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In vitro and *in vivo* antiplasmodial activity of extracts from *Polyalthia suaveolens, Uvaria angolensis* and *Monodora tenuifolia* (Annonaceae)

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ABSTRACT

The present study aimed at investigating the *in vitro* and *in vivo* susceptibility of malaria parasites to crude extracts and fractions from *Polyalthia suaveolens*, *Uvaria angolensis*, and *Monodora tenuifolia*. The ethanolic extracts were partitioned using water, dichloromethane, hexane, and methanol. The most promising fraction was subjected to column chromatography. The antiplasmodial effect of extracts, fractions and sub-fractions against *P. falciparum* Chloroquine resistant (*Pf*K1) strain was determined using SYBR green florescence assay. The promising fraction was assessed for cytotoxicity against Human Foreskin Fibroblast (HFF) cells and further for safety in Swiss albino mice and suppressive effect against *P. berghei.* The methanol sub-fraction of *P. suaveolens* [PStw(Ace)] showed the highest potency with IC_{50} of 3.24 µg/mL. Sub-fraction PS8 from PStw(Ace) was the most active with IC_{50} of 4.42 µg/mL. Oral administration of PStw(Ace) at 5000 mg/kg b.w in mice showed no signs of toxicity. Also, it exerted the highest suppressive effect against *P. berghei* at 400 mg/kg b.w throughout the 4 days experiment. Overall, the results achieved supported the use of the three plants in the traditional treatment of malaria in Cameroon. More interestingly, the PStw(Ace) fraction might be of interest in future development of an antimalarial phytodrug.

Keywords: *Polyalthia suaveolens*, *Uvaria angolensis*, *Monodora tenuifolia*, *Plasmodium falciparum K1,* Toxicity, Antimalarial.

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INTRODUCTION

Malaria mainly caused by *Plasmodium falciparum* and *Plasmodium vivax* represents a serious public health challenge since about 3.3 billion people are at risk worldwide**.** Sub-Saharan Africa is the most affected region with 90% of the 584 000 deaths recorded in 2013**.** Cameroon with 71% of the total population living in high-transmission areas and 4500 deaths recorded annually is one of the most affected countries in Africa (WHO, 2014)**.** The currently prescribed treatments particularly the Artemisinin-based Combination Therapies (ACTs) are threatened by emerging resistance of *P. falciparum* strains (Dondorp et al., 2009). Given these limitations, there is a motivated search for new drugs against *Plasmodium* parasites.

Fortunately, there is evidence that plants traditionally used against malaria are valuable sources of potent antiplasmodial lead compounds (Tsabang et al., 2012; Bele et al., 2011; Ginsburg and Deharo, 2011). It seems logical then to encourage studies on new plants to discover innovative antimalarial leads. Selecting plants based on their indigenous use against fever and/or malaria has been found as better approach to identify plants species with potent antimalarial lead compounds.

An ethnobotanical investigation in four malaria endemic areas of Cameroon revealed people's reliance on *Monodora tenuifolia*, *Uvaria angolensis*, and *Polyathia suaveolens* to treat malaria and related symptoms (Tsabang et al., 2012). Thus, they were selected and studied for *in vitro* and *in vivo* antiplasmodial activities.

Moreover, these plants belong to the Annonaceae family that is well known for biosynthesis of bioactive compounds (Leboeuf et al., 1982), particularly the acetogenins that have a broad spectrum of biological activities including antiplasmodial effect (Rupprecht et al., 1990). Thus, this study was designed to investigate the *in vitro* and *in vivo* antiplasmodial activities of their extracts.

MATERIALS AND METHODS

Plant collection

Leaves, twigs and stem bark of each plant were collected at Mount Kalla (by Nkolbisson-Yaoundé, Mfoundi Division**)** in September 2014 and identified at the National Herbarium of Cameroon where voucher specimens were deposited under the identification numbers HNC 55313, HNC 500594 and HNC 1227 for *M. tenuifolia*, *U. angolensis* and *P. suaveolens* respectively.

