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In vivo antiplasmodial activity of extracts of selected Ghanaian medicinal plants

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Abstract

Background: Malaria is endemic and perennial in all parts of Ghana. Ghana recorded 4,940,270 suspected malaria cases in the first half of the year 2016. This translates into 26,922 as an average daily recorded case of malaria in the country's health facilities during the period.

Methods: This study investigated the *antiplasmodial* activities of aqueous extracts of the stem barks of *Afraegle paniculata*, *Monanthotaxis trichocarpa, Sterculia setigera* and *Strychnos innocua* as well as both aqueous and organic extracts of *Ananas comosus* using pathogen free imprinted control region mice of either sex. The extracts were investigated to establish suppressive and curative antimalarial properties. The curative property was determined by employing two different doses (250mg/kg and 500mg/kg body weight) while suppressive test was done using the later dose in mice infected with *Plasmodium berghei*.

Results: Extracts exhibited significant (P<0.05) reductions in parasitaemia in suppressive test, *Ananas comosus* demonstrated highest activity with percentage suppression of 66.9±1.13 % for a 4-day suppressive test. For curative test, ethyl acetate fraction of *Ananas comosus* and *Afraegle paniculata* were deemed active by showing significant (P<0.05) reductions in parasitaemia which were greater than 40%.

Conclusions: Regarding this study, the parts of the plants used are being reported for the first time of exhibiting antiplasmodial activity against *Plasmodium berghei.* It is therefore possible that, future isolation of lead compounds from *Ananas comosus* and *Afraegle paniculata* may lead to the development of new antimalarial agents.

Keywords: *Afraegle paniculata; Strychnos innocua; Ananas comosus; Sterculia setigera.*

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Background

Malaria is a serious parasitic disease caused by protozoa belonging to the genus *Plasmodium.* Malaria is caused by five species of parasites that affect humans, these parasites are *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*. Of these, *P. falciparum* and *P. vivax* are highly dangerous. Malaria due to *P. falciparum* is the deadliest form, and it is widespread in Africa [1]. Malaria is endemic and perennial in all parts of Ghana, with seasonal variations that are more pronounced in the northern part of Ghana [2].According to the 2010 national census, the entire 24.2 million Ghanaians are at risk of malaria infection. Children under five years and pregnant women however stand a higher risk of severe illness due to declined immunity [2]. Moreover, according to National Malaria Control Programme, Ghana (NMCP), the country recorded 4,940,270 suspected malaria cases in the first half of the year 2016. This translates into 26,922 as an average daily recorded case of malaria in the country's health facilities during the period [3]. This figure means that malaria cases increased by 6.9 % as compared with the previous year. However, malaria under five years case fatality rate dropped from 0.44 % in the first half of 2015 to 0.35 % at the end of June in 2016; but this is not a significant reduction [3]. Although this number has reduced as a result of many malaria control interventions put in place by Ghana Health Service.*Plasmodium falciparum* stillaccounts for 85-90% of all infections [2]. Hence, we need to do more in the area of chemotherapy by getting a new antimalarial agent with different mechanism of action, since it remains a key factor in the fight against malaria [4]. Also, the world-wide escalating problem of resistance of *Plasmodium* to existing antimalarial drugs has emphasized the need for new drugs with alternative mechanisms of action. Moreover, *P. falciparum* resistance to artemisinin has been detected in five (5) countries of the Greater Mekong Sub-region: Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Viet Nam [5].

Fortunately, medicinal plants have invariably been a rich source for new drugs [6].In Ghana, several plant species are used locally to treat malaria and they include *Alstonei boonei*, *Azadirachta indica*, *Cryptolepis sanguinolenta*, *Morinda lucida*, *Nauclea latifolia*, *Ocimum viride, Monanthotaxis sp.* and *Parinari polyandra,* are used in the treatment of malaria [7,8]. There is clearly a need to look into traditional medicines, regarding treatment of malaria in Ghana, to scientifically validate the uses of plants and evaluate their toxicity. Also, little is known about phytochemistry and scientific reports on the *in vivo* antimalarial activities of the plants of current study. Hence, the aim of this study is to investigate antimalarial potential of some Ghanaian medicinal plants by conducting in vivo efficacy, phytochemistry, andacute toxicity studies on the plants.

