The Antimalarial Potential of Three Ghanaian Medicinal Plants

Gustav Komlaga[1,2,3,]
Sandrine Cojean[2],
Mehdi Beniddir A[3],
Rita Dicksona[1],
Pierre Champy[3],
Merlin Lincoln Kwao Mensah[4],
Soulaf Suyyagh-Albouz[2],
Jonathan Jato[1] **and**
Philippe Loiseau M[2]

1   Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
2   Chimiothérapie Antiparasitaire, CNRS UMR 8076 BioCIS, Faculty of Pharmacy, Univ. Paris-Sud, France
3   Laboratoire de Chimie des Substances Naturelles, CNRS UMR 8076 BioCIS, Faculté de Pharmacie, Université Paris-Sud, 5 rue J.-B. Clément, 92296 Châtenay-Malabry Cedex, France
4   Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah Univer-sity of Science and Technology, Kumasi, Ghana
5   Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

Corresponding author:
Komlaga G, Loiseau PM

✉ gustkomla@yahoo.com
  philippe.loiseau@u-psud.fr

Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, Chimiothérapie Antiparasitaire, CNRS UMR 8076 BioCIS, Faculty of Pharmacy, Univ. Paris-Sud, France and Laboratoire de Chimie des Substances Naturelles, CNRS UMR 8076 BioCIS, Faculté de Pharmacie, Université Paris-Sud, 5 rue J.-B. Clément, 92296 Châtenay-Malabry Cedex, France.

Chimiothérapie Antiparasitaire, CNRS UMR 8076 BioCIS, Faculty of Pharmacy, Univ. Paris-Sud, France

Abstract

Objective: Malaria is a major public health problem in Ghana and many indigenes, especially those in rural areas, resort to the use of medicinal plants to treat the disease. The plants: *Persea americana* Mill. (Lauraceae)*, Theobroma cacao* L. (Malvaceae) *and Tridax procumbens* (L.) L. (Compositae) are used solely or in combination with other medicinal plants to manage malaria and its associated conditions. The leaves of the plants which are normally the main parts employed, were studied for their phytochemistry and antiplasmodial activity to establish their chemical profile and verify the antimalarial claim.

Methods: Plant materials were subjected to basic phytochemical screening to identify the major secondary metabolites. The aqueous extracts were evaluated against chloroquine-sensitive 3D7 *P. falciparum* and chloroquine-resistant W2 *P. falciparum* strains, using the fluorescence-based SYBR® green I method to determine their antiplasmodial activity.

Results: Basic phytochemical screening of the leaves revealed the presence of tannins, flavonoids and alkaloids in all three plant materials. *T. cacao* and *P. americana,* in addition, contained purine base alkaloids, triterpenoids including saponins. The aqueous extracts of the leaves showed antiplasmodial activity against the chloroquine-sensitive 3D7 *P. falciparum* ($9.50 \pm 1.38 \leq IC_{50} \leq 10.15 \pm 0.45$ µg/mL) and against chloroquine-resistant W2 *P. falciparum* strains ($6.40 \pm 1.94 \leq IC_{50} \leq 44.94 \pm 1.12$ µg/mL). The aqueous extract of *T. cacao* was the most active and was more active against W2 than 3D7 *P. falciparum.* Only *T. procumbens* displayed cytotoxicity ($CC_{50} < 25$ µg/mL).

Conclusion: *T. cacao, T. procumbens* and *P. americana* possess antiplamodial activity. The activity illustrates their antimalarial potential, and provides rationale for their use in traditional malaria therapy in Ghana. It thus paves the way for further study of these plants for antiplasmodial lead compound(s).