Plants extraction and fractionation

Each plant part was cut into small pieces, dried at room temperature till constant weight and powdered. Five hundred g of each powder were macerated with 2000 mL of ethanol for 72 hours at room temperature. The ethanol extracts were evaporated to dryness under vacuum using a rotary evaporator (Rotavapor Buchi, Swizterland) and weighed. Each extract was further fractionated by liquid-liquid partition as previously described (Alali et al., 1999). Briefly, ethanolic residues were partitioned between dichloromethane and water (1:1) to yield water and dichloromethane fractions as well as interface precipitates. Then, dichloromethane fractions were further partitioned between hexane and methanol (1:1) to yield hexane and methanol fractions (methanol fractions referred to as acetogenin-rich fractions). All extracts and fractions were subjected to antiplasmodial activity screening *in vitro* against *Plasmodium falciparum* chloroquine resistant strain K1 (*PfK1*).

From this screening, the methanol fraction from the twigs of *P. suaveolens* [PStw(Ace)] was found to be the most active

against *P. falciparum*. It was therefore selected and submitted to qualitative phytochemical screening as previously described (Aromdee et al., 2005; Oloyede, 2005; Trease and Evans, 1996; Odebeyi and Sofowora, 1978; Harbone, 1976). Furthermore, it was fractionated using silica gel (70-230 mesh, Merck) column chromatography, eluting with solvent systems of increasing polarities, Hexane-Ethyl Acetate [100:0 – 0:100] and Ethyl Acetate - Methanol [95:5- 0:100]. Four hundred and thirty eight (438) sub-fractions of 100 mL each were collected and subsequently pooled on the basis of their thin layer chromatography (TLC) profiles into twelve (12) major sub-fractions PS1-PS12 [PS1-Hex 100%, PS2-Hex-EtOAc (98 :2), PS3-Hex-EtOAc (97 :3→95: 5), PS4-Hex-EtOAc (95 :5→93 :7), PS5-Hex-EtOAc (93 :7), PS6-Hex-EtOAc (93 :7→85 :15), PS7-Hex-EtOAc (85 :15→78 :22), PS8-Hex-EtOAc (78 :22→60 :40), PS9-Hex-EtOAc (60 :40→ 50 :50), PS10-Hex-EtOAc (50 :50→20 :80), PS11-Hex-EtOAc (20 :80→10 :90) / EtOAc-MeOH (98 :2), PS12-EtOAc-MeOH (95 : 5)/MeOH 100%]. All these sub-fractions were assessed *in vitro* for antiplasmodial activity and cytotoxicity as described below.

In vitro antiplasmodial activity of crude extracts, fractions and sub-fractions

Stock solutions of crude extracts, fractions and sub-fractions were prepared at 1 mg/mL in DMSO 100%, while solution of artemisinin was prepared in distilled water and tested at 1µM highest concentration. The stock solutions were serially diluted in 96-wells plates using RPMI 1640 to achieve the range of tested concentrations from 0.24µg/mL to 125 µg/mL.

PfK1 (*P. falciparum* chloroquine-resistant *K1* strain) was continuously maintained in fresh "O" positive human erythrocytes suspended at 4% (v/v) heamatocrit in complete medium, consisting of 16.2 g/L RPMI 1640 (Sigma) containing 25 mM HEPES, 11.11 mM glucose, 0.2% sodium bicarbonate (Sigma), 0.5% Albumax I (Gibco), 45 µg/L hypoxanthine (Sigma) and 50 µg/L gentamicin (Gibco) and incubated at 37 °C in an atmosphere of 5% O_2, 5% CO_2, 90% N_2. The used medium was replaced daily with fresh complete medium to propagate the culture. Giemsa-stained blood smears were examined microscopically under oil immersion to monitor cell cycle transition and parasitaemia (Trager and Jensen, 1976). Prior the antiplasmodial assay, the culture was synchronized into ring stage parasites by 5% sorbitol (w/v) treatment as previously described (Lambros and Vanderberg, 1979).

