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Assesment of antipasmodial activity of *Cassia siamea* extracts and its pures compounds

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Abstract
Introduction: In order to contribute to the development of new strategies to fight against malaria, we evaluated the antiplasmodial activity of extracts, fractions and molecules of *Cassia siamea* on schizonts of *Plasmodium falciparum*.
Materials and Methods: *Cassia siamea* sterm bark petroleum ether, DCM, ethanol and water extracts sequentially prepared were subjected to bioguided fractionaton by TLC and HPLC coupled to mass on schizonticidal activity on the chloroquinoresistant strain of *P. falciparum* (FcM29 from Cameroon). The MTT test on the KB and Vero cells allowed to evaluate the cytotoxicy of the active ingredients.
Results and Discussion: Fractions Fc (IC50 = 5 ± 1.0 µg / ml) and Fe (IC50 = 1.5 ± 0.98 µg / ml); and molecules P 2 (1.22 ± 0.56 µg / ml), P18 (0.4 ± 0.07 µg / ml), P27 (0.1 ± 0.02 µg / ml) were significantly the most active. These antiplasmodial active principles are not cytotoxic and belong to the groups of alkaloids and triterpenoids.
Conclusion: These results justify the tolerance and use of this plant against malaria in traditional medicine in Congo.

Keywords: *Cassia siamea*, extracts, molecules, activity, schizont, cytotoxicity

1. Introduction

Malaria remains a plague of great importance, with about 1.5 billion people at risk worldwide. The majority of malaria-related clinical cases and deaths occur in areas of high P. falciparum transmission in sub-Saharan Africa (Basco *et al*, 1999, Pradines *et al*., 2003) [2, 18]. It is a public health problem in more than 90 countries, inhabited by about 40% of the world's population (WHO, 2002). Africa accounts for more than 90% of malaria-related deaths (WHO, 2002) [24]. Malaria also has significant economic impacts in endemic areas. In Africa, it is estimated to cost US $ 12 billion in loss of GDP produced each year, this correspond to 40% of all public health expenditure (Sachs *et al*. 2002) [21]. This situation is more dramatic as the strategies developed by states or intergovernmental organizations and NGOs did not benefit the majority of the population at risk, because of the cost too high. To compensate this failure, populations resort to traditional herbal preparations for which safety and efficacy are not scientifically standardized. In Africa, the use of plants plays an important role in the treatment of malaria (Clark *et al*., 1996, Benoit-Vical *et al*., 1998) [3, 5]. A rationalization of the use of antimalarial medicinal plants is very useful.
The development and spread of *Plasmodium falciparum* resistance to most modern antimalarials in general are the main factors behind the worsening of the current malaria situation (Ndounga *et al*., 2008) [13]. In addition active antimalarial drugs nowadays will certainly become inactive in the future; therefore it is necessary to develop new drugs, either to combine with existing ones to minimize the rate of onset of resistance, or to replace drugs that become ineffective on *Plasmodium falciparum*. This study evaluates the antiplasmodial activity of *Cassia siamea* extracts, fractions and molecules in asexual stages of plasmodium.

2 Material and methods

2.1 Plant Material

Stem bark and leaves of *Cassia siamea* Lam were collected in Mindouli (Pool, Congo) in May 2007. This harvest was carried out on the basis of information given by traditional healers. The plant was identified by botanists of the Centre d'Etudes sur les Ressources Végétales (CERVE), Brazzaville-Congo. A specimen of this plant was deposited in the herbarium of the Botanical Laboratory of CERVE under the number 169 P. Sita 1981.

2.2. Methods

2.2.1 Survey on the uses of *Cassia siamea* in traditional medicine in Brazzaville

Twenty (20) traditional healers were interviewed using a questionnaire on the various medicinal preparations based on *Cassia siamea* as well as the diseases concerned by these preparations and their mode of administration (Nsonde

Ntandou *et al*., 2005) [14].

2.2.2 Phytochemical study

The phytochemical study consisted to identify the different chemical families of this plant before to prepare extracts, to divide up and isolate their different molecules in order to identify the active ingredients.

