of ARE. The parasite strain was maintained by serial passage of blood from mouse to mouse.

Parasitic inoculation

The inoculum consisted of 10^7 *P. yoelii* parasitized red blood cells (RBCs)/ml of blood. This was prepared by determining both the percentage parasitaemia and the erythrocyte counts of the donor mouse and diluting the blood with isotonic saline (0.2 mL).

Evaluation of blood schizonticidal activity on an early infection

The 4 day suppressive assay was used to evaluate the blood schizonticidal activity of the ARE (14). The antimalarial activity of the ARE was measured using 3 oral doses (n = 6) of extract 400, 800 and 1200 mg/kg. Chloroquine phosphate (State Pharmaceutical Corporation, Colombo, Sri Lanka) at 10 mg/kg per day was used as the positive control, while DW was used as the negative control. C57BL/6JNcl mice (n = 6) were inoculated intraperitoneally, with 10^7 (in 0.2 ml saline) infected RBC of *P. yoelii* on day zero. The ARE, chloroquine and the vehicle were administered from day zero to day 3. On day 4, blood was obtained from the tail end of each mouse under aseptic conditions and blood smears were made on glass slides. These slides were stained with Giemsa Stain (Fluka Chemie AG CH-9470, Buchs, Switzerland) and the number of parasitized RBC were counted out of at least 3000 erythrocytes in random fields under oil immersion. The degree of parasitaemia (%) was then calculated. The average percentage chemosuppression was also calculated as (A-B) / A x 100, where A was the average percentage of parasitaemia in the control group, and B the average parasitaemia in the ARE treated group (15).

The parasitaemia levels in treated groups were statistically compared with the controls. Further, this assay was extended to days 5 and 6 in order to observe the effects of the ARE on the established infection (16).

Mice used for the 4-day suppressive assay were further observed daily from day 0, for 8 consecutive days and the mean survival time (days) for each group determined.

Evaluation of blood schizonticidal activity in established infection (curative assay)- The mice

were injected intraperitoneally with a 10^7 *P. yoelii* infected RBCs on day 0. Seventy-two h following the injection, mice (n = 6) were orally administered with 800 mg/kg of ARE, DW as the vehicle and chloroquine as the control, daily for 5 consecutive days. Blood smears were prepared daily for 5 days and the parasitaemia was assessed. Further, the mean survival time for each group was determined (17).

Evaluation of sub chronic toxicity

Male mice (n = 6) were treated either, with 1200 mg/kg of ARE or DW daily for 30 consecutive days. Following treatment they were continuously observed for 1 hour on a daily basis for overt signs of toxicity (salivation, diarrhoea, postural abnormalities, and behavioral changes), stress (fur erection, exophthalmia) and aversive behavior (biting and scratching behavior, licking of tail, paw and penis, intense grooming behavior or vocalization) (18). Food and water intake and body weights were evaluated at weekly intervals.

On day 1 post treatment, 0.3 -0.5 mL of a tail bleed from each mouse was collected under mild ether anesthesia using aseptic precautions. The red blood cell (RBC), white blood cell (WBC) and differential (DC) counts of fresh blood was determined using standard techniques (19). Serum parameters (SGOT, SGPT, urea creatinine levels) were determined using Randox kits (Randox Laboratories Ltd., Co. Antrium, U.K) and the spectrophotometer (Jasco V560, Jasco Corporation, Tokyo, Japan) as per manufacturer's instructions. Serum Na^+ and K^+ concentrations were determined using the atomic absorption spectrophotometer (GBC 932, Plus, Victoria, Australia).

Mice were weighed on day 1 post treatment using an animal balance (MP 6000, Chyo Balance Corporation, Tokyo, Japan). Then they were scarified and their liver, spleen, lungs, kidneys, heart and male reproductive organs were immediately excised, the gross morphology examined, blotted free of blood and weights were determined using an electronic balance (SHIMADZU LIBROREB series, SHIMADZU Corporation, Tokyo, Japan). Subsequently, ratios of organs to body weight were calculated.

Statistical analysis

Data obtained from this study were analysed using Mann Whitney U test, Student's test, One way ANOVA and Tukey's HSD. The significant value was set at $p \leq 0.05$.