For drug activity screening, the SYBR green I based fluorescence assay was used as previously described (Smilkstein et al., 2004). This dye interacts with malaria parasite to produce a fluorescent complex. Ninety six microliters of synchronized ring stage parasites at 2% haematocrit and 1% parasitaemia were added into triplicate wells of 96-wells plates containing 4 µL of each inhibitor concentration. Negative control consisted of 0.4% DMSO and positive control of 1 µM Artemisinin (ART) highest concentration. The treated cultures were incubated for 48 hrs at 37 °C in an atmosphere of 5% O_2, 5% CO_2, 90% N_2. Upon incubation, 100 µL of SYBR green I buffer [0.2 µL of 10,000 × SYBR Green I (Invitrogen) per mL of lysis buffer {Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; wt/vol), and Triton X-100 (0.08%; vol/vol)}] were added to each well and mixed gently and further incubated in the dark at 37 °C for 1 h. Fluorescence was subsequently measured using a fluorescence multi-well plate reader (Perkin Elmer) with excitation and emission at 485 and 530 nm respectively. Fluorescence counts for ART were deducted

from counts in each well and a dose–response curve was constructed by plotting fluorescence counts against the drug concentration and activity expressed as 50% inhibitory concentration (IC_{50}) using the IC Estimator-version 1.2 software (http://www.antimalarial-icestimator.net/MethodIntro.htm) where estimated parasite growth in the negative control (0.4% DMSO) is 100 %, and 0% in the positive control (ART) (Le Nagard et al., 2011; Kaddouri et al., 2006).

Cytotoxicity of PStw(Ace) sub-fractions

The cytotoxic effects of the mother fraction [PStw(Ace)] and derived sub-fractions (PS1-12) on mammalian cells was determined using human foreskin fibroblasts (HFF) cells cultured in complete medium containing 13.5 g/L DMEM, 10% fetal bovine serum, 0.2% sodium bicarbonate (w/v) (Sigma) and 50 µg/mL gentamycin (Boyom et al., 2014). Cells ($1x10^4$cells/ml/well) were seeded into 96-well flat-bottom tissue culture plates in complete medium. After 24 h of seeding, 80 µL of each test solution were added and cells incubated for 48 h in a humidified atmosphere at 37 °C and 5% CO_2. For each extract, the concentrations tested were ranged from 0.24-125 µg/mL. 0.4% DMSO v/v was tested as negative control. Twenty microliters of a kit solution of MTS/PMS (Promega) were added to each well, gently mixed and incubated for another 3 h at 37 °C. After gentle supernatant removal and addition of 100 µL DMSO (quench agent) to the cell pellets, formazan formation was measured by recording optical density (OD) in each well using a microtiter plate reader (Biotek EL800, USA) at 490 nm. Mean ODs were plotted versus drugs concentrations and the 50% cytotoxic concentration (CC_{50}) values were determined using the GraphPad Prism5.0 software. Selectivity indices of extracts were subsequently calculated on the basis of their antiplasmodial activities (IC_{50}) and HFF cell cytotoxicity (CC_{50}).

Safety and efficacy of twigs methanol fraction of *P. suaveolens* [PStw(Ace)] in mice

Experimental mice

Six weeks old female Swiss albino mice (20–25 g) provided by the animal house of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I were housed under standard conditions with 12 hours photoperiod and access to water and food *ad libitum* and used for the experiments. Animal welfare and ethical requirements were respected during the experiments according to the American Psychological Association (APA) guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research.

Evaluation of the acute toxicity of fraction PStw(Ace)

The acute toxicity of the methanol fraction from twigs of *P. suaveolens* [PStw(Ace)] was assessed in female Swiss albino mice prior to the *in vivo* antimalarial study. The study was performed according to the Organization for Economic Cooperation and Development guideline 423 (OECD, 2001). Mice were starved for about 4 h but allowed free access to water and weighed prior to test substances administration. Healthy mice were studied in 2 groups of 3 animals each. The test group received orally 1 mL of a single limit dose of 5000 mg/kg b.w of the fraction dissolved in 7% Tween 80/ 3% ethanol/ H_2O. The negative control group received 1 mL of 7% Tween 80/3% Ethanol/ H_2O solution. After the substances were administered, food was further withheld for 1 h, then animals were carefully observed for 4 h, and thereafter daily for 7 days during which several parameters such as mortality, moribund status, body weights, tremors,

convulsions, lethargy, sleep, and other changes in the normal behavior were recorded daily (OECD, 2001).

