

***In vitro* Antiplasmodial Activity of Some Medicinal Plants Used in Folk Medicine in Burkina Faso Against Malaria**

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Abstract: The aim of this study was to evaluate the *in vitro* antiplasmodial activities of four plants used in traditional medicine. Hydroethanolic extract, hydroacetic extract and aqueous extract of *Mitragyna inermis* (Willd.) O. Kuntze (Rubiaceae), *Combretum sericeum* G. Don (Combretaceae), *Alternanthera pungens* H.B. and K (Amaranthaceae) and *Ampelocissus grantii* (Baker) Planch (Vitaceae) have been tested *in vitro* against chloroquine-resistant strain (K1) and chloroquine-sensitive strain (3D7) of *Plasmodium falciparum* using pLDH assay. Aqueous extracts exhibited the best results against K1 with the 50% inhibitory concentration (IC₅₀) values of 0.54±0.18, 1.72±0.99, 1.54±0.04 µg/mL for respectively, *M. inermis* leaves, *C. sericeum* leaves and whole plant of *A. pungens*. Hydroethanolic extract from the leaves of *M. inermis* gave also IC₅₀ value of 0.87±0.10 µg/mL with 3D7. Extracts showed antiplasmodial activity against both chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains. Our study justifies the use of these plants in traditional medicine and leads to further investigations.

Key words: Crude extracts, malaria, medicinal plants, *Plasmodium falciparum*, pLDH assay

INTRODUCTION

The use of plants for health care has existed for centuries and still on going nowadays. History reveals that plants have always been considered as an important source of medicine for human being. Phytomedicines have been an integral part of traditional health care system in most parts of the world for thousands of years (Rath *et al.*, 2009). Despite the development of the pharmaceutical industry, more than 80% of the world's populations continue to use herbal remedies (Zirihi *et al.*, 2005) to treat common diseases including malaria, one of the oldest recorded tropical disease which kills about 1.5 million people each year, the majority of them are children (Greenwood *et al.*, 2005). Nowadays malaria chemotherapy is challenged by the emergence of drug resistant *Plasmodium falciparum* (Hyde, 2005). The most affordable agents, particularly chloroquine and sulfadoxine-pyrimethamine are now ineffective because of the widespread *P. falciparum* resistance to these drugs. Artemisinin-based combinations therapies (ACTs) which are recommended by the World Health Organization (WHO) as the first-line treatment of malaria

(Mutabingwa, 2005) are not affordable for poorest people. Indeed, now more than ever, malaria is both a disease of poverty and a cause of poverty (Sachs and Malaney, 2002; Bourdy *et al.*, 2008). For economic and historic reasons, most affected populations have little access to Western medicine and therefore turn towards traditional medicine for their primary healthcare. There is a need to carry out systematic investigations using modern scientific methods to establish the efficacy and toxicity of the medicinal plants commonly used by traditional medical practitioners (Ayuko *et al.*, 2009). These investigations can lead to new chemical compounds active against *P. falciparum* or lead to ameliorated traditional drugs affordable, disposable and cheaper for people.

Medicinal plants typically contain mixture of different chemical compounds that may act individually, additively or in synergy to improve health. A single plant may, for example, contain bitter substances that stimulate digestion, anti-inflammatory compounds that reduce swellings and pain, phenolic compounds that can act as antioxidant and venotonics, anti-bacterial and anti-fungal that act as natural antibiotics, diuretic substances that

enhance the elimination of waste products and toxins and alkaloids that enhance mood and give a sense of well-being (Gurib-Fakim, 2006).

In Burkina Faso where malaria is endemic, several plants are used by traditional practitioners for their antimalarial properties. Some of them such as *Cochlospermum planchonii*, *Cassia alata* and *Crytoplepis sanguinolenta* have been studied and are disposable and used by populations as complementary and alternative antimalarial drugs (Benoit-vical *et al.*, 2003; Appiah, 2009; Bugyei *et al.*, 2010). However, the effectiveness and safety of many plants remain to be validated. In the present study, the main goal is to evaluate the antiplasmodial properties of some medicinal plants used traditionally against malaria.

