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Antiplasmodial activities of leaves and trunk bark of *Sclerocaria Birea* (A. Ric H.) Hochst (Anacardiaceae), a plant used in traditional medicine of Niger

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In the crude extracts of the bark and leaves of *Sclerocarya birrea*, have been characterized sterols, triterpenes, saponosides, tannins, anthocyanosides, coumarins, reducing compounds, alkaloids and carotenoids. Tests were carried out *in vitro* with extracts from each part of the plant to assess their efficacy against strains of *Plasmodium falciparum* sensitive to chloroquine K1 and that resistant to chloroquine 3D7. The crude alkaloidal extracts of the bark gave IC50 = 2.54 µg / ml with the strain 3D7 and an IC50 = 4.09 µg / ml with the strain K1. On the other hand, the extracts with CH_2Cl_2 showed an IC50 = 36.59 and 37.78 µg / ml respectively with the 3D7 and K1 strains. Those with CH_3OH gave IC50 all greater than 50 µg/ml with both strains. The CH_3OH / H_2O extracts gave IC50 = 21.48 µg / ml with the K1 strain and greater than 50 µg / ml with 3D7. As for the H_2O extracts, the IC50 were = 11.43 µg / ml with the K1 strain and also greater than 50 µg / ml with the 3D7. The alkaloid extracts of leaf gave an IC50 = 9.68 µg / ml with 3D7 and = 3.56 µg / ml with strain K1. With CH_2Cl_2, and IC50 = 6.62 µg / ml was obtained with 3D7 and 4.05 µg / ml with strain K1. The CH_3OH extracts gave an IC50 = 21.12 µg / ml with the 3D7 strain and 21.06 µg / ml with the KI strain. The CH_3OH / H_2O extracts gave with strain 3D7 and IC50 of more than 50 and 32.73 µg / ml with K1. The aqueous extracts gave IC50 greater than 50 µg / ml for 3D7 and 25.17 µg / ml with the K1 strain.

Key words: Medicinal plants, Niger, *Sclerocarya birrea*, Malaria, Antiplasmodial activity, Alkaloids, Dichloromethane.

INTRODUCTION

Malaria is the deadliest parasitic disease caused by a single-celled parasite named Plasmodium. It is now more than a public health problem, but a development one. Niger is no exception, because malaria is the first reason

for consultation in health centers (PNLP, 2018). Indeed, the WHO in its 2020 report indicated that malaria remains a major public health problem worldwide with an average of 229 million cases and 405,000 cases of death reported each year (OMS. Rapport de l'Organisation Mondiale, 2019). Also it emerged from the study carried out in 2019 by the Annual Report of WHO-Niger Representation Office 2.825.329 confirmed malaria cases and 4.106 death principally children under the age of five (OMS-Niger, 2019).

The main antimalarial drugs widely used nowadays are derivatives of quinine and artemisinin. Unfortunately, the development of the parasite's resistance to available drugs challenged the effort to combat malaria, so the search for new active molecules has become a necessity. On the other hand, according to WHO, traditional medicine is still the main recourse for 80% of people in developing countries (Global Malaria Programme: WHO Global, 2019).

Traditional medicine, an element of cultural heritage, is therefore a promising source of investigation for new molecules discovery. This motivated us to conduct our research on this topic. The aim of this studyis to contribute to malaria elimination through the use of antimalarial medicinal plants from the traditional pharmacopoeia of Niger. It consisted of evaluating in vitro the antiplasmodial properties of the trunk bark and leaves of Sclerocaria birrea (A. Ric h.) Hochst (Anacardiaceae).

MATERIALS AND METHODS

Among the plants identified during ethnobotanical investigation in June 2010 at the market of Katako,Niamey, *Sclerocarya birrea* was selected for this work, because of his very large use in Niger for the treatment of malaria and fevers.

Plant collection

The plant was collected in Bougoum, a locality located 25 km west of Niamey, in the Tillaberi Region. This species is found there in abundance. The physical reconnaissance of the plant in the field and the harvesting of its different parts were carried out under the assistance of a technician from the Plant Biology Laboratory of the Abdou Moumouni University (UAM) of Niamey. The harvested organs were transported to the said laboratory where they were washed and dried away from dust and sunlight, and then crushed with a laboratory mortar.