Evaluation of in vivo suppressive activity against Plasmodium berghei

The fraction PStw(Ace) that showed no signs of acute toxicity in mice was studied against *P. berghei* strain B (MRA-406, MR4, ATCC W Manassas Virginia) rodent malaria parasite model that was obtained from BEI Resources (www.beiresources.org)**.** The test was carried out based on the four-day suppressive test described by Fidock et al. (2004). Swiss albino mice weighing 20–25 g were put randomly into test and control groups, each group containing five mice and were supplied with adequate amount of mouse cubes and clean drinking water. The parasitized erythrocytes for each test were collected from an infected donor mouse with rising parasitaemia of 20–30%. The mice were sacrificed after anesthesia with Chloroform and blood was collected in tube containing Heparin (0.5% trisodium citrate) by cardiac puncture. The blood was then diluted with physiological saline (0.9%) in proportion of 1:4. Each mouse was then inoculated with 0.2 mL of blood containing about 10^6 *P. berghei* infected erythrocytes on day 0 through intra-peritoneal route. After four hours of parasite inoculation, four test groups of mice were administered with 100, 200, 300 and 400 mg/kg of fraction PStw(Ace). The negative control group mice were treated with 0.2 mL of the vehicle (7% Tween 80/ 3% Ethanol/ H_2O) and the positive control groups were treated with 24 mg/kg Quinine Sulfate.

Daily, a drop of blood was taken from tail snip of each mouse on frosted slide and smears were prepared, fixed with methanol and stained with 10% Giemsa solution at pH 7.2 for 15 min. Then, three fields were randomly selected on each stained slide and examined under microscope with an oil immersion objective (×100 magnification). The parasitaemia was determined by counting the number of parasitized erythrocytes (RBC) on randomly selected fields of the slide. Percentage of parasitaemia and suppression were calculated using the following formulas:

(1) %Parasitaemia = 100x (Number of parasitized RBCs / Total number of RBCs counted)

(2) % Suppression = 100x [(Parasitaemia in *Nc* – Parasitaemia *Tg*) / Parasitaemia Nc] where *Nc* is negative control, and *Tg* is treated group.

Data analysis

Mean values of triplicate experiments were calculated using MS excel for windows 10.0. The IC_{50} values were obtained using the IC Estimator-version 1.2 software (http://www.antimalarial-icestimator.net/MethodIntro.htm). CC_{50} were calculated using GraphPad Prism 5.0 software. Results are means ± SD (standard deviation).

RESULTS

Plant extraction, partition and antiplasmodial screening

A total of 36 extracts and fractions were prepared from organs collected from *P. suaveolens*, *M. tenuifolia* and *U. angolensis* with yields ranging from 0.02% for the interface precipitate of the twigs of *P. suaveolens* (PStw(I)) to 4.94% (w/w) for the crude ethanolic stem bark extract of *U. angolensis* (UvAsb(EtOH)) (Table 1).

The results of the screening of extracts and fractions against *P. falciparum* (Table 1) indicated that from the 9 crude ethanolic extracts that were tested, 6 [PSl(EtOH), PStw(EtOH), MoTel(EtOH), MoTetw(EtOH), UvAl(EtOH), and UvAtw(EtOH)] exhibited antiplasmodial activity with IC_{50} values ranging from 4.53 to

10 µg/mL. The most active was the crude ethanolic extract from the leaves of *P. suaveolens* [PSl(EtOH), IC_{50}= 4.53 µg/mL]. The subsequent fractions afforded through liquid-liquid partition of all extracts also exhibited varying effects on the parasites. Among the water fractions, only the leaves of *M. tenuifolia* (MoTel(H_2O)) and *U. angolensis* (UvAl(H_2O)) showed moderate antiplasmodial activity at IC_{50} values of 7.75 and 9.78 µg/mL respectively. Also, 2 interface precipitates from the stem bark of *P. suaveolens* (PSsb(I)) and from the leaves of *M. tenuifolia* (MoTel(I)) exerted good activities at the respective IC_{50} values of 3.84 and 4.53 µg/mL.