METHODOLOGY

Parasites strains and *in vitro* culture: Two references *Plasmodium falciparum* strains were used for this study: K1 (chloroquine-resistant strain) and 3D7 (chloroquine-sensitive strain) both obtained from London School of Hygiene and Tropical Medicine (LSHTM), England. Parasites were maintained in continuous culture in the parasitology laboratory of Centre National de Recherche et de Formation sur le Paludisme, using a slightly modified method of Trager and Jensen (1976).

Briefly, parasites were cultured on washed human erythrocytes (blood group O+ obtained from donors) used as host cells, in RPMI 1640 medium supplemented with 0.5% Albumax, 0.05% Hypoxanthine, 2% L-Glutamine (200 mM), 2% N-2-hydroxyethylpiperazine- N-2-ethanesulfonic acid (HEPES 1 M) and 0.5% Gentamicin (10 mg/mL). The culture was performed in 25 cm² sterile flasks containing 5 mL of the culture media with 0.4% hematocrit and were incubated at 37°C in an atmosphere of 93% N₂, 5% CO₂ and 2% O₂ with 90% humidity. Parasitemia was maintained between 1 and 6% and dilutions were done using non-infected O+ erythrocytes when it was upper than 6%. For that, microscopic observations of prepared blood smears fixed with methanol and stained with 5% Giemsa stain were performed daily. Culture medium was also renewed daily. All reagents were provided by Sigma-Aldrich, St. Louis, MO.

Plants collection: Plant parts (leaves and bark of the roots for *Mitragyna inermis* inermis, rhizomes for *Ampelocissus grantii*, whole plant for *Combretum sericeum*) *Alternanthera pungens* and leaves for *Combretum sericeum* were collected in the area of Saponé (Department of Bazèga Province in central Burkina Faso) located at about 45 Km south Ouagadougou, in March 2009. These plants were taxonomically authenticated at the Department of Plant Biology and Ecology of the University of Ouagadougou.

Plant parts were air-dried during 2 weeks in the laboratory under continuous ventilation, away from sun light and dust. The samples were then pulverized into fine powder with a mechanical crusher and the powder was hermetically sealed in polythene bags and stored away from light and moisture until the time of extraction and analysis.

Plant extracts preparation: Acetone-water (70/30) extract and ethanol-water (70/30) extract were obtained as described previously (Zongo *et al.*, 2010). For water extracts, 25 g of each sample boiled in distilled water for 30 min and cooled at room temperature. The extracts were then decanted and filtered through Whatman filter paper N° 1 and the resulting filtrates were concentrated at low pressure with a rotary evaporator (BÜCHI, Labortechnik, Switzerland) and lyophilized. All extracts were stored at 4°C until analysis. Total alkaloids of *M. inermis* were extracted using liquid-liquid partition as described previously (Zongo *et al.*, 2009).

***In vitro* antiplasmodial activity assessment:** Stock solutions of the crude extracts were prepared at 10 mg/mL. Lyophilized water extracts were dissolved in de-ionised water. Hydroacetic and hydroethanolic extracts were dissolved in dimethylsulphoxide (DMSO) and distilled water (1:1 in volume). Total alkaloids were dissolved in 100% DMSO. All stock solutions were sterilized by filtration through 0.22 µm syringe micro-filters (Fisherbrand). Stock solutions of the reference drugs [chloroquine diphosphate (CQ) and Dihydroartemisinin (DHA)] were similarly prepared in sterile de-ionised water at respectively 1600 and 256 nM. The stock solutions were further diluted with culture media to achieve the required concentrations before being tested in culture.