Keywords: *Persia americana*; *Theobroma cacao*; *Tridax procumbens*; Antiplasmodial; Phytochemistry; Malaria; Traditional; Medicinal plants

Tel: 233207823018
+33607114308

Introduction

The use of medicinal plants in the treatment of diseases has a long history worldwide. In developing countries, about 80% of the population relies on traditional medicine for the treatment of various ailments including life-threatening ones such as malaria. In Sub-Saharan Africa, medicinal plants, which constitute the mainstay of traditional medicines, have significant medicinal usages, though with little or no supportive scientific data. Plants used in malaria and fever account for 6% of the medicinal plants of the Ghanaian domestic market [1] and some of these plants have been formulated into commercial phytomedicines [2]. According to Komlaga et al. [2], about 6%, 8% and 7% of herbalists in the Bosomtwe and Sekyere East Districts of Ghana, respectively, employ *Persea americana* Mill. (Lauraceae)*, Theobroma cacao* L. (Malvaceae) and *Tridax procumbens* (L.) L. (Compositae) in the traditional treatment of malaria. We therefore evaluated the antiplasmodial activity of the aqueous extracts of the three plants in order to provide scientific validation for their antiplasmodial use in traditional medicine. *Persea americana* is cultivated for its nutritious fruit which contains vitamins and minerals [3]. The leaf is used for the treatment of malaria [3-5], dysentery, coughs, liver obstructions and high blood pressure [6]. It is used to enhance menstrual flow and reduce high levels of uric acid in the body [6]. The skin of the fruit is used to treat intestinal parasitic worms infestation, cancer of the labia and other tumours [3]. The fruit pulp has emollient and carminative properties, and lowers blood cholesterol levels. The mashed fruit pulp has aphrodisiac properties, and the unripe fruit used to induce abortion. The pulp is used externally against suppurating wounds and for hair growth. The seed ointment is used to treat various skin diseases including scabies, purulent wounds, lesions of the scalp and dandruff. The seed oil has astringent properties [6]. The leaf extract induced weight reduction, displayed antihepatotoxic activity, antiulcer and anticonvulsant effects [7]. Aqueous extract of the leaf showed hypoglycaemic activity [8] and inhibitory effect on acyclovir and PAA-resistant herpes simplex virus [9]. Methanol extracts of the leaf exhibited antimycobacterial activity [10] while the leaf and stem bark extracts showed antibacterial activity and the fruit extract wound healing activity [11]. *Theobroma cacao* has been used for an array of medicinal purposes. The unfermented seeds and the seed coat are used to treat diabetes, digestive and chest complaints. Cocoa powder from fermented cocoa seeds is used to prevent heart disease. Cocoa butter helps lower cholesterol levels [12]. The leaf extracts showed antiproliferative activity [13] and blocked glycolytic pathway of glucose oxidation resulting in accumulation of glucose and pyruvate [14]. *Tridax procumbens*

is used as food and medicine [15]. It is used to treat high blood pressure, bronchial catarrh, malaria, dysentery, diarrhoea, stomach ache, headache and wound healing [2,16,17]. It also prevents hair loss and stops hemorrhage from cuts and bruises [16,17]. The aqueous extract of the plant exhibited antiplasmodial activity [18], hypotensive activity and bradycardia in normal rats [19]. Various extracts showed anti-inflammatory [20], anti-arthritic activities [21] and antimicrobial activity [22]. The aqueous extract did not show cytotoxicity against HepG2 cells [23].

Materials and Methods

Reagents and chemicals

Chemicals including RPMI (Roswell Park Memorial Institute) 1640, hypoxanthine, N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid (HEPES), glucose, albumax II, SYBR® green I-lysis buffer, DMEM-F12 (Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12), streptomycin, fetal bovine serum (FBS), Trypsin, Ethylenediaminetetraacetic acid (EDTA) were obtained from GIBCO/Invitrogen life Technologies, France. Non-infected O+ blood was kindly donated by Bichat Hospital (Paris, France).

Selection, collection and preparation of plant materials

The leaves of *Persea americana* Mill. (Lauraceae)*, Theobroma cacao* L. (Malvaceae) and *Tridax procumbens* (L.) L. (Compositae) **(Table 1),** selected from an earlier survey, were harvested in July 2013 in Kumasi, Ghana. They were selected because high percentage of herbalists employ them in the traditional treatment of malaria in the Bosomtwe and Sekyere East Districts of Ghana [2]. The plant materials were authenticated by Dr G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Sciences and Technology (KNUST), Kumasi, Ghana, and herbarium specimen with Voucher numbers **(Table 1)** deposited in the same Department. The harvested plant materials were air dried under shade at ambient temperature (28-35°C) for 7 days. They were then coarsely powdered using an electric mill and stored in airtight containers under room temperature until required for use.