The acetogenin-rich (methanolic) fractions globally showed better potency compared to other types of fractions, and were the most active with 6 out 9 fractions [PStw(Ace), PSsb(Ace), MoTel(Ace), MoTetw(Ace), UvAl(Ace), and UvAtw(Ace)] showing IC_{50} values ranging from 3.24 to 7.78 µg/mL. Overall, the acetogenin-rich fraction of the twig extract of *P. suaveolens* [PStw(Ace)] showed the best antiplasmodial activity (IC_{50} = 3.24 µg/mL) and was therefore selected for further studies.

A qualitative phytochemical screening of the promising *P. suaveolens* [PStw(Ace)] fraction indicated the presence of three main classes of secondary metabolites, including alkaloids, phenols, and lactones.

This fraction was further fractionated to afford 12 major sub-fractions that were screened for antiplasmodial activity and cell cytotoxicity.

Antiplasmodial activity of sub-fractions from the twigs acetogenin-rich fraction of *P. suaveolens* [PStw (Ace)]

The results presented in Table 2 indicated that from the 12 sub-fractions afforded from the twigs acetogenin-rich fraction of *P. suaveolens*, only PS7 and PS8 showed antiplasmodial activity. They were respectively eluted with Hex-EtOAc (85:15→78:22) and Hex-EtOAc (78:22→60:40). PS8 exerted the more potent inhibition of the growth of malaria parasites (IC_{50} = 4.42 µg/mL), compared to PS7 that rather exhibited a moderate antiplasmodial inhibition with IC_{50} of 8.55µg/mL. Apart from these two sub-fractions, all the others were inactive against *P. falciparum* in culture.

Overall, from the bio-guided studies of the mother *P. suaveolens* twigs acetogenin-rich fraction [PStw(Ace)], it appeared that fractionation led to activity decline. Of note, PStw(Ace) (IC_{50}= 3.24 µg/mL) was more active and selective than the subsequent sub-fractions afforded from column chromatography. This potency decrease through fractionation might result from rupture of synergistic interactions between the components, and also suggests that if left unfractionated, this fraction might be further developed as potential antimalarial ameliorated plant extract. It is within this framework that the PStw(Ace) fraction was evaluated for safety in mice, and for antimalarial efficacy using the rodent model *Plasmodium berghei* parasites.

***In vivo* activities of the twigs acetogenin-rich fraction of *P. suaveolens* [PStw(Ace)].**

Acute toxicity profile of PStw(Ace) fraction

The study of the promising twigs methanolic fraction of *P. suaveolens* showed safety at the dose of 5000 mg/kg b.w. through oral administration in mice. Briefly, no death was recorded among experimental animals. Moreover, no major behavioral changes (moribund status, body weight loss, tremors, convulsions, salivation, diarrhea, persistent lethargy or sleep) were recorded during the 7 days observation beyond plant

fraction administration. Overall, test animals globally behaved like controls (untreated mice). Therefore, the 50% lethal dose (LD_{50}) was > 5000 mg/Kg b.w, and the plant fraction categorized as nontoxic according to the OECD guidelines (OECD, 2001).

Suppressive antiplasmodial activity of PStw(Ace) fraction in mice

From the four days activity assessment, the suppressive effect of the twigs acetogenin-rich fraction of *P. suaveolens* against *P. berghei* in mice was determined as presented in Figure 1. On day one of the experiment, the percent parasitaemia was globally similar in all six groups of mice, indicating that infection was establishing in all inoculated animals. Subsequently, the quality control assay (dose 0) indicated rising parasitaemia from day 1 to day 4 of the experiment (Figure 1). Besides, PStw(Ace) fraction exerted a dose-dependent suppression of the parasitaemia, and the more pronounced effect was observed at the highest dose of 400 mg/kg b.w. At this highest dose (400 mg/kg b.w), PStw(Ace) exhibited a strongest suppressive effect throughout the study period compared to the positive control. These results further emphasized the promising feature of fraction PStw(Ace) that otherwise showed to be safe in mice at doses below 5000 mg/kg b.w.