2.2.2.1 Phytochemical extraction

Extraction was performed at room temperature. 1100 g of stem bark powder was successively macerated with 4 solvents of increasing polarity (petroleum ether, chloroform, ethanol and water), in order to obtain the maximum of compounds. Each maceration was carried out by keeping the sample in 5 L of solvent. The operation were repeated three (3) time for a total of 48 hours corresponding to each type of prepared extract. After filtration and concentration, the following yields were obtained: 0.62% CSE 1 (6.84 g extracted with petroleum ether), 0.92 % CSE 2 (9.67 g extracted with chloroform), 0.8 % CSE 3 (8.59 g ethanol extract). The mash resulting from the maceration with ethanol was again used for extraction with boiling water for 10 minutes at 100°C. The aqueous solution obtained was cooled for 4 h and then filtered before being centrifuged (30 min, 7000 g) and finally lyophilized to give a dry extract 1.1% CSE4 (26 g extracted with water). CSE1 and CSE2 were solubilized in DMSO, while CSE3 and CSE4 were solubilized in distilled water. The ethanol extract of the leaves (CSF) was prepared by maceration of 20 g of leaf powder in 50 ml of alcohol at 90 ° for 48 h, the operation was repeated twice to have a yield of 10%.

2.2.2.2 Identification of chemical groups by tube reactions

The traditional phytochemical tests for the detection of the chemical families present in plants long used for Congolese plants by Bouquet have been use (Nsonde Ntandou *et al*., 2005) [14].

2.2.2.3 Splitting and isolation

a) **Preparative plate chromatography:** The most used supports are silica in normal phase (sometimes reversed). This type of separation is used for the purification of the compounds. Preparative plates (silica gel 60, F254, 0.2 × 20 × 20 cm, Merck) were used. The deposition of 30 mg of sample is carried out by an automatic depositor (Camag automatic TLC sampler 4).

b) **High Performance Liquid Chromatography (HPLC):** HPLC analyzes were carried out with 40 g on pre-filled silica column using a Merck Hitachi Lachrom HPLC chain equipped with 7100 Lachrom pump, UV and DEDL diode array detector (Merck Hitachi 7455 Lachrom) driven by software D-7000 HSM (Merck) with a flow rate of 17 ml / min for a variable gradient in the ratio % A (H2O) /% B (ACN) respectively at the rate of (0% / 100%) at t = 0 min, (95% / 5%) at t = 20 min, (0% / 100%) at t = 30 and 35 min. The chromatograms of the aqueous extract were carried out in reverse phase on a Sunfire HPLC column (9 mm × 350 mm) with a flow rate set at 1 ml / min. The analytical and preparative HPLCs were carried out on Sunfire-type reverse phase columns (9 mm × 350 mm) under the same conditions as simple HPLC (Budzikiewicz *et al*., 1976) [4].

c) **Thin layer chromatography (TLC):** Thin-layer chromatography is used to monitor purifications and to verify presence and degree of purity of the products studied (Khan *et al*., 2005) [8]. These analyzes are carried out in normal phase on aluminum plates covered with silica (Silicagel 60 F254, Merck) and dehydrated in an oven. These analyzes concern extracts. The migration solvent used is dichloromethane and the developing reagent is p-anisaldehyde. A storage solution was prepared with each organic extract. 10 mg of each of three organic extracts studied (CSE1, CSE2 and CSE3) were dissolved in 1 ml of its preparation solvent. Using a micropipette 10 µl of each storage solution were removed and deposited on a silica gel plate (60 F254 Merck) with an aluminum support. These plates were then placed in an oven set at 37 ° C for drying until complete evaporation of the traces of the solvent, before to be placed in the development tanks in glass, containing the eluent. Then, we observed the migration of the eluting solvent resulting in the substances contained in the extracts of the plant at various speeds. Then these plates were removed from the tanks as soon as the front of the solvent had reached about 10 cm; then dried again in an oven set at 37 ° C. The revelation was carried out by p-anisaldehyde which makes it possible to characterize several chemical groups. The aqueous extract CSE4 could not be visualized correctly in the different chromatographic conditions because it does not migrate. The analysis was performed by HPLC C18 (reverse phase). TLCs were analyzed in visible light and U.V. (254 and 356 nm), before and after revelation with p-anisaldehyde. The different spots observed, are the characteristics attesting the existence of chemical substances present in the various extracts of the plant. At the end of this analysis, fractions having the same chromatographic profile were grouped together.