Methods

Sample collection and identification

Fresh, unripe but mature pineapples (*Ananas comosus*) were collected from Ejisu in Ashanti Region, in June, 2015; with voucher number KNUST/HM/2016/007.Moreover,*Monanthotaxis trichocarpa* (stem bark) with voucher number KNUST/HM/2015/5005,*Afraegle paniculata* (stem bark), voucher number KNUST/HM/2015/5006,*Strychnos innocua* (stem bark), voucher number KNUST/HM/2015/5007 and*Sterculia setigera* (stem bark),voucher number KNUST/HM/2015/5008 were also collected at Asakraka-Kwahu in the Eastern Region of Ghana, in April, 2015. All the plant samples wereidentified and authenticated in the Department of Herbal Medicine, KNUST, before use and specimen placed in the Department Herbarium.

Preparation of plant samples and extraction

Preparation of the plant samples mimicked that of the traditional modes of preparation of plant materials for malaria treatment. Aqueous extracts were obtained from milled stem bark of the plant samples by separately boiling each of the powered samples in distilled water for 15 minutes and filtered. The filtrates were concentrated by freeze-drying to obtain dried extracts. Also, chopped pineapples (both pericarp and mesocarp) was blended and filtered. The filtrate was concentrated by freeze-drying to obtain a dried extract. In addition, 30 g of lyophilized extract from the pineapple was dissolved in a separatory funnel containing 500 ml of distilled water and then washed exhaustively with petroleum ether (3×400 mL), chloroform and ethyl acetate each with (3×400 mL) sequentially. All the extract residues were used in *in-vivo* studies.

Phytochemical screenings

Phytochemicals such as alkaloids, flavonoids, tannins, triterpenes, sterols, coumarins, saponins and cyanogenic glycosides were tested for by performing tests on the aqueous extracts using standard methods described [9-11].

Animals used for in vivo assay

The animals used in the study were pathogen free Imprinted Control Region (ICR) mice of either sex. The mice were obtained from the Animal House Department of Centre for Plant Medicine Research (CPMR), Mampong-Akuapem of Ghana. The mice were between 8-12 weeks old with their weights ranged between 25-33 g. The study was carried out under the surveillance of the Centre for Plant Medicine Research ethical committee, Mampong-Akuapem of Ghana; on September 20th 2015 with the certificate number CPMR-ET/M.02 2015.

Malaria parasite strain

The *Plasmodium berghei* (NK 65) strain was used in the in-vivo study. It was donated by the Department of Immunology of the Noguchi Memorial Institute for Medical Research, of the University of Ghana.

Inoculation of parasite in mice and determination of parasitemia

Plasmodium berghei parasitized erythrocytes was determined from adonor mouse using a standard method [12]. The total inoculum concentration of 5.0× 10^7 per mL was prepared by diluting the blood collected into a heparinized tube from the infected mouse with phosphate buffered saline after estimating the total number of erythrocytes/ml of the blood.Each mouse was then intraperitoneally inoculated on day 0with 0.2 mL of the diluted infected erythrocytes containing 1.0× 10^7 *P. berghei* parasitized red blood cells [12]. The percentage parasitaemia was determined by counting infected erythrocytes in five fields, divided by the total erythrocytes in the five fields then multiplied by hundred [13].

$$\text{Percentage parasitaemia} = \frac{\text{number of infected red blood cells /field}}{\text{total number of red blood cells /field}} \times 100\%$$

Treatment of mice using suppressive test

The test was used to assess the schizontocidal activity of the plant extracts against *Plasmodium berghei* infected mice using the test protocol [12]. The mice were divided into seven groups of five mice per group. The mice in negative and positive control groups were given 0.2 mL distilled water and arthemether- lumenfatrine 4 mg/kg body weight once daily respectively. For the remaining five groups, five different plant extracts of 500 mg/kg body weight were used to treat them once daily. The first day of inoculation was named D_0. Treatment with the test drugs and standard drug commenced 3hours after inoculation and continued daily for four days from day 0 to day 3. After the treatment, thin blood film was prepared from the tail of each experimental mouse on day four in order to determine the average percentage parasitaemia and chemosuppression of parasitaemia [12,14]. Also, the mean survival time for each group was evaluated by computing the average survival days of mice over a period of 30 days. Results were statistically analyzed using One-WayANOVA from GraphPad Prism v 5 at 95% confidence interval.