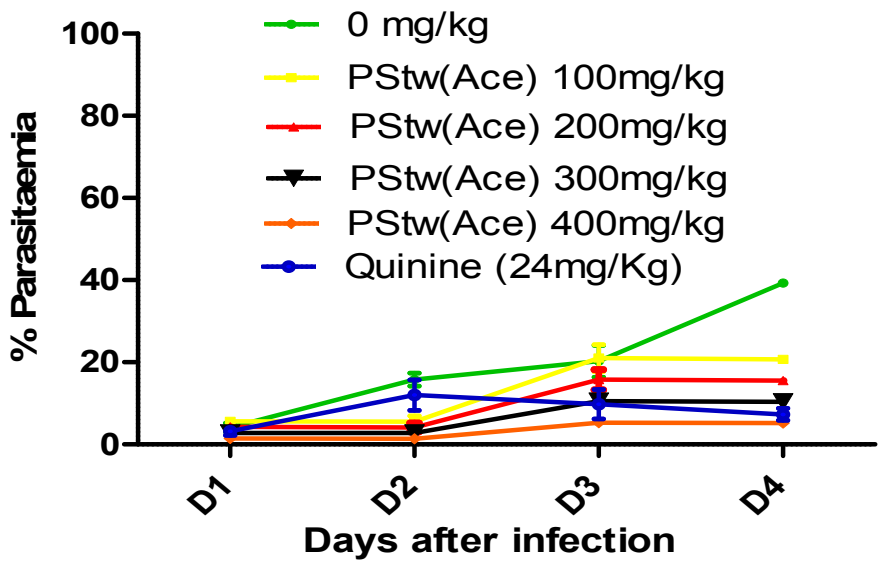

Figure 1: Effect of PStw(Ace) at different doses on parasitemia level in *P. berghei* infected mice.
Mice were treated with four doses of the fraction for four consecutive days. Mean % parasitaemias in each experimental group was determined daily by microscopic observation of tail blood thin smears stained with 5% Giemsa, and determination of % erythrocytes infected with *P. berghei*.

Table 1: Extraction yield and antiplasmodial activity of extracts and fractions from *P. suaveolens*, *M. tenuifolia* and *U. angolensis* (Annonaceae).

Plant & voucher specimen number	Extract/fraction	Plant part	Extract code	*Extraction Yield (% w/w)	**IC_{50} (µg/mL±SD) against *PfK1*
Polyalthia suaveolens HNC 1227/SRF/CAM	Crude Ethanolic	Leaf	PSl(EtOH)	4.28	4.53± 0.82
		Twig	PStw(EtOH)	1.80	5.75 ±1.83
		Stem bark	PSsb(EtOH)	6.30	>10
	Water	Leaf	PSl(H_2O)	0.90	>10
		Twig	PStw(H_2O)	0.34	>10
		Stem bark	PSsb(H_2O)	0.46	>10
	Interface precipitates	Leaf	PSl(I)	0.07	>10
		Twig	PStw(I)	0.02	>10
		Stem bark	PSsb(I)	0.06	3.84±0.86
	Methanolic Acetogenin-rich fractions	Leaf	PSl(Ace)	1.63	>10
		Twig	PStw(Ace)	0.45	3.24± 0.1
		Stem bark	PSsb(Ace)	1.45	4.90 ± 0.73
Monodora tenuifolia HNC55313/SRF/ CAM	Crude ethanolic	Leaf	MoTel(EtOH)	3.98	5.48±0.19
		Twig	MoTetw(EtOH)	1.79	8.93±0.19
		Stem bark	MoTesb(EtOH)	4.03	>10
	Water	Leaf	MoTel(H_2O)	2.11	7.75± 0.33
		Twig	MoTetw(H_2O)	1.24	>10
		Stem bark	MoTesb(H_2O)	1.94	>10
	Interface precipitates	Leaf	MoTel(I)	0.33	4.53±0.44
		Twig	MoTetw(I)	0.82	>10
		Stem bark	MoTesb(I)	0.12	>10
	Methanolic Acetogenin-rich fractions	Leaf	MoTel (Ace)	0.82	3.84± 0.37
		Twig	MoTetw (Ace)	1.23	5.02 ± 0.80
		Stem bark	MoTesb (Ace)	1.21	>10
Uvaria angolensis HNC50059/SRF/ CAM	Crude ethanolic	Leaf	UvAl(EtOH)	3.27	9.98± 2.87
		Twig	UvAtw(EtOH)	3.83	10.00± 0.87
		Stem bark	UvAsb(EtOH)	4.94	>10
	Water	Leaf	UvAl(H_2O)	0.76	9.78 ± 1.87
		Twig	UvAtw(H_2O)	0.06	>10