The antiplasmodial assays were carried out on 96-well flat-bottomed micro-culture plates (Costar Glass Works, Cambridge, UK) using the Plasmodium lactate dehydrogenase (pLDH) method (Makler *et al.*, 1993; Kaddouri *et al.*, 2006). Serial two fold dilutions of the extracts and the reference drugs (CQ and DHA) were made in duplicate in the plate. The concentrations of the tested extracts ranged from 50 to 0.78 µg/mL, those of CQ from 800 to 12.5 nM and those of DHA from 12.8 to 0.2 nM. 100 µL of the parasitized blood adjusted at 2% hematocrit with parasitemia between 1.5 and 2% were added to each well containing 100 µL of extracts or drug dilution. Final volume in the wells was 200 µL at 1% hematocrit and around 1% parasitemia. Positive control (parasitized blood only) and negative control (non parasitized blood) were prepared in plate. The plates were incubated at 37°C in an atmosphere of 93% N₂, 5% CO₂ and 2% O₂ with 90% humidity for 72 h. Parasite

Table 1: *In vitro* antiplasmodial activity of the extracts against *Plasmodium falciparum* K1 (CQ-resistant) and 3D7 (CQ-sensitive) strains of by pLDH assay

	IC ₅₀ (µg/mL) ± SD					
	Aqueous extract		70% acetone extract		70% ethanol extract	
	K1	3D7	K1	3D7	K1	3D7
<i>M. inermis</i> (leaves)	0.54±0.18	2.77±0.41	1.98 ±0.26	2.03±0.35	3.02±0.60	0.87±0.10
<i>M. inermis</i> (roots)	ND	5.54±0.93	1.82 ±1.50	2.32±1.32	9.97±4.78	9.97±4.78
<i>C. sericeum</i> (leaves)	1.72±0.99	ND	12.58±5.60	8.52±3.28	7.81±0.57	ND
<i>A. grantii</i> (rhizomes)	5.77±3.50	3.3 6±1.82	20.21±4.71	4.95±0.33	3.47±1.25	8.43±3.27
<i>A. pungens</i> (whole plant)	1.50±0.04	7.53±0.87	ND	ND	17.66±0.02	ND

ND: Not determinate

inhibition was then determined spectrophotometrically at 650 nm, by measuring the activity of the pLDH in control and drug treated cultures, using a microplate reader (BioTek Instrument, USA). At the end of incubation, another 96 well microtiter plate was carefully labeled and filed with 100 µL of the Malstat reagent, 25 µL of NBT/PES (Nitro Blue Tetrazolium/Phenazine Ethosulphate). The cultures were resuspended and 20 µL were transferred into each corresponding well and the plate was incubated in the dark for 10 min before the spectrophotometric assessment. Parasite growth which is correlated with the pLDH activity is considered to be 100% in the positive control wells. The pLDH activity expressed as a percentage of growth for each extracts or drug concentration was calculated using the following formula:

$$\text{Growth (\%)} = (\text{Odext} - \text{Odnc}) / \text{ODpc} \times 100$$

where, Growth % is the percentage of the growth of the parasites in contact with the extract, ODext the Optical Density of each tested well, ODnc the Optical Density of negative control and ODpc the Optical Density of positive control.

The anti-malarial activity of the each extract was then expressed as IC₅₀ [mean±Standard Deviation (S.D)] of two separate experiments performed in duplicate. The data analyzing software, TableCurve 2Dv5 was used for IC₅₀ determination.

Resistance Indexes (RI) for extracts and reference drugs were calculated as (K1 IC₅₀)/(3D7 IC₅₀).

Plant extracts were prepared in the Laboratory of Pharmacology and Biochemistry of the CRSBAN (Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles) and the antiplasmodial assays were performed in Laboratory of Parasitology of The CNRFP (Centre de National de Recherche et de Formation sur le Paludisme). Both centers are in Ouagadougou, Burkina Faso.

RESULTS

The *in vitro* activity of the different extracts from the plants against CQ-sensitive (3D7) and CQ-resistant (K1)

P. falciparum strains are summarized in Table 1. According to criteria established by Deharo *et al.* (2001), an extract can be considered active when its IC₅₀ is less than 5 µg/mL, moderately active when its IC₅₀ is between 5 and 10 µg/mL, inactive when its IC₅₀ is over 10 µg/mL.