2.2.3 Studies associating phytochemistry and pharmacology

The bio-guided fractionation and isolation of the extracts consisted of complementary integration of phytochemical fractionation and pharmacological study in vitro of schizonticidal activity to guide separation, purification and isolation, with a view to select fractions and active chemical compounds against the chloroquine-resistant strain of *Plasmodium falciparum* (FcM29) / Cameroon. For this strain, the IC50 value for chloroquine is 0.1 M (Frappier *et al*., 1996) [6]. To fractionate the extracts and isolate certain molecules, the following techniques were employed: flash chromatography, preparative HPLC, preparative TLC, precipitation and crystallization. 150 mg of CSE1 was placed in a mixture of dichloromethane and methanol (20/80, v / v). We observed the formation of a precipitate. After filtration, the solid phase (70 mg) and the liquid phase (56 mg) obtained were subjected to chemical fractionation by application of the following tests: flash chromatography, HPLC, TLC. From the solid phase it was possible to isolate P1 to P9 molecules. While from the liquid phase the P10 to P12 molecules were

isolated with F1 to F4 fractions. 150 mg of CSE2 subjected to chemical fractionation by the use of flash chromatography, HPLC and TLC allowed the isolation of molecules from P13 to P25 with fractions F14 to F23. The fractionation of the 150 mg of CSE3 was carried out on the use of a single technique, flash chromatography thanks to which fractions ranging from F24 to F38 were obtained. The 150 mg of CSE4 was first passed on polyamine in order to eliminate the tannins.

After this removal, preparative HPLC isolated the molecules from P26 to P29. After TLC analysis coupled to mass spectroscopy, the fractions and molecules having the same chromatographic profiles were grouped as follows: Fa (F1,2,3,4,5,6) = 10 mg; Fb (F8,12,23,24,25,28,29,32) = 20 mg; Fc (F 13.18, 20.37.38) = 8mg); Fd (F22, 27, 31, 33, 34) = 7mg; Fe (F7.8,9,10,11,21,30,35,36) = 17 mg); Pa (P1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29) = 34 mg; Pb (P18.20) = 5 mg; Pc (P2) = 5 mg. The pure molecules (lupeol, betulinic acid, betulin, Beta-sitosterol, emodin and apigenin) identified and already isolated in this plant were provided by Dr. Jean Théophile Banzouzi to be evaluated for their antiplasmodial activity (Kagamate *et al*., 2014) [7].

2.2.4 Pharmacological study

2.2.4.1 Study of schizonticidal activity on *P. falciparum in vitro*

This study consisted in evaluating the antiplasmodial activity of extracts, fractions and isolated molecules of *Cassia siamea* bark on the *P. falciparum* isolates of patients and on the FcM 29 reference strain from Cameroon.

a) **Collection of isolates:** A sterile sample of 2 to 5 ml of the patient's blood was made on EDTA using a vacutainer. Patients with uncomplicated malaria were selected at Centre de Santé Intégré de Tenrikyo Integrated, located in the southern district of Brazzaville (Congo). In this area, the prevalence of chloroquine-resistant of *Plasmodium falciparum* malaria was very high (Ndounga *et al*., 2007) [13]. The sample taken was immediately placed at 4 ° C until the cultivation took place within a maximum of 8 hours later than the time at which the sample was taken.

b) **Preparation of culture medium:** The standard medium is RPMI 1640 (Ruswell Park Memerial) powdered with L-glutamine, without bicarbonate (Gibco) at a dose of 10.40 g per 1 liter of water. 8 g of powdered HEPES were dissolved in 900 ml of bidistilled water. The resulting mixture was made up to 960 ml. The whole mixture was then magnetically stirred for 2 hours in the ambient atmosphere to ensure complete dissolution. A solution of NaHCO3 at 5 g / ml was prepared extemporaneously. From this latter solution, 4.2 ml was taken to be added to the first mixture. The solution obtained was first sterilized by addition of 10 µg / l of gentamicin. The second sterilization was performed by membrane (0.22 µm) filtration. The medium obtained were stored at 4 ° C. At the time of use, the medium was each supplemented with 0.5% of albumax and then filtered with a membrane (0.22 µm).