	Stem bark	UvAsb(H_2O)	0.05	>10
Interface precipitates	Leaf	UvAl(I)	0.74	>10
	Twig	UvAtw(I)	0.18	>10
	Stem bark	UvAsb(I)	0.23	>10
Methanolic Acetogenin-rich fractions	Leaf	UvAl(Ace)	1.39	5.78 ± 0.75
	Twig	UvAtw(Ace)	1.31	7.78 ± 0.13
	Stem bark	UvAsb(Ace)	1.98	>10

*Plant parts were extracted by maceration in ethanol and further partition using different solvents and the yield calculated in percentage relative to the weight of the starting materials. **50% inhibitory concentration; the susceptibility of *P. falciparum* K1 strain to plant extracts was assessed in culture using triplicate experiments; S.D. = standard deviation.

Table 2: Antiplasmodial and cytotoxic activities of sub-fractions of the *P. suaveolens* twigs acetogenin-rich fraction [PStw(Ace)].

Fraction/[a]sub-fractions	[b]IC_{50} (µg/mL± SD) against *PfK1*	[c]CC_{50} against HFF (µg/mL)	[d]SI (CC_{50}/IC_{50})
PStw(Ace)	3.24± 0.10	>30	>9.26
PS1	>10	-	-
PS2	>10	-	-
PS3	>10	-	-
PS4	>10	-	-
PS5	>10	-	-
PS6	>10	-	-
PS7	8.55 ± 2.05	>30	> 3.51
PS8	4.42 ± 0.87	>30	> 6.79
PS9	>10	-	-
PS10	>10	-	-
PS11	>10	-	-
PS12	>10	-	-
ART	0.005±0.0008	>30	>6000

[a]The 12 sub-fractions were afforded by column chromatography eluting with solvent systems of increasing polarity; [b]50% inhibitory concentration; the susceptibility of *P. falciparum* K1 strain to sub-fractions was assessed in culture using triplicate experiments; S.D. = standard deviation; [c]Cell cytotoxicity of sub-fractions was assessed on normal human foreskin fibroblast cells (HFF) using triplicate experiments; [d]Selectivity indices were calculated using the IC_{50} and CC_{50} of each sub-fraction; ART (Artemisinin) was tested as positive control.

DISCUSSION

The present study aimed at investigating the *in vitro* and *in vivo* susceptibility of malaria parasites to crude extracts and fractions from *Polyalthia suaveolens*, *Uvaria angolensis*, and *Monodora tenuifolia*. The crude ethanolic extracts exerted varying degrees of inhibition against *P. falciparum* K1 *in vitro.* Following a bio-guided approach, the extracts were partitioned using the acetogenin extraction protocol designed by Alali et al. (1999). The results achieved in this investigation indicated that methanol fractions (acetogenin-rich fractions) have the overall best activity against *P. falciparum* K1. This potency of acetogenin-rich fractions has previously been reported (Boyom et al., 2011) in a similar study targeting the Chloroquine-resistant *P. falciparum* W2 strain. Globally, the observed inhibitory effect of methanolic fractions could be attributed to the presence of acetogenins. Indeed, these compounds have been shown to inhibit complex I (NADH: ubiquinone oxidoreductase) in mitochondrial electron transport systems (Lewis et al., 1993); in addition, they are potent inhibitors of NADH oxidase of plasma membranes (Morré et al., 1995); these enzymes are all found in *P. falciparum* and the effects of inhibitors impair parasite oxidative and cytosolic ATP production, leading to apoptosis. Acetogenins have been also reported as potent cytotoxics with insecticidal, ascaricidal, fungicidal, antiparasitic, bactericidal and antiplasmodial activities (Rakotomanga et al., 2004; Guadaño et al., 2000). Beside the few previous works reported above, Boyom (2004) also showed that essential oils from the stem bark of *P. suaveolens* and *U. angolensis* have *in vitro* inhibitory potential against *P. falciparum* W2**.** Also, extracts from closely related *P. longifolia* were recently found to display moderate to good antiplasmodial activity against chloroquine sensitive *P. falciparum* 3D7 strain (Sunita et al., 2016).