The activities obtained (Table 1) indicate that most of our extracts can be classified active against both *P. falciparum* strains. However, 70% ethanol extract from *M. inermis* roots and *A. pungens*, just as 70% acetone extract from *C. sericeum* and *A. grantii* were inactive against *P. falciparum* K1 strain (IC₅₀>10 µg/mL) but have moderate activity towards 3D7 strain (5 µg/mL<IC₅₀<10 µg/mL). All extracts from *M. inermis* leaves were active (IC₅₀ ranged from 0.54 to 3.02 µg/mL). The most active extract was the aqueous extract (IC₅₀ = 0.54 µg/mL)

The IC₅₀ values recorded with *P. falciparum* K1 and 3D7 strains are respectively 304.50±20.50 nM and 74.87±0.69 nM for chloroquine diphosphate (CQ), 2.53±0.53 nM and 1.61±0.48 nM for dihydroartemisinin (DHA) (Table 2). Total alkaloids of *M. inermis* already tested in several previous studies and used here as reference gave 2.02±0.60 µg/mL and 1.61±0.28 µg/mL for respectively K1 and 3D7 *P. falciparum* strains.

Table 3 summarizes the Resistance Indexes (RI) of extracts and reference drugs. All calculated RI were low except those of 70% ethanol extract from *M. inermis* leaves (RI = 3.47), acetone extract of *A. grantii* (RI = 4.08) and CQ (RI = 4.06).

Figure 1 (a-f) shows parasites growth inhibition by extracts. As it can be seen, the curves showed similar growth inhibition by aqueous, hydroethanolic and hydroacetonic extracts.

Table 2: *In vitro* antiplasmodial activity of the reference drugs (CQ and DHA) and total alkaloids of *Mitragyna inermis* against *P. falciparum* K1 (CQ-resistant) and 3D7 (CQ-sensitive) strains by pLDH assay

Reference drugs	IC ₅₀	
	K1	3D7
Chloroquine diphosphate (CQ)	304.50±20.50 nM	74.87±0.69 nM
Dihydroartemisinin (DHA)	3.15±1.00 nM	1.61±0.48 nM
Total alkaloids of <i>M. inermis</i> *	2.02±0.60 µg/mL	1.61±0.28 µg/mL

*: Total alkaloids of *M. inermis* are used here as reference compound

Table 3: Comparison of Resistance Index (RI) of CQ, DHA with the extracts

Plants	Resistance index (RI)		
	Aqueous extract	70% acetone extract	70% ethanol extract
<i>M. inermis</i> (leaves)	0.18	0.97	3.47
<i>M. inermis</i> (roots)	ND	0.78	1.33
<i>C. sericeum</i> (leaves)	ND	1.47	ND
<i>A. grantii</i> (rhizomes)	1.71	4.08	0.41
<i>A. pungens</i> (whole plant)	0.21	ND	ND
CQ	4.06		
DHA	1.96		
Total alkaloids	1.25		

ND: Not determine

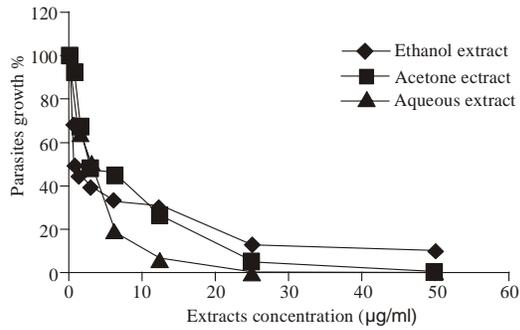


Fig. 1a: 3D7 growth inhibition by *Mitragyna inermis* leaves extracts by pLDH assay