c) **Preparation of the suspension of red blood cells:** Blood was centrifuged for 10 min at 2000 rpm to separate the red blood cells from the plasma. The red cell pellet was subsequently washed twice with pure RPMI 1640 for 10 min by centrifugation at a speed of 2000 rpm. The third wash was done as the first two with the only difference that the latter uses a complete medium (RPMI + albumax). In cases where the initial parasitaemia was greater than 1%, the sample was diluted with red blood cells from a Group A blood donor, having taken no antibiotic or anti-malarial for a month, in order to reduce parasitaemia in the range of 0.5 to 1%. The red cell suspension required for a plate was 20 ml of a 1.5% hematocrit suspension (Rai *et al*., 1984) [19].

d) **Performing the test on isolates:** The aqueous extracts and reference products were solubilized in DMSO 4%. Extracts stock solutions were prepared at a concentration of 20 mg / ml. The chloroquine diphosphate powder from the Sanofi-aventis laboratory was used for the preparation of 1.5 mg / ml of stock solution. The quinine powder from the Sanofi-aventis laboratory was used to prepare 1.5 mg/kg of stock solution. The culture was conducted according to the technique described by Trager and Jensen (1976).Tests were carried out in 96-well flat bottom plates (Costar) with lids. The last dilution was made directly in the plate by placing 50 µl of the initial dilution of the product to be tested in the first two rows of wells No. 1 and No. 2, and then 50 µl of complete culture medium (RPMI-10% albumax) in row 2 to row 12. Culture was subjected to a ½-½ dilution from row 2 to row 12. Each concentration was tested in two wells. The rows of wells n° 11 and n° 12 served as controls without product. After diluting, we added 200 µl of the suspension of parasitized red blood cells.

This culture was made in an atmosphere depleted of oxygen and enriched in CO_2.To do this, we placed the microtitration plate in a desiccator containing water on its bottom and equipped with a tap. After placing a lighted candle and adjusting the lid, the tap was closed, the candle automatically extinguished after consumed oxygen and produced CO_2. The desiccator was then placed in a regular oven and set at 37 ° C. After 42 hours of incubation, the plate was removed from the incubator and the recovered red blood cell pellet was conditioned as a thick drop. After drying and staining with Giemsa, the schizont count was performed in each well compared to the control wells using an electric microscope. The results were presented as inhibitory concentration 50 (IC 50). Five isolates were tested during this study.

e) Performing the test on the reference strain: FcM29: The products were tested on chloroquine-resistant strains of *Plasmodium falciparum* FcM29 / Cameroon. For this strain, the IC50 value of chloroquine is 0.1 M (Frappier *et al*., 1996) [6]. Antiplasmodial activity was determined using the isotopic method of Desjardins *et al*. (1979) [19].

The drugs to be tested were dissolved in DMSO at a concentration of 10 mg / ml and serially diluted with the culture medium in a 96-well costar microplate (Koukouikila Koussounda, 2012) [9], before to add an asynchronous culture of parasites (1% of the final parasitaemia to 1% hematochritis). Plates were maintained for 24 hours at 37 ° C. Then 0.5 µCi of hypoxanthine [^{3}H] added to each well. The parasites were further incubated for more than 24 hours. Inhibition of growth of each product was expressed as IC50. The concentration was determined by comparing the radioactivity incorporated in the treated groups with those of control cultures (Mbatchi *et al*., 2006) [11].

2.2.4.2 Cytotoxicity study

The cytotoxicity of the extracts was evaluated on KB cells (cancer cells of the human epidermis) and Vero (cells of the African green monkey kidney). These cells are cultured in DMEM (modified Dulbecco culture medium) enriched with 25 mM glucose, 10% (v / v) fetal calf serum, 100 IU penicillin, 100 µg / ml streptomycin and 1.5 µg / ml of fungizone, all stored under 5% CO2 at 37 ° C (Lekana Douki *et al*., 2013) [10]. Plates of 96 wells were seeded at a rate of 600 cells per well in 200 µl of culture medium. After 24 hours, plant extracts dissolved in DMSO at a concentration of 10 mg / ml are added to the cells and left for 72 hours at the final concentration of 1% in a fixed volume of DMSO. Each extract is tested three times. The witnesses received an equivalent volume of DMSO. After 2 h of incubation with the MTS reagent (Promega, Madison, WI), the number of viable cells is determined by measuring the optical density of each well at 490 nm with a spectrophotometer, then the percentage inhibition of cell growth is calculated. The reference used is the taxotere at the concentration of 2.5x10-10 M.