The curative test

The curative test evaluated the curative capability of the extracts on established infections and it was done according to the standard method described by Ryley and Peters [15]. On the first day (Do), mice were infected as in 4-day suppressive test. However, oral treatment with the test drugs of two different doses of 250mg/kg and 500mg/kg commenced 72 hours after infection and continued once daily for five days. Arthemether- lumenfatrine 4mg/kg was given to the positive control and negative control groups received 0.2 ml each of distilled water (for test using aqueous extracts only) and 5% dimethyl sulfoxide (DMSO) in normal saline (for test using organic extracts and fractions). Parasitaemia was monitored daily using microscopic examination of Giemsa-stained thin blood smears prepared from the tail of each mouse daily for five days. Parameters like the percentage parasitaemia and parasitaemia suppression were calculated. Results were statistically analyzed using Two-Way ANOVA followed with Tukey and Bonferroni post-tests from GraphPad Prism v 5 at 95% confidence interval.

$$\text{Percentage suppression} = 100 - \frac{\text{Mean parasitaemia of treated group}}{\text{Mean parasitaemia of control group}} \times 100$$

Acute toxicity test

Acute toxicity test was carried out according to the Organization for Economic Cooperation and Development (OECD) 423 guideline for testing chemicals [16]. Thirty-six mice used for the study were grouped into six groups of six mice each of either sex. The individual group members were given one of the five different crude extracts at a dose of 5000 mg/kg body weight once except the normal control group which received only 0.2 mL of distilled water. The extracts were administered orally and the animals were observed clinically for a period of 14

days. Parameters such as survival and general health indicators were used to assess acute toxicity [16, 17].

Results and discussion

Phytochemical constituents

Phytochemical screening of the various aqueous extracts of the plant samples revealed the presence of many secondary metabolites such as flavonoids, alkaloids, saponins, tannins, coumarins, triterpenes and cyanogenic glycosides as presented in Table 1. This confirms the study that some of the secondary metabolites produced by plants include alkaloids, flavonoids, tannins, coumarins, triterpenes, sterols, cyanogenic glycosides among others [11]. Several studies have confirmed different biological activities of these secondary metabolites [18-20]. Thus, an initial confirmation of these metabolites creates a good platform to link up their presence with the bioactive effects of the plants being investigated.

Suppressive test

The plants at a dose of 500 mg/kg, revealed that the extracts possessed *in-vivo* antiplasmodial activity. From One-Way ANOVA analysis (Figure 1) and Tukey's post hoc test analysis, extracts showed higher efficacy at 95 % confidence interval when compared to the negative control group. Regarding the four-day suppressive test, the results saw reduction in parasitaemia of the experimental mice where WP demonstrated highest activity among the extracts with percentage suppressions of 66.9±1.13 %. Moreover, % parasitaemia suppressions of the other extracts were 40.6±1.66 %, 49.5±1.20 %, 36.0±1.91 % and 44.8±1.42 % for MTB, APB, SSB and SIB respectively. The standard drug (A/L) recorded 100% parasitaemia suppression for four-day suppressive tests (Figure 1). In addition, drugs or extracts with percentage suppression ≥ 40% by day three and onwards are deemed active [14]. It therefore means that the extracts WP, APB, MTB and SIB were considered active against *Plasmodium berghei* strain used in the experiment. It could be explained that the extracts possessed high activity against asexual stages and early gametocytes of plasmodial infections. Surprisedly, the average day of survival of the mice that were administered with APB at 500 mg/kg body weight is less than that of MTB (Table 2), even though APB has high percentage suppression than MTB. The extract APB could be toxic at that dose. Also, from the phytochemical screening of the plant samples, it has been identified that the plant samples contain a range of phytochemical constituents such as flavonoids, alkaloids, saponins, tannins, glycosides coumarins and terpenoids. The above phytochemicals play key roles in amelioration of diseases [21].

It can be argued that, secondary metabolites are responsible for the observed antimalarial activities of the extracts although the active constituents are yet to be identified. Some of the extracts contain alkaloids, and alkaloids have been documented as one group of compounds responsible for antimalarial activity, and one of the oldest and important antimalarial drugs, quinine, belongs to this group of compounds [22]. Flavonoids have also been shown in *in vivo* and in vitro tests to exhibit antimalarial activity [23]. The presence of alkaloids and flavonoids in *Ananas comosus* (WP) extracts might have contributed to drastic antimalarial activity demonstrated by the fruit extracts. In addition, their presence in *Ananas comosus* and some of the other extracts justify the antimalarial activities exhibited by the plant extracts.