Little is known about the antiplasmodial activity of extracts from *M. tenuifolia.* However, the methanol (acetogenin-rich) fractions from a closely related species, *M. myristica* have previously showed moderate antiplasmodial activities with IC_{50} values ranging 5.52-9.03 µg/mL (Boyom et al., 2011)**.** Also, recent studies on other *Uvaria* spp., *viz. Uvaria lucida* and *Uvaria scheffleri* showed inhibition of *P. falciparum* W2 with IC_{50} values of 10.3 and 6.8 µg/mL respectively (Muthaura et al., 2015)**.**

To find out which components might be eliciting the antiplasmodial activity in the promising PStw(Ace) fraction, a qualitative phytochemical screening indicated the presence of three main classes of secondary metabolites, namely alkaloids, phenols, and lactones. They might be individually and/or collectively responsible for the exhibited antiplasmodial activity. Indeed, these groups of compounds were previously reported to have significant inhibitory effects on *P. falciparum* (Kaur et al., 2009; Jimenez-Romero et al., 2008; Tasdemir et al., 2006). Functional terminal lactones or butenolide often characterize the acetogenins (Li et al., 2008). To further purify sub-fractions of the promising fraction with improved activity, PStw(Ace) was submitted to bio-guided column chromatography. Two sub-fractions (PS7 and PS8) exhibited antiplasmodial activity. They might contain lactones that are readily extracted using solvents systems in the same range as those used to afford sub-fractions PS7 and PS8 (Khetarpal et al., 1991). Also, alkaloids were previously shown to be suitably extracted using Hex-EtOAc (80:20 v/v) solvent system (Khalik et al., 2016). It is therefore assumable that components of the active fraction PStw(Ace) might be alkaloid-like and or bear lactone functional groups. However, the subsequent sub-fractions PS7 and PS8 showed to be less

active than PStw(Ace). This latter was therefore progressed and showed to be also active *in vivo* against the rodent model *P. berghei*. Furthermore, it also showed to be selective and safe in mice through oral administration, emphasizing its potential for antimalarial drug development.

Conclusion

The results achieved from the investigation of *P. suaveolens*, *M. tenuifolia,* and *U. angolensis* have indicated that some of the plant extracts possess antiplasmodial activity. Of particular note, the methanol fraction PStw(Ace) (acetogenin-rich) obtained from liquid-liquid partition of the *P. suaveolens* twigs ethanol crude extract has shown the overall best antiplasmodial activity. Further study of this fraction has also indicated its safety in mice, and suppressive potential of *P. berghei* parasitaemia over a four days study. This acetogenin-rich fraction should be studied in details to determine its qualitative and quantitative compositions in an attempt to formulate a standardized phytodrug against malaria.

COMPETING INTERESTS

The authors declare no competing interest.

AUTHORS' CONTRIBUTIONS

FFB designed and supervised the study; ANM, LRYT, CDJM, MATT, RMTK, PVTF, EAMK, and RGK performed the chemical and biological (*in vitro* and *in vivo* studies) parts of the study and drafted the manuscript; FFB, EAMK, and RGK critically revised the manuscript. All authors agreed on the final version of the manuscript.

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