2.3. Statistical analysis

Data collected was analyzed using dose-response curves, probit analysis. Some of the results were expressed with 95% confidence intervals. Statistical significance was calculated using an analysis of variance (ANOVA). Significant differences were determined by the use of the Duncan's multiple-range test. Values of $p < 0.05$ were considered significant.

3. Results

3.1 Uses of *Cassia siamea* in the Congolese pharmacopoeia

The result of this investigation showed that leaves, bark and roots are the three organs involved in these preparations. The decoction, the maceration and the infusion are the different modes of traditional preparations based on this plant. This plant is used against the following pathologies and symptoms: malaria, fever, various pains, edema, urogenital infections, influenza infection, HIV / AIDS, gastrointestinal disorders (Table 1).

3.2 Phytochemical study

Cassia siamea bark extracts contain mainly triterpenes and polyphenols followed by saponins, tannins, and alkaloids (Table 2). The chromatographic profile on TLC shows that CSE1, CSE2, CSE3 extracts have almost the same types of chemical constituents (Figure 1). The results of the HPLC analysis of the different extracts show both similar and distinct peaks (Figure 2). We can notice the similarity of the profiles for CES1 and CES2, this confirms the results obtained by CCM.

Table 2

Secondaries metabolites	Estimated content
Polyphenols	+ + +
terpenoids	+++
Saponines	+ +
Tannins	+ +
Alkaloids	+

+++: Strong presence
++: Average presence
+: Low presence

Table 1: Use of *Cassia siamea* Lam in traditional medicine in Brazzaville

Symptoms	Organs	Preparation	Instructions for use	Frequency
Fever	Leaves	Aqueous decoction or infusion	½ glass per os, 3 times/day	7
	Barks/roots	Aqueous decoction or infusion	½ glass or chewing a few quantity per os, 3 times/day	10
Pain	Barks/roots	Aqueous decoction or maceration	½ glass or chewing a few quantity per os, 3 times/day	10
	Leaves	Aqueous decoction or infusion	½ glass per os, 3 times/day	4

Flu (influenza)	Barks/roots/leaves	Aqueous decoction or maceration	½ glass or chewing a few quantity per os, 3 times/day	4
HIV/AIDS	Barks/roots	Aqueous decoction or maceration	½ glass or chewing a few quantity per os, 3 times/day	7
	Leaves	Aqueous decoction or infusion	½ glass per os, 3 times/day	4
Malaria	Leaves	Aqueous decoction or infusion	½ glass per os, 3 times/day	8
	Barks	Aqueous decoction or infusion	½ glass or chewing a few quantity per os, 3 times/day	8
Urogenital Infections	Leaves	Décoction et infusion aqueuses	½ glass per os, 3 times/day	6
	Barks/roots	Aqueous decoction or maceration	½ glass or chewing a few quantity per os, 3 times/day	7

n = 20 peoples interviewed

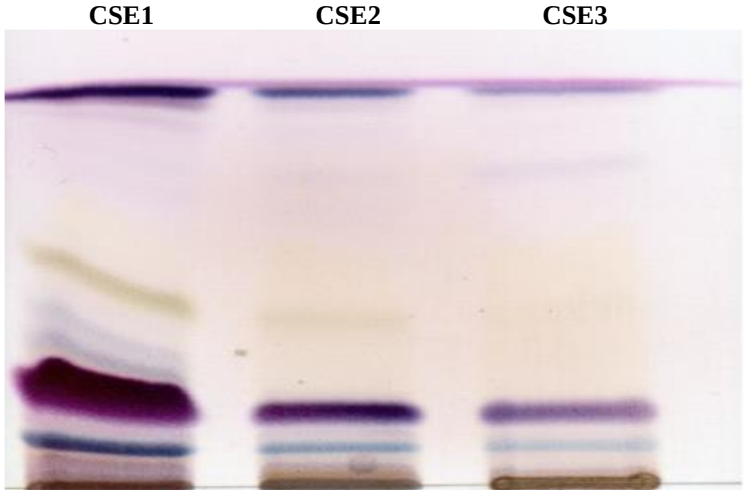
3.3 Cytotoxic study

The values relating to the cytotoxicity study are presented in Table 3. None of the 4 extracts or schizanticide molecules is cytotoxic. Extracts CSE3 and CSE4 are completely devoid of toxicity against KB and Vero cell lines.