In-vivo assay of curative effects of plant extracts

In curative activities, the various percentage parasitaemia in mice reduced to different degrees from the first day of treatment to the last day. Day one after treatment, all the samples saw no significant (P> 0.05) reduction in the level of parasitaemia. However, from day two after treatment appreciable levels of decrease in the parasitaemia were achieved with the highest activity on the last day (Tables 3, 4, 5 and Figures 2,3 and 4). The % parasitaemia reductions by the sample WP at 500 mg/kg were 8.9±0.71 %, 24.7±0.96%, 43.4±1.24%, 52.2±2.02% and 62.1±1.73% from day 1 to day 5 after treatment, respectively. When the dose was changed to 250 mg/kg the percentage parasitaemia reductions recorded were 8.7±1.20 %, 21.7±1.05 %, 42.4±1.25 %, 49.1±1.02 %, 55.5±0.79 % from day 1 to day 5 after treatment, respectively. Moreover, % parasitaemia suppressions exhibited by APB at 500 mg/kg and 250 mg/kgfrom day 1 to day 5 after treatment were 7.1±0.61%, 19.3±0.99 %, 33.8±1.25 %, 42.9±1.97 % ,46.5±1.50 % and 6.8±0.89 %, 17.4±1.14 %, 40.4±0.97 %, 43.4±1.51 %, 51.4±0.89 % respectively. Also, % parasitaemia reductions by MTB at 500 mg/kg were recorded as 4.3±0.88%, 5.3±1.10 %, 4.6±1.88 %, 14.1±2.19 % and 21.3±2.92 % from day1 to day 5 after treatment, respectively. But that of 250 mg/kg were 1.2±1.09 %, 2.2±0.74 %, 4.3±1.32 %, 5.4±1.67 %, 4.40±0.68 %. Similarly, percentage suppressions showed by SSB at the two different doses (500 mg and 250 mg) as shown in Figures 2 and 3, were 3.9±0.42 %, 4.5±1.21%, 9.1±3.62 %, 25.2±1.19 %, 27.2±0.94 %; and 3.1±0.91%, 5.5±0.68 %, 6.0±2.89 %, 8.0±1.83 %, 7.5±1.00 % respectively. The sample SIB at 500 mg/kg and 250 mg/kg gave the following percentage parasitaemia: 6.8±1.15 %, 12.2±0.50 %, 25.9±1.80 %, 32.8±1.63 %, 40.4±1.29 %; and 4.7±0.66 %, 10.9±0.97 %, 20.7±1.66 %, 31.8±1.40 %, 37.4±1.61 % respectively.

Also, regarding the fractions from *Ananas comosus,* only ethyl acetate fraction (WPEA) was deemed active with percentage suppressions recorded as 10.7±1.67 %, 27.3±1.50 %, 42.4±1.13 %, 49.5±1.04 % and 61.2±0.74 % for day one to day five post-treatment respectively (Figure 4 and Table 5). Although the aqueous fraction also exhibited the following percentage reductions 4.1±0.67%, 7.5±0.77 %, 14.6±0.89 %, 23.0±1.30 % and 26.5±2.07 %; the fraction was deemed inactive in that according to Jimenez-Dıaz et al. [14], drugs or extracts with percentage suppression ≥ 40% by day three and onwards are deemed active.

Arguably, one can say that, the presence or absence of some of secondary metabolites in their concentrations or proportions in the various extracts could also affect the level of inhibition of the growth of malaria parasite. Also, synergic property, potentiation and antagonistic effects might play significant roles in the antimalarial activity of the plants as well as the various fractions, hence the activity levels were not identical [24-26]. However, the standard antimalarial drug A/L recorded the highest percentage of parasitaemia reductions from day one to day five after treatment. A close look at the results in Figures 2, 3 and 4 has revealed that, the effects of the extracts at the stated doses produced significant differences between them and the negative control group from day 5 to day 7 at p< 0.05. This could be explained on the basis that the test drugs were effective against *Plasmodium berghei* and the parasites failed to replicate from day 5 to day 7 therapy period. However, experimental mice died after the withdrawal of test drugs due to parasite multiplication in the red blood cells. Notwithstanding that, the extracts of *Ananas comosus* and *Afraegle paniculata* were deemed active for curative test at a dose of 500 mg/kg body weight and only *Ananas comosus* was deemed active at a dose of 250 mg/kg body weight. This is because drugs or extracts with percentage suppression ≥ 40% by day three and onwards are deemed active [14]. For *Ananas comosus,* it can be argued that, there is not much difference between the results of the two doses indicating that the active compound in the extract is potent even at a lower dose. General appearance and behavioural observations for control and tested groups,showed that the mice which received *Afraegle paniculata* extract recorded severe side effects such as decreased locomotion, skin and erect fur position, salivation and lethargy. The severity of the side effects could be due to the toxicity of the extract.