Extraction of plant materials

For each plant material, 100 g of powder was boiled in 2 L of water in accordance with traditional preparations for 30 minutes. The decoction was then strained through double-layered white cotton material and subsequently through filter paper. The filtrate was lyophilized and the dry material kept at 4°C until needed for analysis.

Preliminary phytochemical analysis of materials

Plant materials were subjected to qualitative phytochemical screening to determine the presence of the major phytochemical constituents. The screening was performed according to standard procedures [21,22].

Plasmodium culture maintenance

Parasite cultures consisting of chloroquine-sensitive 3D7 *P. falciparum* and chloroquine-resistant W2 *P. falciparum* were maintained according to the method described by Trager & Jensen [24,25]. The parasites were separately cultivated at 37°C in a candle jar on complete culture medium of RPMI 1640, containing 2.5% hematocrit, hypoxanthine, HEPES, glucose, albumax II, and buffered with $NaHCO_3$. Fresh culture was maintained for at least 96 hours (2 complete life cycles) before being used for assays. Cultures were synchronized with 5% D-sorbitol (Sigma) and at least 90% ring forms were obtained before assays were run. Prior to the launch of assays, the level of parasitaemia of an aliquot of a stock culture was determined by light microscopy following Giemsa staining [26].

Antiplasmodial activity

The antimalarial activity of aqueous extracts was evaluated against 3D7 *P. falciparum* and W2 *P. falciparum* strains using the fluorescence-based SYBR® green I approach in 96-well microplates as described by Smilkstein et al. [27] with some modifications. Positive control wells for each assay contained no inhibitor while negative control contained chloroquine (CQ). Experiments were run in duplicate with both test and control drugs employed at varying concentrations. Test solutions were prepared in dimethyl sulfoxide (DMSO) and diluted with culture medium to give a maximum DMSO concentration of 0.5% in a final well volume of 200 μL, containing 1% parasitemia and 2.5% haematocrit. Extracts and negative control [chloroquine (CQ)] were prepared by two-fold dilution, in a dose-titration range of 0.098 - 100 μg/mL, to obtain 11 concentrations each in triplicate. After 42 h incubation in a candle jar at 37°C, the plates were frozen at -80°C overnight. The frozen plates were then taken through a 3 freeze-thaw cycles of 1 h thawing at room temperature, 5 minutes homogenization

and 1 h freezing at -80°C. Five microlitres (5 μL) of homogenate was added to 45 μL of SYBR® green I in lysis buffer in a PCR plate. The PCR plate was incubated at room temperature for an hour and the fluorescence measured using a multi-well plate reader (Eppendorf realplex mastercycler epgradient S) with excitation and emission wavelength bands centered at 485 and 530 nm, respectively. The IC_{50}, defines as drug concentration that inhiits the growth of *P. falciparum* by 50%, was estimated using the web based antimalarial estimator, ICEstimtor, at http://www.antimalarial-icestimator.net/MethodIntro.htm [26].

Cytotoxicity on HUVEC using MTT assay

Human umbilical vein endothelial cells (HUVECs) were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM-F12) medium in the presence of fetal bovine serum (FBS; 10%) plus streptomycin (1%) and incubated in 5% CO_2 at 37°C [28,29]. The cytotoxicity of aqueous plant extracts was evaluated using MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) colorimetric method [30]. HUVEC were seeded in a 96 well plate at 15000 cells/ well and incubated for 24 h when cells reached >80% confluence. After discarding the old medium, the cells were incubated in a medium containing aqueous extracts of the plant materials in a dose-titration range of 0.78-100 μg/mL. After 24 h incubation, 20 μL MTT (5 mg/mL) was added to each well and cells were incubated for another 3 h. Finally, the culture medium containing MTT solution was removed and the formazan crystals were dissolved in 100 μL of dimethylsulfoxide (DMSO). Absorbance was read with an Eppendorf plate reader at 546 nm. Drug concentration that reduced the number of viable cells by 50%, CC_{50}, was calculated using GraphPad Prism Software (Version 5.0, San Diego, CA, USA).

Results

Phytochemical screening

All three plant materials tested positive for flavonoids, tannins, and alkaloids. However, *Theobroma cacao* and *Persea americana* also showed the presence of purine base alkaloids and terpenoids **(Table 2)**.