RESULTS - ***Results of phytochemical screening-*** Phytochemical screening of the ARE revealed the presence of triterpenes, saponins and polyphenolic compounds, but alkaloids, flavanoids and tannins were absent.

Evaluation of blood schizonticidal activity on an early infection

The results obtained from the 4 day suppressive assay are summarized in Table 1. Oral administration of ARE at 800 mg/kg dosage significantly ($p< 0.05$) suppressed the parasitaemia in *P. yoelii* by 60.88 % compared with the control (Figure 1). The suppression in parasitaemia compared with the control induced by the 400 and 1200 mg/kg doses was not significant ($p> 0.05$). The suppressive effect of the ARE was curvilinearly dose dependent ($r^2 = 0.96$, $p< 0.05$). Chloroquine as the positive control significantly ($P < 0.05$) suppressed the parasitaemia by 97.72% (Figure 1). The 800mg/kg dose of the ARE, therefore was 40% less potent than chloroquine.

The 4 day suppressive assay was continued and the parasitaemias of these mice were monitored on days 5 and 6 (Table 1). In this model, the level of parasitaemia was significantly ($p< 0.05$) lower compared with the control in mice treated with 800 mg/kg of the ARE on days 4 and 6 of the infection. However, there was significant ($p < 0.05$) daily reduction of parasitaemia in mice treated with chloroquine in *P. yoelii* model.

The mean survival time of mice infected with *P. yoelii* are summarized in Table 2. There was a significant survival ($p < 0.05$) of mice treated with the 800 mg/kg dosage of ARE and chloroquine compared with the vehicle in *P. yoelii* infected mice.

Evaluation of blood schizonticidal activity in established infection (curative assay)

There was a daily increase in parasitaemia in the ARE (800 mg/kg) treated group as well as in the control group subjected to the curative assay (Table 3). However, there was a daily decrease in parasitaemia level in the chloroquine treated group. Although, the mean survival time of the treated group was not significantly ($p > 0.05$) different from the control group, the chloroquine treated group showed significant survival ($p< 0.05$) compared with the control group (Table 4).

Toxicological studies

Treatment with *B. acutangula* ARE (1200 mg/kg) did not elicit any overt signs of toxicity, stress and aversive behavior. However, yellowing of fur was observed in two treated mice. Both serum and haematological parameters showed no significant

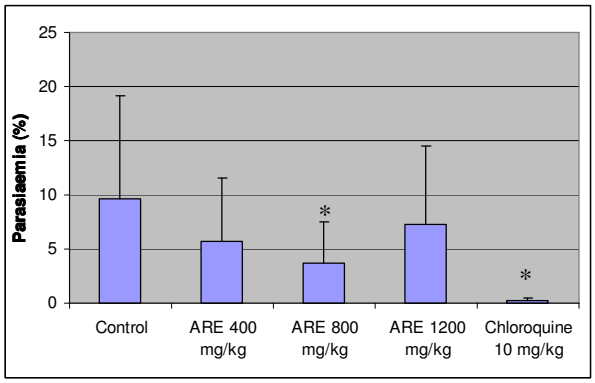

Figure 1. In vivo blood schizonticidal activity of Barringtonia actunagula aqueous root extract in the 4-day suppressive assay (means ± SEM)*p<0.05 as compared with the control (Mann Whitney U test)

Table 1 : In- vivo blood schizonticidal activity of Barringtonia acutangula aqueous root extract (ARE) in the 4-day suppressive assay

Treatment	**Parasitaemia (%)**		
	Day 1	Day 2	Day 3
Control (Distilled water)	9.60 ± 2.25	16.94 ± 4.94	26.43± 5.03
ARE			
400 mg/kg	5.79± 0.52	9.76 ± 1.71	19.73 ± 2.65
800 mg/kg	3.75 ± 0.91*	8.90 ± 1.97	9.15± 7.50*
1200 mg/kg	7.26± 5.43	15.80 ± 9.09	17.72 ± 7.50
chloroquine (10 mg/kg)	0.218 ± 0.16**	0.00014 ± 0.00039**	0**