Acute oral toxicity-LD$_{50}$

The preliminary acute oral toxicity study, to check behavioral toxicity signs and also mortality of experimental mice has shown that only *Afraegle paniculata* bark that has LD$_{50}$value less than 5000 mg/kg (Table 6), hence it is toxic at that dose. The other test drugs have their LD$_{50}$ values greater than 5000 mg/kg (Table 6), therefore they have high degree of safety [16]. According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, a crude extract of LD$_{50}$> 5000 mg/kg has its lowest toxicity and this justifies the claim that the plant parts used in the study had high degree of safety except *Afraegle paniculata* stem bark.

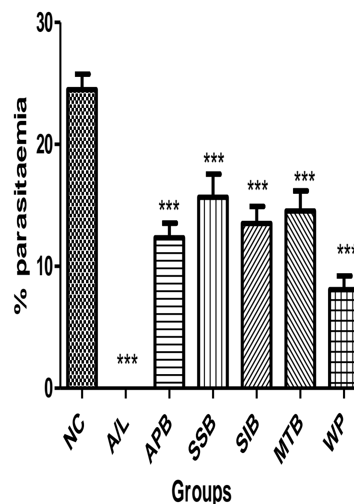

Figure 1. 4-daysuppressive test
4-Day Suppressive Test of WP, MTB, SSB, APB and SIB at 500mg/kg as well as 4mg/kg of A-L on the Plasmodium berghei infection in mice. The treatment started 3 hours after infection. Estimation of parasitaemia for the experimental groups was done on day 4.Each bar represents Mean ± SEM values calculated per group. Results were statistically analyzed with One-Way ANOVA followed by Tukey's *post hoc* test from GraphPad Prism v 5 at 95% confidence interval.$***P$< 0.001 denotes significance level when compared to Negative Control group.