Table 1 Plant for antiplasmodial assessment.

Plant name	Family	Local name (Ewe/Twi)	Voucher specimen number	Part investigated
Persea americana Mill.	Lauraceae	Peya	HM/032/13	Leaf
Theobroma cacao L.	Malvaceae	Koko	HM/033/13	Leaf
Tridax procumbens (L.) L.	Compositae	Nantwi bini	HM/034/13	Whole plant

Table 2: Phytochemical screening of the leaves of the plants.

Constituents	P. americana	T. cacao	T. procumbens
Triterpenoids	+	+	-
Saponins	+	+	-
Tannins	+	+	+
Alkaloids	+	+	+
Purine base alkaloids	+	+	-
Flavonoids	+	+	+

Keys: +: present; -: absent, in the conditions used.

In vitro antiplasmodial activity of aqueous extracts

The extracts were assessed at concentrations up to 100 µg/mL against 3D7 and W2 strains of *Plasmodium falciparum*. All three extracts showed antiplasmodial activity. Activity against 3D7 *P. falciparum* strain was generally around 10 µg/mL. The extract of *T. cacao* was particularly more active against the chloroquine-resistant W2 *P. falciparum* strain (IC$_{50}$: 6.40 ± 1.94 µg/mL). However, *P. americana* and *T. procumbens* extracts were respectively 3 and 4 folds less active when compared to their activity against 3D7 *P. falciparum*. Resistant index (RI) was generally low and exceptionally low (RI<1) for *T. cacao.* Only *T. procumbens* demonstrated cytotoxicity (CC$_{50}$: 24.89 ± 3.68). The extracts of *P. americana* and *T. cacao* generally showed high selectivity index (SI>2.9) whereas *T. procumbens* displayed largely low SI **(Table 3)**.

Discussion

The use of medicinal plants in malaria therapy and also, in the therapy of many other diseases is commonplace at the primary healthcare level in many developing countries. In Ghana, medicinal plants have been used traditionally to manage malaria; some without scientific evidence of efficacy or safety. *Theobroma cacao, Persea americana* and *Tridax procumbens,* examples of such plants, were evaluated for their phytochemical profile, antiplasmodial activity and cytotoxicity.

The leaves contained various secondary metabolites including, tannins, alkaloids, flavonoids, and terpenoids **(Table 2)**. Many compounds belonging to these phytochemical groups had shown antiplasmodial activity [31] and may indicate the same for constituents of the plants studied.

The aqueous extracts of the three plants demonstrated varying degrees of activity against chloroquine-sensitive 3D7 and chloroquine-resistant W2 strains of *P. falciparum* (**Table 3**). According to Philippe et al. [32], the antiplasmodial activity of plant extracts can be classified as highly active (IC$_{50}$ ≤ 5 µg/mL); moderately active (5<IC$_{50}$ ≤ 15 µg/mL); weakly activity (15<IC$_{50}$ ≤ 50 µg/mL) and inactive (IC$_{50}$>50 µg/mL). Against this backdrop, all the extracts were considered to be moderately active against 3D7 *P. falciparum*. On the other hand, only the leaf extract of *T. cacao* showed moderate activity against W2 *P. falciparum*; that of *P. americana* and *T. procumbens* were only weakly active against the same strain.

P. americana and *T. cacao* leaf extracts exhibited no cytotoxicity against HUVECs up to the highest concentration (100 µg/mL) tested, and displayed good selectivity for the parasite (SI>2.9). Conversely, *T. procumbens* showed significant cytotoxicity and very low selectivity especially for the chloroquine-resistant parasite **(Table 2)**. *T. procumbens* can therefore be described as generally cytotoxic since it demonstrated cytotoxicity to both parasite and human cells. This extract needs further investigation regarding potential toxicity and evaluation of benefit/risk ratio in man. Until this is done, *T. procumbens* should be used in traditional treatment of malaria with caution since it could be harmful to the host especially in high doses. Extracts with high selectivity index offer the potential of safer therapy [27,28]. Therefore with appropriate dosage regimen, *T. cacao* in particular could serve as an important malaria therapy. However, preclinical studies and clinical studies would be required to ascertain potential usefulness.