Preparation of raw extracts

Ten gram of powder of each raw material were used for maceration by successive extractions for 16 h with 100 ml of dichloromethane, then with methanol and methanol/water. The extract obtained with dichloromethane (DCM) was filtered and concentrated after total evaporation. The methanol and methanol/water extracts were concentrated dry under vacuum and then freeze-dried with the BRAND freeze dryer. Crude aqueous extracts were made by putting 10 g of plant powder in 100 ml of boiling water for 30 min. After cooling, the solution was filtered and then concentrated under vacuum using the rotary evaporator and frozen at -20°C for 24 h and then freeze-dried.

The total alkaloid extract was obtained as follows: the raw material powder (10 g) is basified with 28% ammonia at pH 9 and then macerated with dichloromethane in a chromatography column for 16 h. The organic phase obtained after elution (100 ml) was concentrated and subjected to liquid-liquid extraction with 3% H_2SO_4 until pH 2-3. The aqueous phase was alkalized again with 28% ammonia up to pH 9 to 10 and extracted again with dichloromethane. Extracts of total alkaloids were thus obtained after concentration. All extracts were then weighed to determine their yield.

Characterization of chemical groups

Phytochemical screening

Phytochemical screening of these extracts was done according to the Ciulei (1982) method for the characterization of the chemical groups. Hydrolyzed and non-hydrolyzed extracts were prepared from the crude extracts.

One gram of each lyophilisate was mixed with 100 ml of distilled water then settled in a bottle to obtain non-hydrolyzed extracts. 25 ml of the non-hydrolyzed extract solutions were added to 15 ml of a 10% HCl solution. The mixture was refluxed for 30 min. After cooling, the hydrolyzed solutions were transferred to a separating bulb and then extracted with dichloromethane (3 × 10 ml). The organic phases were collected and dried with anhydrous sodium sulfate. After filtration on Wattman paper, the extracts were concentrated under vacuum. Thus were used the following tests:

(i) the Shibata test for flavonoids;
(ii) the Bornsträger reaction for the f anthracenosides;
(iii) the Liebermann-Burchard test for triterpene and steroidal aglycones;
(iv) the Dragendorff and Mayer test for alkaloids;
(v) the Carr and Price reaction for carotenoids;
(vi) NH4OH 10% for coumarins
(vii) Fehling Liquor test for carbohydrates;
(viii) The soda pellet test for anthocyanosides;
(ix) The test of the foam index for saponosides;
(x) The $FeCl_3$ test for polyphenols (tannins).

Thin layer chromatography (TLC) characterization

Five microliters of hydrolyzed and non-hydrolyzed extracts were deposited on TLC plates (aluminum support; silica gel adsorbent (CAMAG lot 990220). The plates were dried at room temperature and then placed in the migration tanks containing the eluant. The distance to be covered by the solvent is 8 cm from the line of the spots. The removed plates were dried and then read with UV (254/366 nm) and revealed with a reagent specific to each chemical group.

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Revelations

Coumarins: Eluant: n-hexane-ethyl-toluene acetate (6:2:2 v/v/v); developer: a solution of KOH (1N), UV lamp (254 or 366 nm). The appearance of blue or green spots indicated their presence.

Steroidique and / or triterpenic glycosides: Eluant: n-hexane-toluene- ethyl acetate (3: 1:2 v / v / v). Revelation with the mixture solution of anisaldehyde sulfuric acid, concentrated sulfuric acid and ethanol (0.5:5:85 v/v/v) after drying the plates at 100°C for 5 min. Tetra cyclic triterpenes and phytosterols gave a purple coloration turning blue.

Penta cyclic triterpenes were identified by persistent purple coloration

Saponosides: Eluant: mixture chloroform - glacial acetic acid-methanol-water (16:8:3:2 v/v/v/v). Eluent : sulfuric anisaldehyde, concentrated sulfuric acid and ethanol (0.5:5:85 v/v/v); after drying the plates at 100°C for 5 min, brown or purple spots are observed.

Flavonoids: Eluent: mixture toluene, acetone and formic acid (5:5:1 v/v/v). Revelation under UV at 366 nm; solution of diphenylborinate-ethanol-ethylglycol 4000 (100 mg: 50 ml: 400 mg) with an appearance of spots of yellow or yellow-orange color.