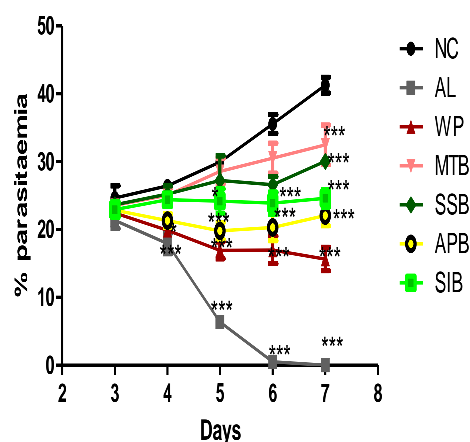

Figure 2. Curative effects at 500mg/kg

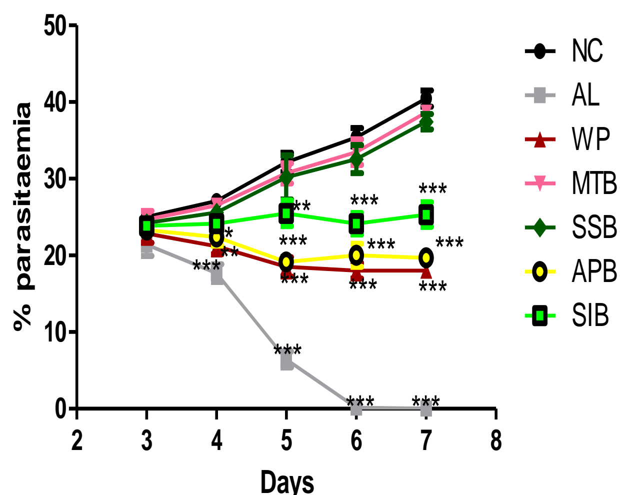

Figure 3. Curative effects at 250mg/kg

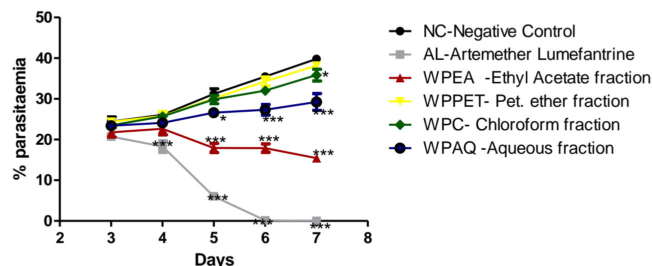

Figure 4. Curative effects of Extracts of WP fractions at 250 mg/kg

Curative effects of WP, MTB, SSB, APB and SIB at 500mg/kg and 250mg/kg; WPEA, WPPET, WPC and WPAQ at 250 mg/ kg as well as 4mg/kg of AL on the Plasmodium berghei infection in mice. The treatment started on day 3 post infection, up to day-7. Daily estimation of parasitaemia for the experimental groups was done.Each point represents Mean ± SEM. $*P< 0.05$, $**P< 0.01$, $***P< 0.001$ denote significance level when compared to NC group. Results obtained were analyzed using Two-Way ANOVA from GraphPad Prism v 5 followed by Bonferroni's post hoc test at 95% confidence level.

Table 1. Phytochemical constituents

Test	*Afraegle paniculata* (Aqueous extract)	*Monanthotaxis trichocarpa* (Aqueous extract)	*Sterculia setigera* (Aqueous extract)	*Strychnos innocua* (Aqueous extract)	Pineapples
Flavonoids	-	+	+	-	+
Alkaloids	-	-	+	-	-
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Coumarins	+	-	-	+	+
Triterpenes (salkowski's Test	-	-	+	-	+
Sterols (Libermann Burchard's Test)	-	-	+	-	+
Cyanogenic glycosides	-	+	+	+	-

key: present (+), absent (-)

Table 2. Four-day suppressive test (500mg/Kg)

Groups	NC	A/L	WP	MTB	APB	SSB	SIB
Survival days	8±0.200	Above 30±0.00	17±2.70	13±1.35	12±0.96	10±1.20	12±1.48

Values are expressed as mean ± S.E.M. (n = 5).

Table 3. Curative effects at 500mg/kg body weight

Day after treatment	NC	A/L	WP	MTB	SSB	APB	SIB
	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression
Day 1	0.0±1.83	13.2±1.21	8.9±0.71	4.3±0.88	3.9±0.42	7.1±0.61	6.8±1.15
Day 2	0.0±0.77	32.1±1.52	24.7±0.96	5.3±1.10	4.5±1.21	19.3±0.99	12.2±0.50
Day 3	0.0±0.37	78.7±0.96	43.4±1.24	8.6±1.88	9.1±3.62	33.8±1.25	25.9±1.80
Day 4	0.0±1.38	98.6±0.12	52.2±2.02	14.1±2.19	25.2±1.19	42.9±1.97	32.8±1.63
Day 5	0.0±1.17	100.0±0.00	62.1±1.73	21.3±2.92	27.2±0.94	46.5±1.50	40.4±1.29

Values are expressed as mean ± S.E.M. (n = 5).

Table 4. Curative effects at 250mg/kg body weight

Day after treatment	NC	A/L	WP	MTB	SSB	APB	SIB
	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression
Day 1	0.0±0.70	14.5±1.49	8.7±1.20	1.2±1.09	3.1±0.91	6.8±0.89	4.7±0.66
Day 2	0.0±0.60	34.8±1.17	21.7±1.05	2.2±0.74	5.5±0.68	17.4±1.14	10.9±0.97
Day 3	0.0±1.28	79.9±0.16	42.4±1.25	4.3±1.32	6.0±2.89	40.4±0.97	20.7±1.66
Day 4	0.0±1.19	99.6±0.08	49.1±1.02	5.4±1.67	8.0±1.83	43.4±1.51	31.8±1.40
Day 5	0.0±1.05	100±0.00	55.5±0.79	4.4±0.68	7.5±1.00	51.4±0.89	37.4±1.61

Values are expressed as mean ± S.E.M. (n = 5).

Table 5. Curative effects at 250mg/kg body weight

Day after treatment	NC	A/L	WPEA	WPA	WPPET	WPC
	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression	% Suppression
Day 1	0.0±1.15	15.2±0.75	10.7±1.67	4.1±0.67	0.1±0.34	3.8±0.52
Day 2	0.0±0.46	29.8±1.45	27.3±1.50	7.5±0.77	1.2±1.13	4.0±0.69
Day 3	0.0±1.31	80.6±0.81	42.4±1.13	14.6±0.89	3.5±0.89	4.3±1.01
Day 4	0.0±0.68	99.6±0.10	49.5±1.04	23.0±1.30	3.4±1.06	9.7±0.96
Day 5	0.0±0.69	100±0.00	61.2±0.74	26.5±2.07	4.1±0.33	10.0±1.43

Values are expressed as mean ± S.E.M. (n = 5).