The resistance index (RI) was determined to assess the levels of resistance demonstrated by the resistant strain of the parasite against the respective extracts. The chloroquine-resistant W2 *P. falciparum* displayed no resistance to *T. cacao* while it showed low level of resistance to *P. americana* and *T. procumbens* extracts. This may suggest *T. cacao* a more appropriate remedy in the traditional treatment of malaria particularly at a time that *P. falciparum* resistance is a challenge in the treatment of the disease [33,34].

Appiah-Opong et al. [18] reported an inferior antiplasmodial activity (IC$_{50}$>500 µg/mL) for the aqueous extract of *T. procumbens* against chloroquine-resistant Dd2 *P. falciparum* strain while in this study, a more stronger activity (IC$_{50}$: IC$_{50}$ 44.94 ± 1.12 µg/mL) was established against the chloroquine-resistant W2 *P. falciparum* strains. Many factors could account for this difference among which could be the strain of *P. falciparum* used and plant-associated factors such as the geographical source of plant, the season of collection, the age when collected, preparation and storage condition (where applicable) of extract.

The aqueous extract of *P. americana* leaf is reported for the first time for antiplasmodial activity; likewise, the leaf aqueous extract of *T. cacao.* However, the bean (cocoa) powder has been shown to have antiplasmodial activity [35].

Aside the direct effects of drugs on the malaria parasite in

Table 3 *In vitro* antiplasmodial activity and cytotoxicity of aqueous extracts of Ghanaian medicinal plants against 3D7 and W2 strains of *P. falciparum* and HUVECs respectively.

Extract	Antiplasmodial activity		RI	Cytotoxicity	SI, 3D7	SI, W2
	IC$_{50}$: 3D7	IC$_{50}$: W2	(IC$_{50}$: W2/IC$_{50}$: 3D7)	CC$_{50}$	(CC$_{50}$/IC$_{50}$)	(CC$_{50}$/IC$_{50}$)
PA	9.93 ± 0,86	34.20 ± 5.80	3.4	>100	>10.1	>2.9
TC	9.50 ± 1.38	6.40 ± 1.94	0.7	>100	>10.5	>15.6
TP	10.15 ± 0.45	44.94 ± 1.12	4.4	24.89 ± 3.68	2.5	0.6
CQ	0.02 ± 0.00	ND	ND	ND	ND	ND

Keys: PA: aqueous extract of *Persea americana* leaf, TC: aqueous extract of *Theobroma cacao* leaf, TP: aqueous extract of aerial part of *Tridax procumbens*; 3D7: choloroquine-sensitive *P. falciparum* strain; W2: choloroquine-resistant *P. falciparum* strain; ND: not determined; CQ: chloroquine; Selectivity Index (SI) is define as the ratio of CC$_{50}$ to IC$_{50}$; Resistance Index (RI) is defined as the ratio of the IC$_{50}$ of the choloroquine-resistant *P. falciparum* line (W2 *P. falciparum*) to that of the parent (choloroquine-sensitive) strain (3D7 *P. falciparum*); the concentration of CQ is expressed in µM; IC$_{50}$ and CC$_{50}$ are expressed in µg/mL ± SD.

chemotherapy of the disease, alleviation of the feverish symptoms, such as joint pains, body weakness and headache among other associated symptoms are important. Adeyemi et al. [36] reported the analgesic and anti-inflammatory activities of the leaf aqueous extract of *P. americana*. Also *T. procumbens* has shown anti-inflammatory [20] and anti-arthritic activities [21]. These symptomatic non-parasite specific effects of the extracts together with their antiplasmodial activity could account for the relief from the disease during therapy. This study thus justifies the traditional use of these plants in malaria treatment in Ghana.

Conclusion

The aqueous extracts of *Theobroma cacao* (leaf), *Tridax procumbens* (whole plant) and *Persea americana* (leaf) possess antimalarial effect. They were active against both chloroquine-sensitive 3D7 and chloroquine-resistant W2 *P. falciparum*. Only *T. procumbens* displayed cytotoxicity. The study supports the traditional use of the three plants as a therapy for malaria in Ghana, and provides scientific justification for further study of the plants [36].