Tannins: Eluent: mixture of toluene-acetone-formic acid (5:5:1 v/v/v). Revelation by a 2% ferric chloride solution in 80% ethanol (1: 1 v / v); appearance of spots of blue coloration for hydrolyzable tannins (gallic), greenish for catechic (condensed) tannins.

Alkaloids: Eluent: mixture of toluene-ethyl acetate-diethylamine (7:2:1 v/v/v). Draggendorf's reactive developer.

Emodols and anthracenosides: Eluent: mixture of n-hexane-ethyl acetate-toluene (3:1:1 v/v/v). Revealation: a solution of KOH (1N), appearance of red or orange-yellow spots.

Bioassays

The biological material consisted of strains of *Plasmodium falciparum*; a species responsible for the majority of malaria cases in sub-Saharan Africa (Sanon et al., 2013). The chloroquino-resistant *P. falciparum* strains K1 and Chloroquino-sensitive 3D7 strains were used. K1 strains were supplied by the London School of Hygiene and Tropical Medicine, London, United Kingdom (LSHTM) in London (England). The 3D7 strains were supplied by the NMIMR Laboratory (Nuguchi Memorial Institute on Medical Research) (Ghana). They were kept in continuous culture in human blood at the Pharmacology Laboratory of the National Centre for Research and Training on Malaria (CNRFP) in Ouagadougou, Burkina Faso.

Continuous cultivation of parasites *in vitro*

The strains were maintained in continuous culture using a simplified culture technique for Plasmodium falciparum according to Trager and Jensen (1976). The renewal of the culture medium was carried out every 24 h accompanied by the control of parasitaemia.
The parasites were thawed and cultured in vials containing Complete Culture Medium of RPMI 1640, L glutamine 2%, Timpon Hepes 2% Gentamicin 0.5% Albumax 5% and hypoxanthin 9.5%. The vials containing the culture were gasified with the mixed gas composed of (2% O_2, 5% CO_2, 93% N_2). The vials were then incubated in the CO_2 incubator at 37°C under conditions allowing maximum growth of parasites. After the renewal of the culture medium, the parasitaemia was controlled by the making of blood smears. When parasitaemia reached 6%, a subculture was made using new unparasitized blood of blood group A+.

Evaluation of antiplasmodial activity *in vitro*

The evaluation of antiplasmodial activity was made by the technique of pLDH. The products to be tested consisted of plant extracts and reference products, Dihydroartemisinin and Chloroquine (DHA and QC) solubilized in dimethylsulfoxide (DMSO) or distilled water. Beforehand, the solutions of the extracts were prepared at the concentration of 10 mg /ml.

After dilutions to 1/100, the concentrations were reduced to 100 µg/ml. The tests were performed on 96-well plates filled with a fixed volume of parasitized red blood cells (2% parasitaemia). The fractions to be tested (at different concentrations) were then added in duplicate in the wells. After 72 h of incubation, plates homologous to the test plates were prepared and the different substrates and coenzymes were added to them. The MALSTAT solution (160 ml of distilled water, 200 ml of Triton X100, 2 g of L-Lactato, 0.66 g Trizma base, 66 mg APAD (3-acetylpiridine adenine dinucleotide), at pH 9 was distributed for a volume of 100 mµl / cupula.

To this solution, 25 mµl of the NBT / PES solution (100 ml of distilled water, 160 mg of NTB and 8 mg of PES) were added to each cup and 20 µl of blood of the test plate were added to each cup including positive and negative controls. After 10 min of incubation, the plates were immediately read to the spectrophotometer at a wavelength of 650 nm. The IC_{50} values were determined graphically by the percentage of inhibition of parasite growth as a function of the positive control. The IC_{50} of the different extracts obtained were analyzed according to the criteria of Deharo et al. (2001).

RESULTS

Phytochemical screening

In the bark sterols, triterpenes, saponosides, tannins, coumarins, anthocyanosides, flavonoids, reducing compounds and alkaloids have been detected (Table 1 and 2). In the leaves the same compounds as those of the barks were found with in addition carotenoids. TLC tests were used to confirm or disprove liquid media tests.

Results of *in vitro* antiparasitic tests

Figures 1 to 6 represent the growth inhibition curves of strains 3D7 and K1 by the active extracts of the plant (Table 3).

DISCUSSION

Phytochemical characterizations

Sterols, triterpenes, saponosides, tannins,

Table 1. Screening of chemical groups.