*Values are expressed as means ± SEM (n = 6) ; * p < 0.05 as compared with the control (Mann Whitney U test) ;** P < 0.01 as compared with the control (Mann Whitney U test)*
ARE- Aqueous root extract

Table 2 : Mean survival time of mice treated with the Barringtonia acutangula aqueous root extract (ARE) of the 4-day assay

Drugs/extracts	**Mean survival time (days)**
Control (distilled water)	6.5 ± 0.55
ARE	
400 mg/kg	7.3 ± 0.51
800 mg/kg	8.0 ± 0.00*
1200 mg/kg	6.8 ± 0.75
chloroquine (10 mg/kg)	8.0 ± 0.00*

*Values are expressed as means ± SD (n = 6); *p< 0.05 when compared with the control (1 way ANOVA and Tukey's HSD)* ARE- Aqueous root extract

Table 3 : In vivo blood schizonticidal activity of Barringtonia acutangula aqueous root extract (ARE) in the curative assay

	Parasiatemia (%)			
Treatment	**Day 3**	**Day 4**	**Day 5**	**Day 6**
Control (Distilled water)	42.3	53.4	78.2	97
ARE (800 mg/kg)	35.8	50.4	68.9	80.5
chloroquine (10 mg/kg)	38.9	25.4	14.9	10.7

Values are expressed as means ± SEM (n = 6); Values are not significant, p > 0.05 ; ARE- Aqueous root extract

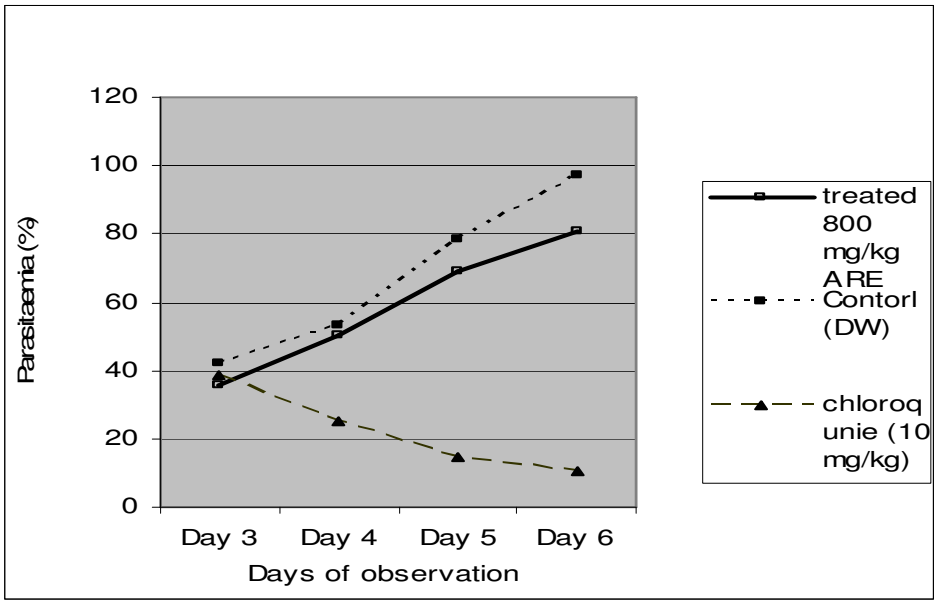

Figure 2. ***Effect of oral administration of Barringtonia acutagula aqueous root extract (ARE) on established infection (curative assay). (mean ± SEM)***

Table 4 : The mean survival time of mice treated with Barringtonia acutangula aqueous root extract (ARE) in the curative assay

Drugs/extracts	Mean survival time (days)
Control (distilled water)	4.00 ± 0.89
ARE (800 mg/kg)	4.833± 1.32
chloroquine (10 mg/kg)	12.33 ± 1.50*

*Data are expressed as mean ± SD (n = 6); *P < 0.05 when compared with the control (Student'test);* ARE- Aqueous root extract

Table 5 : Effect of oral administration of Barringtonia acutangula aqueous root extract on toxicological parameter