P. falciparum has displayed resistance to existing antimalarial drugs including artermisinin and its derivatives [37], emphasizing the need for new effective but affordable antimalarials. The three plants could be considered for formulations into standardized antimalarial phytomedicines using the 'reversed pharmacology' approach [38] to afford cheaper alternatives to malaria therapy. Also, different organic solvent extracts of the plant materials could be evaluated to identify solvent specific extracts with promising antiplasmodial activity. Such extracts can become a subject of activity-directed fractionation to isolate active compounds. Compound showing significant activity could be modified chemically to afford molecule with high efficacy and safety. Isolated compounds can also be assessed in combination with themselves and classical antimalarial drugs for synergistic effect against *P. falciparum*.

References

1   Van Andel T, Myren B, Van Onselen S (2012) Ghana's herbal market. J Ethnopharmacol 140: 368-378.

2   Komlaga G, Agyare C, Dickson RA, Mensah MLK, Annan K, et al. (2015) Medicinal plants and finished marketed herbal products used in the treatment of malaria in the Ashanti region, Ghana. J Ethnopharmacol 172: 333-346.

3   KEW Botanical Garden, (2015) Persea americana KEW. [Online] Available: http://www.kew.org/science-conservation/plants-fungi/persea-americana-avocado.

4   Asase A, Asafo-Agyei T (2011) Plants Used for Treatment of Malaria in Communities Around the Bobiri Forest Reserve in Ghana. J Herbs Spices Med. Plants 17: 85-106.

5   Betti J, Iponga D, Yongo OD, Mbomio D, Yobo C M, et al. (2013) Ethnobotanical study of medicinal plants of the Ipassa-Makokou Biosphere Reserve, Gabon: Plants used for treating malaria. J Med Plants Res 7: 2300-2318.

6   Ken F (2015) Persea americana, Useful Tropical Plants Database. [Online]. Available: http://tropical.theferns.info/viewtropical.php?id=Persea+americana.

7   Yasir M, Das S, Kharya MD (2010) The phytochemical and pharmacological profile of Persea americana Mill. Pharmacogn Rev 4: 77-84.

8   Antia BS, Okokon JE, Okon PA (2005) Hypoglycemic activity of aqueous leaf extract of Persea americana Mill. Indian J Pharmacol 37: 325-326.

9   Miranda MM, Almeida AP, Costa SS, Santos MG, Lagrota MH, et al. (1997) In vitro activity of extracts of Persea americana leaves on acyclovir-resistant and phosphonoacetic resistant Herpes simplex virus. Phytomedicine 4: 347-352.

10 Gomez-Flores R, Arzate-Quintana C, Quintanilla-Licea R, Tamez-Guerra P, Tamez-Guerra R, et al. (2008) Antimicrobial activity of Persea americana Mill (Lauraceae) (Avocado) and gymnosperma glutinosum (Spreng.) Less (Asteraceae) leaf extracts and active fractions against Mycobacterium tuberculosis. Am J Sci Res 3: 188-194.

11 Nayak BS, Raju SS, Chalapathi Rao AV (2008) Wound healing activity of Persea americana (avocado) fruit: a preclinical study on rats. J Wound Care 17: 123-126.

12 KEW Botanical Garden (2015) Theobroma cacao, KEW. [Online]. Available: http://www.kew.org/science-conservation/plants-fungi/theobroma-cacao-cocoa-tree.

13 Baharum Z, Akim AM, Taufiq-Yap YH, Hamid RA, Kasran R (2014) In Vitro Antioxidant and Antiproliferative Activities of Methanolic Plant Part Extracts of Theobroma cacao. Molecules 19: 18317-18331.

14 Casikar V, Mujica E, Mongelli M, Aliaga J, Lopez N, et al. (2010) Does chewing coca leaves influence physiology at high altitude. Indian J Clin Biochem 25: 311-314.

15 Ken F (2014) Tridax procumbens, Useful tropical plants. [Online]. Available: http://tropical.theferns.info/viewtropical.php?id=Tridax+procumbens.

16 Ankita J, Jain A (2012) Ttridax procumbens (L.): A weed woth immense medicinal importance: A Review. Int J Pharma Bio Sci 3: 544-552.