Solvent	Chemical groups	Plant	
		S. birrea B	***S. birrea L***
CH_2Cl_2	Sterols and Triterpens	+	**+**
	Aglicones flavoniques	-	**-**
	Emodols	+	**-**
	Alkaloids	+	**+**
	Coumarines	+	**-**
	Carotenoïdes	-	**+**
MeOH	Phenols (Tannins)	+	**+**
	-Alkaloids	+	**+**
	-Reducing compounds	+	**+**
	-Saponosides	+	**+**
	-Glycosides sterols and Triterpenes	+	**+**
	-Flavonoids	-	**-**
	-Anthracnoses	-	**-**
	-Coumarin derivatives	+	**+**
	-Anthocyanidins	+	**+**
MeOH /H_2O	Phenols (Tannins)	**+**	**+**
	-Alkaloids salts	**-**	**-**
	-Reducing compounds	**+**	**+**
	-Saponosides	**+**	**+**
	-Glycosides sterols and Triterpenes	**+**	**+**
	-Flavonosides	**-**	**-**
	-Anthracenosides	**-**	**-**
	-Coumarin derivatives	**+**	**-**
	-Anthocyanosides	**+**	**+**

(+): Presence: (–) : not detected; (nr): not researched.

Table 2. Chemical groups sought in extracts by decoction.

Solvent	Chimical groups	Plants	
		S. birrea B	***S. birrea L***
H_2O	**-**Polyphenols (Tannins)	**+**	**+**
	-Alkaloids salts	**+**	**+**
	-Reducing compounds	**+**	**+**
	-Saponosides	**+**	**±**
	-Sterols and Triterpenes	**±**	**±**
	-Flavonosides	**-**	**-**
	-Anthracenosides	**-**	**-**
	-Coumarin derivatives	**+**	**+**
	-Anthocyanidins	**+**	**+**

(+): Presence; (–) : not detected; (nr): not researched.

anthocyanosides, coumarins and reducing compounds were found in both the trunk bark and the leaves of *S. birrea*. Virginie et al. (2016) highlighted polyphenols, tannins, coumarins, flavonoids, triterpenes, and phytosterols as the main compounds of this plant. These same authors announced that *S. birrea* is has anti-

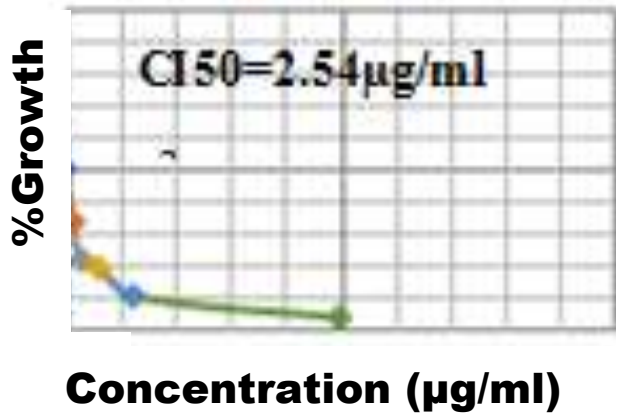

Figure 1. Curves of inhibition of the growth of strains by the alkaloid extract of the barks of *S. birrea* (Stump 3D7).

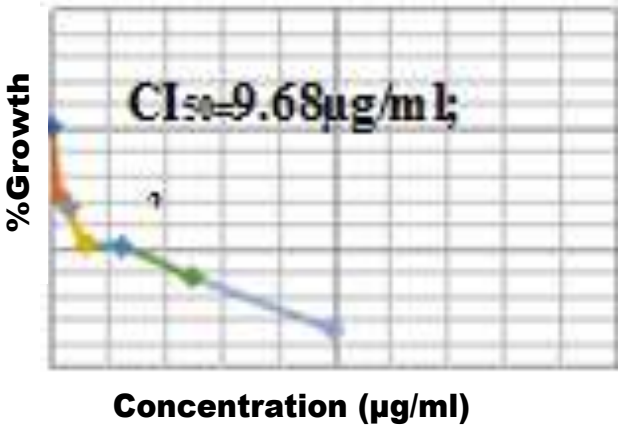

Figure 3. Inhibition curves of growth of strain K1 by leaf extracts of *S. birrea* (alkaloid extracts).