Parameters	Control (Distilled water)	ARE (1200 mg/kg)
Serum parameters		
SGOT (U/L)	30.967 ± 0.472	30.950 ± 0.749
SGPT (U/L)	14.217 ± 0.283	13.833 ± 0.209
Urea (mg/dl)	48.31 ± 4.61	44.54 ± 3.57
Creatinine (mg/dl)	2.03 ± 0.253	1.00 ± 0.194
Serum Na+ (mol/L)	0.00743 ± 0.00029	0.01 ± 0.0061
Serum K+ (mol/L)	0.30937 ± 0.00305	0.3360± 0.0112
Blood parameters		
WBC count cells/ mm^3	1546 ± 25.9	1656.7 ± 85.6
RBC counts cells/ mm^3	$6.46x\ 10^6 \pm 5.71\ x10^5$	$6.27x10^6 \pm 4.46x10^5$

Values are expressed as means ± SEM (n = 6); Values are not significant, p>0.05 vs. control

ARE- Aqueous root extract *;* SGOT- serum glutamic-oxaloacetic transaminase WBC- white blood cell count ; SGPT- serum glutamic- pyruvate transaminase; RBC- red blood cell count

Table 6 : Effect of oral administration of Barringtonia acutangula aqueous root extract on differential count of white blood cells

Parameters	Control (distilled water)	ARE (1200 mg/kg)
Neutrophils (%)	26.67 ± 2.33	36.67 ± 8.86
Eosinophils(%)	3.38 ± 0.422	3.33 ±0.422
Monoctyes(%)	4.33 ± 0.715	3.83 ± 0.543
Lymphocytes(%)	65.67 ± 2.29	56.67 ± 8.70

Values are expressed as means ± SEM (n = 6); Values are not significant, p > 0.05 vs. control

ARE- Aqueous root extract

Table 7 : Effect of oral administration Barringtonia acutangula aqueous root extract (ARE) on body weights

Treatment	1st week	2nd week	3rd week	4th week
Control (DW)	32.40 ± 1.32	35.29 ± 1.14	41.05 ± 0.489	41.45 ± 0.630
ARE (1200 mg/kg)	35.38 ± 1.10	36.33 ± 3.21	39.75 ± 4.55	40.10 ± 5.28

Values are expressed as means ± S.E.M (n = 6) ; Values are not significant p>0.05 vs. control ;
DW – Distilled water ; ARE- Aqueous root extract

Table 8 : Effect of oral administration Barringtonia acutangula aqueous root extract (ARE) on food intake

Treatment	1st week	2nd week	3rd week	4th week
Control (DW)	0.065 ± 0.272	0.052 ± 0.229	0.044 ±0.229	0.046 ± 0.173
ARE (1200 mg/kg)	0.051 ± 0.007	0.036 ± 0.005*	0.032 ±0.005*	0.047± 0.002

*Values are expressed as means ± SEM (n = 6) ; *p< 0.05 as compared with the control (Mann-Whitney U test)*
DW – Distilled water ; ARE- Aqueous root extract

Table 9 : Effect of oral administration Barringtonia acutangula aqueous root extract (ARE) on water intake

Treatment	1st week	2nd week	3rd week	4th week
Control (DW)	0.053 ±0.006	0.058 ± 0.005	0.03 ± 0.004	0.028 ± 0.004
ARE (1200 mg/kg)	0.03 ± 0.003	0.023± 0.002*	0.018 ± 0.003*	0.023 ±0.006

*Values are expressed as means ± SEM (n = 6) ; *p < 0.05 as compared with the control (Mann-Whitney U test)*
DW – Distilled water ; ARE- Aqueous root extract

(p<0.05) difference between the treated and control mice (Table 5 and Table 6). Treatment did not significantly alter the body weights (Table 7) but there was a significant (p< 0.05) reduction in food intake (Table 8) and water intake (Table 9) during the 2nd and 3rd week of the experiment.

DISCUSSION

In this study the blood schizonticidal activity of the ARE on early infection and established infection was investigated using 4-day suppressive and the curative assay, respectively, in the *P. yoelii* murine model.