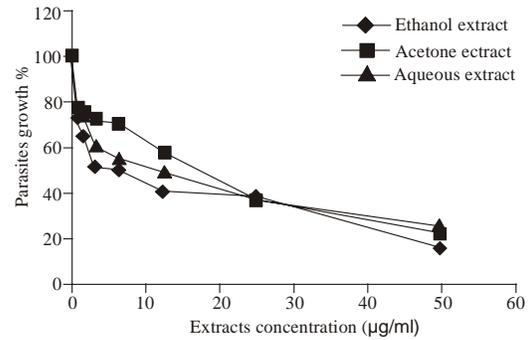


Fig. 1d: K1 growth inhibition by *Ampelocissus grantii* rhizome extracts by pLDH assay

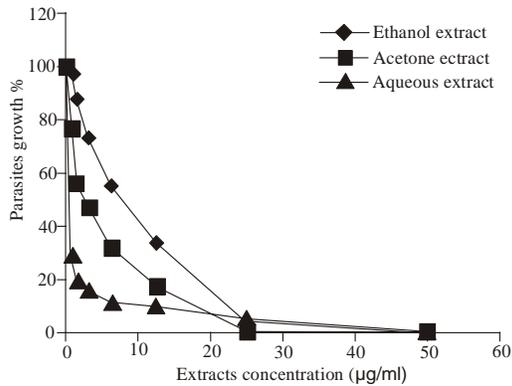


Fig. 1b: K1 growth inhibition by *Mitragyna inermis* leaves extracts by pLDH assay

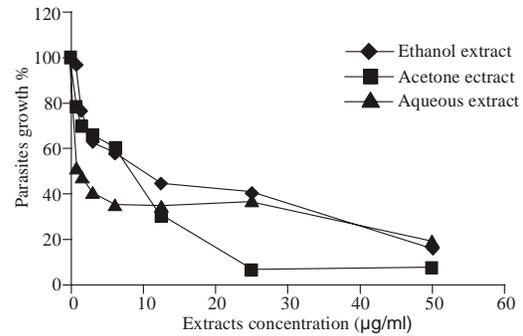


Fig. 1e: K1 growth inhibition by *Combretum sericeum* leaves extracts by pLDH assay

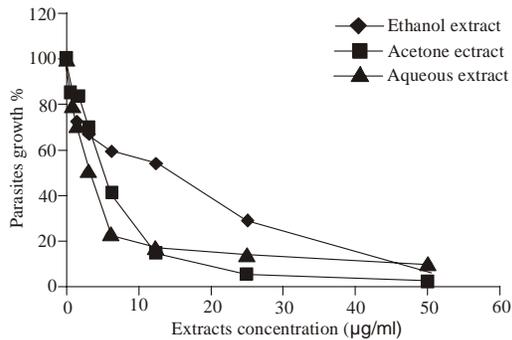


Fig. 1c: 3D7 growth inhibition by *Ampelocissus grantii* rhizome extracts by pLDH assay

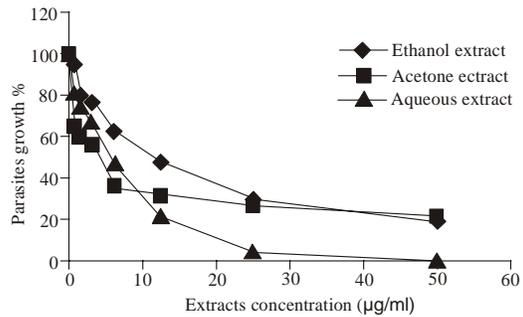


Fig. 1f: 3D7 growth inhibition by *Mitragyna inermis* roots extracts by pLDH assay

DISCUSSION

In Burkina Faso, as in all developing countries, plants are regularly solicited by healers to treat recurrent fever and malaria. In this study, we evaluated the *in vitro* antiplasmodial activities of crude extracts from four African traditional medicinal plants using pLDH assay. According to our results, most of extract including aqueous extracts exhibited good antiplasmodial activity against 3D7 (CQ-sensitive) and K1 (CQ-resistant) *P. falciparum* strains. There were no significant differences in the growth inhibition of the two kinds of parasites (Fig. 1). This suggests that extracts act on resistant parasites like on sensitive parasites. This is confirmed by RI calculation. Indeed, it can be seen from the Table 3 that most of RI values are low (<2), indicating activity of these extracts on CQ-sensitive strains as well on CQ-resistant strains. It may be explained by the fact that crude extracts contain mixtures of different chemical compounds that may act individually, additively or in synergy against parasites. Besides, in order to limit increasing parasite resistance to antimalarial drugs, the World Health Organization (WHO) recommended that all countries experiencing resistance to conventional monotherapies, such as chloroquine, amodiaquine or sulfadoxine-pyrimethamine, should use combination therapies, preferably ACTs (Artemisinin-based Combination Therapies) for *P. falciparum* malaria (WHO, 2001a, b).