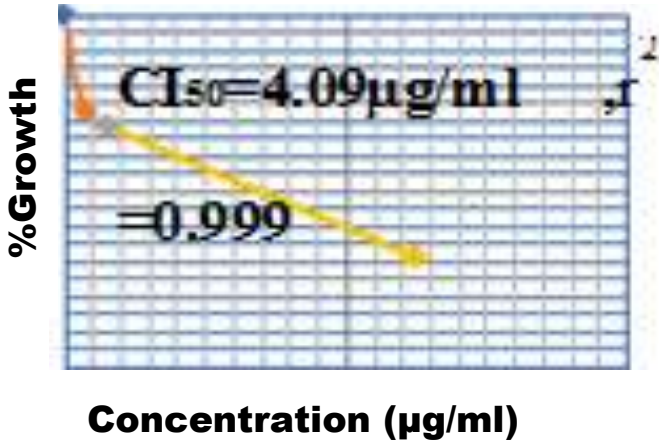

Figure 2. Curves of inhibition of the growth of strains by the alkaloid extract of the barks of *S. birrea* (Stump K1).

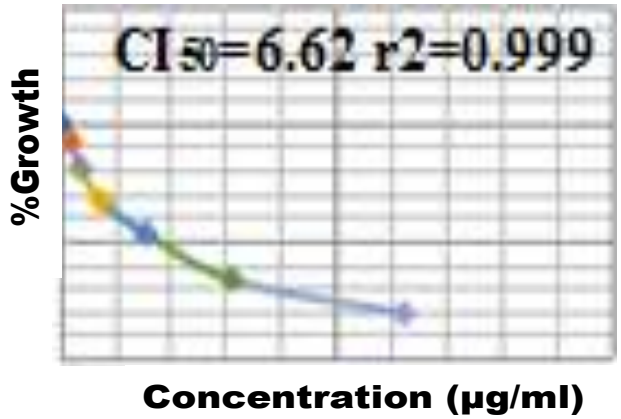

Figure 4. Inhibition curves of growth of strain K1 by leaf extracts of *S. birrea* (DCM extract).

diarrheal, antidiabetic, anti-inflammatory, antimicrobial, antiplasmodial, antihypertensive, anticonvulsant and antioxidant pharmacological properties.

The results obtained with trunk bark joined those of other authors. Indeed, Nacoulma (1996) had found tannins, sterols, saponosides and alkaloids in the trunk bark of *S. birrea*. Previous studies had shown that saponosides are endowed with antiplasmodial activities (Nassirou et al., 2015).

Furthermore, Ojewole et al, 2010 had found the same compounds in the trunk bark of *S. birrea* except for the saponosides that were obtained in the case of our study. This can be explained by the edaphic conditions of the harvesting area. The results corroborate those obtained by Fotio et al. (2009). These authors also attributed anti-inflammatory properties to methanol and aqueous extracts from the trunk bark of *S. birrea*. Asres et al.

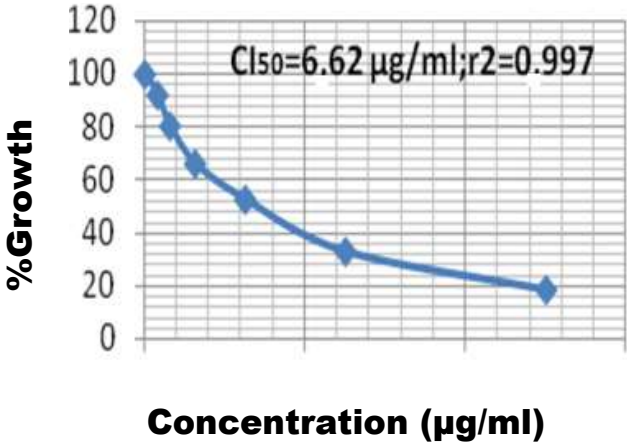

Figure 5. Growth inhibition curves of strain 3D7 by extracts of *S. birrea* leaves (alkaloid extracts).

Table 3. Antiplasmodial activities: IC_{50} values of extracts on strains 3D7 and K1.

Plant species (parts used)	Strain	Crude extracts (µg/ml)					Standard product (nM)	
		DCM	MeOH	MeOH/H_2O	H_2O	Alk. Ext	CQ	DHA
Sclerocarya birrea (B)	3D7	36.59	>50	21.48	>50	**2.54**	13.33	2.31
	K1	37.78	>50	>50	11.43	**4.09**	612.28	3.82
Sclerocarya birrea (L)	3D7	**6.62**	21.12	>50	>50	**9.68**	80.22	1.90
	K1	**4.05**	21.06	32.73	25.17	**3.56**	101.84	0.62

B= bark; L= leaves, DCM= dichloro-methane, CQ= Chloroquine, DHA= Dihydroarthemisinine.