Both rodent and simian *in vivo* malaria models are widely used for the detection of antimalarial activities of various therapeutic agents including natural products (20). Among these, *P. yoelii* rodent models have proved to be a convenient and reliable method for the detection of antimalarial activity (21). Antiparasitic activity of *B. acutangula* ARE against *P. yoelii* model, may indicate that, the ARE is possibly effective against *P. falciparum* human parasites (21).

The oral administration of ARE exhibited a curvilinear dose dependent blood schizonticidal activity in the *P.yoelii* model. The highest antiplasmodial activity of ARE was elicited at 800 mg/kg dosage, in the 4 day suppressive assay murine model. However, it was 40 % less potent than chloroquine (10 mg/kg) in this model. The curative potential of the ARE investigated using the mean survival time in mice showed similar properties between mice treated with the ARE and chloroquine.

Mode of action of many antimalarial agents correlates with the growth inhibition and/or invasion inhibition of the trophozoites and schizont stages (22). These parasite stages are in an active metabolic state, hydrolyzing haemoglobin for essential amino acids. It has been postulated that most antimalarial therapies work by binding free haem, thereby preventing toxic membrane lytic effects (23). Some drugs (pyrimethamine and sulfadoxine) inhibit dihydrofolate reductase of plasmodia and thereby block the biosynthesis of purine and pyrimidines, which are so essential for DNA synthesis and cell multiplication. This leads to failure of nuclear division at the time of schizont formation (24). However, the ARE has to be tested in *in vitro* studies to specify the mode of action and the most effective stage of malaria life cycle (22).

The ARE of *B. acutangula* was found to contain triterpens, saponins and polyphenolic compounds. These compounds could have elicited the observed antiplasmodial activity either singly or in synergy. The triterpenes of various plant species have been extensively studied for antiplasmodial activity (25). One of the compounds that has been explored further was the quassinoids which are degraded triterpenes founds in various plant species (25). Preliminary phytochemical analysis performed in this study showed the presences of triterpenoids in the ARE, which may

have played a vital/ key role in inducing antiparasitic activity of the ARE.

Toxicity has been addressed as the main draw back of indigenous preparations. Toxicity studies of the ARE were therefore carried out to evaluate its safety for oral administration. The ARE was well tolerated showing no overt signs of toxicity, stress, aversive behavior or behavioral changes. Further, hepatotoxicity, renal toxicity, haematotoxicity could be ruled out. Also, the ARE failed to alter the weights and the gross morphology of vital organs of test mice. Nevertheless, slightly significant reduction was observed in food intake and water intake during the 2nd and 3rd week of the experiment. Though the ARE was found to be less potent than chloroquine with regard to antimalarial activity, it has a distinct advantage of being relatively non-toxic when compared with widely used antimalarials.

The results accrued from this study encourage pursuing further investigations to identify the active compound responsible for the observed antimalarial effects of the root extract of B. *acutangula*. Development of herbal antimalarials is critical considering the emergence and spread of drug resistance. The present study must be continued to investigate its effects against drug resistant rodent parasites or drug resistant *P. falciparum* to evaluate its effectiveness against drug resistant malaria.

In conclusion, this study demonstrated that the ARE of *B. acutangula* is orally active, non toxic and possesses antimalarial corroborating the claim of indigenous medicine in Sri Lanka.