IC₅₀ values of CQ, DHA and total alkaloids of *M. inermis* (Table 3) used here as references are not far from those reported in literature in antimalarials assessment. The commonly accepted IC₅₀ on 3D7 is <4 nM (Savini *et al.*, 2010). Previous studies reported IC₅₀ values less than 5 (IC₅₀<5 µg/mL) for total alkaloids (Traore-Keita *et al.*, 2000; Azas *et al.*, 2002). Azas *et al.* (2002) reported 2.4±0.5 µg/mL for W2 (CQ-resistant strain) and 2.11±1.00 for D6 (CQ-sensitive strain). Reference values are very important in a test for this test validation.

Our results show good antimalarial activity for all *M. inermis* extracts with the lowest value of IC₅₀ for aqueous extract (IC₅₀ = 0.54±0.18 µg/mL). This result didn't much with those of Mustofa *et al.* (2000) who reported IC₅₀ values of 40.71±7.21, 44.86±0.93 and 45.49±2.16 µg/mL with aqueous extract of *M. inermis* respectively on FcM29-Cameroon (CQ-resistant strain), FcB1-Columbia (CQ-resistant strain) and Nigerian (CQ-sensitive strain) using the radioactive micromethod of Desjardins *et al.* (1979) with some modifications. In our study, *M. inermis* leaves extracts showed better antiplasmodial activity than roots extracts. For roots extracts, only hydroethanolic extract showed good antiplasmodial activity (IC₅₀= 1.82±1.5 µg/mL for K1 and 2.32±1.32 µg/mL for 3D7). Antimalarial compounds seem to be more concentrated in leaves of this plant than in its roots.

For *C. sericeum*, *A. pungens* and *A. grantii*, their use in traditional medicine for malaria, fever and other diseases treatment has been reported by several ethnobotanical studies (Koné *et al.*, 2004; De Albuquerque *et al.*, 2007; Ayodele *et al.*, 2010; Zirih *et al.*, 2010). Our study reports for the first time their *in vitro* evaluation for antiplasmodial activity. As shown in Table 1, aqueous extracts from *C. sericeum* leaves and *A. pungens* exhibited good antiplasmodial activity on K1 strain (IC₅₀ = 1.72±0.99 µg/mL and IC₅₀ = 1.5±0.04 µg/mL, respectively). The results also indicated that extract from *A. grantii* which showed good antibacterial activity (Zongo *et al.*, 2010) have globally moderate antiplasmodial activity (IC₅₀ value ranged from 3.47±1.25 to 20.21±4.71 µg/mL).

Roughly speaking, the plants tested in this study showed antiplasmodial activity. The classification of the activity as good, moderate and inactive depends on the chosen criteria. For Rasoanaivo *et al.* (2004) the antiplasmodial activities of the extracts are qualified as "inactive" when IC₅₀ value is above 50 µg/mL. It has been noticed that aqueous extracts were more active against parasites. This suggests that most antiplasmodial compounds of these plants are extractible in hot water. Water is the main solvent used in traditional medicine to prepare remedies and most of time, this preparation is made by decoction similarly to what we done in this study.

In conclusion, our study justifies the use of these plants in folk medicine for malaria /fever treatment. This study is a preliminary approach. Further investigations guided on cytotoxicity, acute and chronic toxicity, genotoxicity and LD₅₀ (Dose that kills 50% of experimented animals) determination can lead to efficient, safely, cheaper and disposable enhanced traditional medicines. Bioguided fractionation of these crude extracts can also lead to new active antiplasmodial compounds.