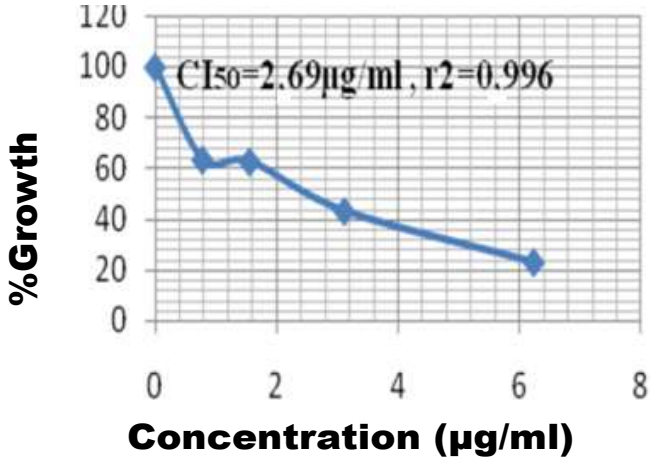

Figure 6. Growth inhibition curves of strain 3D7 by extracts of *S. birrea* leaves (DCM extracts).

(2001) attributed antiplasmodial activity to tannins present in *Combretum molle*. TLC confirmed the presence of the chemical groups identified by the general characterization tests in liquid media. The presence of alkaloids in both parts of the plant may explain their antiplasmodial activity. Flavonoids not detected by the liquid characterization test in the leaves and bark of the plant were revealed by the CCM. This result showed that the characterization tests have limits, because depending on the concentration of the compounds present in the extracts.

Antiplasmodial activities

The crude extracts tested their antiplasmodial activity on the reference strains K1 and 3D7. Table 2 shows that the total alkaloids extracts of the bark have very good antimalarial effects on strains K1 and 3D7 with an IC50 of less than 5 µg / ml (Figures 1 and 2). DCM extracts and leaf alkaloids were also identified as moderately active on strain K1 with IC5O greater than 5 and less than 10 µg/ml, (Figures 3 and 4). On strain 3D7, the alkaloid extracts of the leaves were also moderately active (Figure 5). On the other hand, the DCM extract was very active on the same strain (Figure 6).

In addition, aqueous, methanol and methanol/water extracts from leaves and bark were noted inactive on both strains with IC50s over than 10 µg/ml (Table 3). When we consider the criteria established by Deharo et al. (2001), the best antimalarial activities were obtained with the alkaloid extracts from the bark and the DCM extracts and alkaloids from leaves. Then our study showed a good antiplasmodial activities against the both of two parasites strains such as demonstrated previously study of Soma et al. (2017) in Burkina Faso and Hout et al. (2006) in Cambodia.

The inhibition of the growth of parasites could be due to a possible synergistic effect of the phytochemicals of the DCM extracts, possibly sterols-triterpenes, flavonoids, anthracenosides on which previous studies have demonstrated their antiplasmodial properties. The antiplasmodial properties of *S. birrea* were reported by several authors including Ojewole et al. (2010), Gathirwa et al. (2008). The antiplasmodial activity mentioned above about the two parts of the plant would partly justify their use in traditional medicine in Niger.

Some authors demonstrated the antiplasmodial activity of alkaloids, triterpenes and flavonoids in West Africa medicinal plants selected on the basis of their use in the treatment of malaria and or fever (Ouattara et al., 2014; Serge et al, 2012; Traoré et al., 2009). These plants include *Esenbeckian febrifuga* (Rubaceae), *Lisianthus speciosum* (Gentianaceae) and *Tachia quianensis* (Gentianaceae). Also the antipyretic, often analgesic and anti-inflammatory properties of sterols and triterpenes have been reported (Bruneton, 1993). Chloroquine IC50 attests to the sensitivity of strain 3D7 and the resistance of strain K1. The IC50 values obtained by the DHA ranged from 0.62 to 3.82 nM. This confirmed the validity of the tests carried out (Sanon et al., 2003).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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