Table 6. Acute oral toxicity-LD_{50}

Observation	WP			MTB			APB		
	d_1	d_2	d_3	d_1	d_2	d_3	d_1	d_2	d_3
Skin & fur position	-/+	-/+	-/+	-/+	-/+	-/+	++	+++	+++
Eye colour	-/+	-/+	-/+	-/+	-/+	-/+	-/+	++	+++
Decreased Movement	-	-	-	-	-	-	+	++	+++
Salivation	-	-	-	-	-	-	+	+++	+++
Drowsiness	-	-	-	-	-	-	++	++	++
Diarrhea	-	-	-	-	-	-	++	+++	+++
Coma	-	-	-	-	-	-	++	++	++
Lethargy	-	-	-	-	-	-	+++	+++	+++
Weight loss	-	-	+	-	-	-	++	+++	+++
Death	-	-	-	-	-	-	++	+++	+++

Acute Oral Toxicity-LD_{50} (Continuation)

Observation	SSB			SIB			NAC		
	d_1	d_2	d_3	d_1	d_2	d_3	d_1	d_2	d_3
Skin & fur position	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
Eye colour	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+	-/+
Decreased Movement	+	+	+	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-	-
Drowsiness	+	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-
Coma	-	-	-	-	-	-	-	-	-
Lethargy	+	+	+	+	+	+	-	-	-
Weight loss	+	++	++	+	+	+	-	-	-
Death	+	-	-	-	-	-	-	-	-

Physical appearance and behavioural observations of the experimental ICR mice were monitored to determine acute toxicity level, LD_{50}. N = 5. KEY: (-/+) Normal, (-) absent; (+) mild symptom; (++) moderate symptom and (+++) severe symptom. Also, mild symptom= symptom observed in only one mouse; moderate symptom= symptoms observed in two or three mice; severe symptom= symptom observed in four, five or six mice. Moreover, d_1= day1to day 3, d_2= day 4 to day 7 and d_3= day 8 to day 14. NAC=Normal control

Conclusions

Regarding this study, the parts of the plants used are scientifically reported for the first time of exhibiting antiplasmodial activity against *Plasmodium berghei.*The results of this study have shown that the stem barks of *Afraegle paniculata, Monanthotaxis trichocarpa, Sterculia setigera* and *Strychnos innocua*as well as *Ananas comosus* possess curative and suppressive antimalarial properties on P. *berghei* in ICR mice. The study also revealed that *Afraegle paniculata* and *Ananas comosus* were deemed active for both tests.It is therefore possible that, future isolation of lead compounds from WP and APB may lead to the development of new antimalarial agents. Also, the preliminary acute oral toxicity study indicated that the plant parts used in the study had high degree of safety except *Afraegle paniculata* stem bark which was toxic at higher doses. Phytochemical screening of the various aqueous extracts of the plant samples revealed the presence of many secondary metabolites such as flavonoids, alkaloids, saponins, tannins, coumarins, triterpenes and cyanogenic glycosides.

Abbreviations

APB- *Afraegle paniculata* stem bark
MTB- *Monanthotaxis trichocarpa* stembark
SSB- *Sterculia setigera*stembark
SIB- *Strychnos innocua* stem bark
WP-Whole pineapple or *Ananas comosus*
A/L-Artemether lumefantrine
OECD -Organization for Economic Cooperation and Development
DMSO- Dimethyl Sulfoxide.
ICR- Imprinted Control Region
C.P.M.R- Centre for Plant Medicine Research
NMCP- National Malaria Control Programme.

Authors' Contribution

RKA, IA and NT investigated phytochemical screening, extraction of plant materials and partitioning of crude extracts in various solvents; JA-L, AO, MFO, SA and NT carried out the *in-vivo antiplasmodial* activity using mice model. All authors discussed the results and contributed to the final manuscript. Also, all authors contributed to the interpretation of the results by providing critical feedback which helped shape the research, analysis and manuscript. RKA, IA, NT, JA-L, AO, MFO and SA contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

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