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REFERENCES

- Appiah, A.A., 2009. The Golden Roots of *Cryptolepis sanguinolenta*. In: Juliani, H.R., J.E. Simon and C. Ho, (Eds.), African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality. ACS Sym. Ser., pp: 231-239.

- Ayodele, S.M., G. Alpheus and O.M. Iruaga, 2010. Antibacterial screening of the root, stem and leaf extracts of *Terminalia albida* sc. elliot on selected pathogenic bacteria. Afr. J. Microbiol. Res., 4(13): 1457-1459.
- Ayuko, T.A., R.N. Njau, W. Cornelius, N. Leah and I.O. Ndiege, 2009. *In vitro* antiplasmodial activity and toxicity assessment of plant extracts used in traditional malaria therapy in the Lake Victoria Region. Mem. Inst. Oswaldo Cruz., 104(5): 689-694.
- Azas, N., N. Laurencin, F. Delmas, C. Di Giorgio, M. Gasquet, M. Laget and P. Timon-David, 2002. Synergistic *in vitro* antimalarial activity of plant extracts used as traditional herbal remedies in Mali. Parasitol. Res., 88(2): 165-171.
- Benoit-Vical, F., A. Valentina, B. Da, Z. Dakuyo, L. Descamps and M. Mallié, 2003. N'Dribala (*Cochlospermum planchonii*) versus chloroquine for treatment of uncomplicated *Plasmodium falciparum* malaria. J. Ethnopharmacol., 89(1): 111-114.
- Bourdy, M., L. Willcox, H. Ginsburg, P. Rasoanaivo, B. Graz and E. Deharo, 2008. Ethnopharmacology and malaria: New hypothetical leads or old efficient antimalarials? Int. J. Parasitol., 38(1): 33-41.
- Bugyei, K.A., G.L. Boye and M.E. Addy, 2010. Clinical efficacy of a tea-bag formulation of *Cryptolepis sanguinolenta* root in the treatment of acute uncomplicated falciparum malaria. Ghana Med. J., 44(1): 1-7.
- De Albuquerque, U.P., P.M. De Medeiros, A.L.S. De Almeida, J.M. Monteiro, E.M.F.L. Neto, J.G. De Melo and J.P. Dos Santos, 2007. Medicinal plants of the *caatinga* (semi-arid) vegetation of NE Brazil: A quantitative approach. J. Ethnopharmacol., 114(3): 325-354.
- Deharo, E., G. Bourdy, C. Quenevo, V. Munoz, G. Ruiz and M. Sauvain, 2001. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. J. Ethnopharmacol., 77(1): 91-98.
- Desjardins, R.E., C.J. Canfield, J.D. Haynes and J.D. Chulay, 1979. Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. Antimicrob. Agents Chemother., 16(6): 710-718.
- Greenwood, B.M., K. Bojang, C.J. Whitty and G.A. Targett, 2005. Malaria. Lancet, 365(9469): 1487-1498.
- Gurib-Fakim, A., 2006. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects Med., 27(1): 1-93.
- Hyde, J.E., 2005. Drug-resistant Malaria. Trends Parasitol., 21(11): 494-498.
- Kaddouri, H., S. Nakache, S. Houzé, F. Mentré and J. Le Bras, 2006. Assessment of the drug susceptibility of *Plasmodium falciparum* clinical isolates from africa by using a *Plasmodium lactate dehydrogenase* immunodetection assay and an inhibitory maximum effect model for precise measurement of the 50-percent inhibitory concentration. Antimicrob. Agents Chemother., 50(10): 3343-3349.
- Koné, W.M., K.K. Atindehou, C. Terreaux, K. Hostettmann, D. Traoré and M. Dosso, 2004. Traditional medicine in North Côte d'Ivoire: screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacol., 93(1): 43-49.
- Makler, M.T., J.M. Ries, J.A. Williams, J.E. Bancroft, R.C. Piper, B.L. Gibbins and D.J. Hinrichs, 1993. Parasite lactate dehydrogenase as an Assay for *Plasmodium falciparum* drug sensitivity. Am. J. Trop. Med. Hyg., 48(6): 739-741.
- Mustofa, V.A., F. Benoit-Vical, Y. Péliissier, D. Kone-Bamba and M. Mallié, 2000. Antiplasmodial activity of plant extracts used in West African traditional medicine. J. Ethnopharmacol., 73(1-2): 145-151.
- Mutabingwa, T.K., 2005. Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy. Acta Trop., 95(3): 305-315.
- Rasoanaivo, P., E. Deharo, U.S. Rastimamanga and F. Frappier, 2004. Guidelines for the Nonclinical Evaluation of the Efficacy of Traditional Antimalarials. In: Willcox, M., G. Bodeker, P. Rasoanaivo and J. Addae-Kyereme, (Eds.), Traditional Medicinal Plants and Malaria, CRC Press, Boca Raton, pp: 255-270.
- Rath, S.K., N. Mohapatra, D. Dubey, S.K. Panda, H.N. Thatoi and S.K. Dutta, 2009. Antimicrobial activity of *Diospyros melanoxylon* bark from simlipal biosphere reserve, Orissa, India. Afr. J. Biotechnol., 8(9): 1924-1928.
- Sachs, J. and P. Malaney, 2002. The economic and social burden of malaria. Nature, 415(6872): 680-685.
- Savini, H., J.B. Souraud, S. Briolant, E. Baret, R. Amalvict, C. Rogier and B. Pradines, 2010. Atorvastatin as a potential antimalarial drug: *In vitro* synergy in combinational therapy with dihydroartemisinin. Antimicrob. Agents Chemother., 54(2): 966-967.
- Trager, W. and J.B. Jensen, 1976. Human malarial parasites in continuous culture. Science, 193(4254): 673-675.
- Traore-Keita, F., M. Gasquet, C. Di-Giorgio, E. Ollivier, F. Delmas, A. Keita, O. Doumbo, G. Balansard and P. Timon-David, 2000. Antimalarial activity of four plants used in traditional medicine in Mali. Phytoter. Res., 14(1): 45-47.