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REFERNCES

1. World Health Organization, World Health Report 2000, (WHO Press, Geneva. 2000).
2. PI Trigg, AV Kondrrachine. The current global malaria situation. In: Sherman IW eds. *Malaria: Parasite Biology, Pathogenesis, and Protection*. Washington, DC: ASM Press; 11-22 (1999).
3. R.S. Phillips. Current status of malaria and potential for control. *Clin. Microbiol Rev.* 208–226 (2001).
4. E. Omulokoli, B. Khan and S.C Chhabra. Antiplasmoidal activity of four Kenyan medicinal plants. *J Ethnopharmacol*. **56**:133-137 (1997).
5. S. Sanon, N. Azas, M. Gasquet, E. Ollivier, V. Mahiou, N. Barro, N. Cuzin- Ouattara, A.S. Traore, E. Esposito, G. Balansard and P. Timon-David. Antiplasmodial activity of alkaloid extracts from P*avetta crassipes* (K.Schum) and *Acanthospermum hispidum* (DC), two plants used in traditional medicine in Burkina Fasco. *Parasitol Res*. **90**: 314-317 (2003).
6. G.C. Kirby. Medical plants and the control of protozoal disease, with particular reference to malaria. *Trans. Royal Soc. Trop. Med. Hygine* **90**: 605-609 (1996).
7. R. Mahindapala. Sri Lanka's medicinal plants. *Loris*. **22**: 32-36 (2000)
8. N.W. Veralupitiya. *Osuthuru Visituru III,* (Publication of the Department of Aurveda, Colombo, Sri Lanka, 1994) pp. 35.
9. D.M.A. Jayaweera. *Medical Plants used in Ceylon, IV*. (National Science Council of Sri Lanka, Colombo, Sri Lanka, 1981) pp. 125.
10. A. Uawonggul, S. Chaveerach, T. Thammasirirak, C. Arkararichien, S. Chuachan and Daduang. Screening of plants acting against *Heterometrus laoticus* scorpion venom activity on fibroblast cell lysis. *J Ethnopharmacol.* **103**: 201-207 (2006).
11. D.M. Jayasinghe, *Ayurveda Pharmacoepia*, (Department Ayurveda, Colombo, Sri Lanka, 1979) pp. 30-35.
12. B.N. Dhawan and R.C. Srimal, *Laboratory Manual for Pharmacology Evaluation of Natural Products*, (International Centre for Science and High Technology, Trieste, Italy, 2000) pp. 7-55.
13. N.R. Farnsworth, *Phytochemical Screening*, (Chicago College of Pharmacy, University of Illinois, Chicago, 1996) pp. 32-65.
14. W. Peters, J.H. Protus and B.L. Robinson. The chemotherapy of rodent malaria, XXII: The value of drug resistant strains of *P. berghei* in screening for blood schizonticidal activity. Annals of Tropical Med Parasitol. **69**: 155-171 (1975).
15. J. E. Okokon, B. Ita and A.E Udokpoh. Antiplasmodial activity of *Homalium letestui. Phytotherapy Res* **20**: 949-951 (2006).
16. E.O. Ajaiyeoba, U.I. Abalogu, H.C. Krebs and A.M.J. Oduola. *In vivo* antimalarial activities of *Quassia amara* and *Quassia undulate* plant extracts in mice. *J Ethnopharmacol*. **67**: 321-325 (1999).
17. J.F Ryley and W. Peters. The antimalarial activity of some quinine esters. *Ann. Trop. Med. Parasitol*. **84**: 209-222 (1970).
18. S.A. Deraniyagala, W.D. Ratnasooriya and C.L Goonasekara. Antinociceptive effect and toxicological study of the aqueous bark extract of B*arringtoina racemosa* on rats. *J Ethnopharmacol*. **86**: 21-26 (2003).
19. C.L. Ghai. *A Textbook of Practical Physiology*. (Jaypee Brothers Medical Publishers Ltd., New Delhi, 1993) pp.119-202.
20. World Health Organization, Chemotherapy of malaria and resistance to antimalarials, (World Health Organization Technical Report Series, 1973) pp.529.
21. W. Peters. Rational methods in the search for antimalarial drugs. *Trans. Royal Soc. Trop. Med. Hygine*. **61**: 400-410 (1967).

22. M. Mazier, M. Franetich, M. Carraz, O. Silvie, P. Pino. Models for studying the effects of herbal antimalarials at different stages of the *Plasmodium* life cycle. In: Willox M, Bodeker G, Rasoanaivo P, eds. *Traditional Medicinal Plants and Malaria*. Boca Raton: CRC Press; 271-278 (2004).
23. M. Foley and L. Tilley. Quinoline antimalarials: mechanisms of action and resistance and prospects for new agents. *Pharmacol. Therap*. **79**: 55-87 (1998).
24. H.P. Rang. M.M. Dale and J.M. Ritter. *Pharmacology*. (Churchill, Livingston, London, 1995) pp.673-682.
25. C.W. Wright. Traditional antimalarials and the development of novel antimalarial drugs. *J Ethnopharmacol*. **100**: 67-71 (2005).