17 Mshana RN, Abbiw DK, Addae-Mensah I, Adjanouhoun E, Ahyi MRA, et al. (2001) Traditional medicine and Pharmacopoeia; Contribution to the revision of ethnobotanical and floristic studies in Ghana. Accra: Science and Technology Press, CSIR.

18 Appiah-Opong R, Nyarko AK, Dodoo D, Gyang FN, Koram KA, et al. (2011) Antiplasmodial activity of extracts of Tridax procumbens and Phyllanthus amarus in in vitro Plasmodium falciparum culture systems. Ghana Med. J 45: 143-150.

19 Salahdeen H, Yemitan OK, Alada ARA (2004) Bioline International Official Site (site up-dated regularly). African J. Biomed Res 7: 27-29.

20 Jachak SM, Gautam R, Selvam C, Madhan H, Srivastava A, et al. (2011) Anti-inflammatory, cyclooxygenase inhibitory and antioxidant activities of standardized extracts of Tridax procumbens L. Fitoterapia 82: 173-177.

21 Petchi Rr, Vijaya C, Parasuraman S (2013) Anti-arthritic activity of ethanolic extract of Tridax procumbens (Linn.) in Sprague Dawley rats. Pharmacognosy Res 5: 113.

22 Bharathi V, Varalakshmi B, Gomathi S, ShanmugaPriya A, Karpagam T (2012) Antibacterial activity of tridax pro-cumbens linn. Int J Pharma Sci Res 3: 364-367.

23 Koukoui O, Cokou PAD, Medoatinsa E, Prigent S, Nusse O, et al. (2015) Chemical Profile, Cytotoxicity Antiradical and Hypolipidemic Activities of Tridax Procumbens of Benin. Res J Pharm Biol Chem 6: 1441-1449.

24 Harborne J (1991) Phytochemical analysis: A Guide to Modern Techniques of Plant Analysis. 2nd edn. Chapman and Hall, London.

25 Evans W (2006) Trease and Evans Pharmacognosy Textbook. 15th edn. Elsevier Limited, United Kingdom.

26 Trager W, Jensen JB (1976) Human malaria parasites in continuous culture. Science 193: 673-675.

27 Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M (2004) Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. Antimicrob Agents Chemother 48: 1803-1806.

28 Kaddouri H, Nakache S, Houzé S, Mentré F, Le Bras J (2006) Drug Susceptibility of Plasmodium falciparum Clinical Isolates from Africa using Plasmodium Lactate Dehydrogenase Immunodetection Assay and inhibitory Emax model for precise IC50 measurement. Antimicrob Agents Chemother 50: 3343-3349.

29 Le Nagard H, Vincent C, Mentré F, Le Bras J (2010) Online analysis of in vitro resistance to antimalarial drugs through nonlinear regression. Comput Methods Programs Biomed.

30 Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

31 Batista R, De Jesus Silva Júnior A, De Oliveira AB (2009) Plant-derived antimalarial agents: New leads and efficient phytomedicines. Part II. non-alkaloidal natural products. Molecules 14: 3037-3072.

32 Philippe G, Angenot L, De Mol P, Goffin E, Hayette MP, et al. (2005) In vitro screening of some Strychnos species for antiplasmodial activity. J Ethnopharmacol 97: 535-539.

33 Scholar EM, Pratt WB (2000) The Antimicrobial Drugs. Oxford University Press, UK.

34 Wright CW, Phillipson JD (1990) Natural products and the development of selective antiprotozoal drugs. Phyther Res 4: 127-139.

35 Amponsah SK, Bugyei KA, Osei-Safo D, Addai FK, Asare G, et al. (2012) In vitro activity of extract and fractions of natural cocoa powder on Plasmodium falciparum. J Med Food 15: 476-482.

36 Adeyemi OO, Okpo SO, Ogunti OO (2002) Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana Mill (Lauraceae). Fitoterapia 73: 375-380.

37 Jambou R, Legrand E, Niang M, Khim N, Lim P, et al. (2005) Resistance of Plasmodium falciparum field isolates to in-vitro artemether and point mutations of the SERCA-type. Lancet 366: 1960-1963.

38 Willcox M, Graz B, Falquet J, Diakite C, Giani S, et al. (2011) A 'reverse pharmacology' approach for developing an anti-malarial phytomedicine. Malar J 10: S8.