- World Health Organization (WHO), 2001a. Antimalarial drug combination therapy: Report of a WHO technical consultation, 4-5 April 2001. Geneva (WHO/CDS/RBM/2001.35). Retrieved from: http://www.rollbackmalaria.org/cmc_upload/0/000/015/082/use_of_antimalarials2.pdf.
- World Health Organization (WHO), 2001b. The use of Antimalarial Drugs. Part II: 1.8 Artemisinin and its derivatives. Retrieved from: http://www.rbm.who.int/cmc_upload/0/000/014/923/am2_1-8.htm.
- Zirihi, G.N., L. Mambu, F. Guédé-Guina, B. Bodo and P. Grellier, 2005. In vitro antiplasmodial activity of 33 West African plants used for treatment of malaria. *J. Ethnopharmacol.*, 98(3): 281-285.
- Zirihi, G.N., K. N'guessan, E.T. Dibié and P. Grellier, 2010. Ethnopharmacological study of plants used to treat malaria, in traditional medicine, by Bete Populations of Issia (Côte d'Ivoire). *J. Pharm. Sci. Res.*, 2(4): 216-227.
- Zongo, C., O.E.F. Akomo, A. Savadogo, L.C. Obame, K. Jean and S.A. Traore, 2009. *In vitro* antibacterial properties of total alkaloids extract from *Mitragyna inermis* (Willd.) O. Kuntze, a West African traditional medicinal plant. *Asian J. Plant Sci.*, 8(2): 172-177.
- Zongo, C., A. Savadogo, L. Ouattara, I.H.N. Bassole, C.A.T. Ouattara, A.S. Ouattara, N. Barro, J. Koudou and A.S. Traore, 2010. Polyphenols content antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae), a medicinal plant from Burkina Faso. *Int. J. Pharmacol.*, 6(6): 880-887.