Table 3: Cytotoxicity of extracts and molecules on KB and Vero cell lines

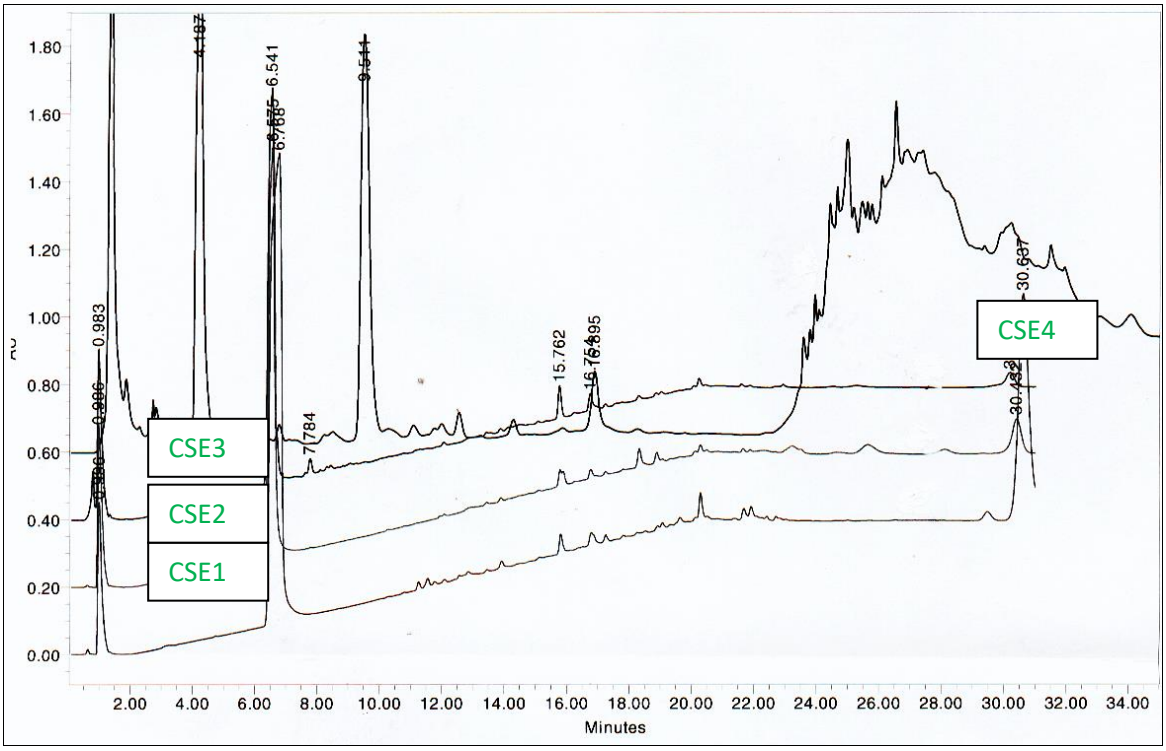
Products	LD50 µg/ml	
	KB	Vero
CSE1	72,0±4,3	56,9±
CSE2	67,0±6,1	58,1±5.8
CSE3	> 100	> 100
CSE4	> 100	> 100
P2 et P27	> 100	> 100
P18	> 100	> 100
Taxotere	0.00017	0.01

n = 5
KB: cancer cells of the human epidermis
Vero: African green monkey kidney cells
CSE 1: bark extract of *Cassia siamea* with petroleum ether;
CSE 2: *Cassia siamea* bark extract with dichloromethane;
CSE 3: *Cassia siamea* bark extract with ethanol;
CSE4: *Cassia siamea* bark extract with distilled water;
P: molecule.



CSE 1: bark extract of Cassia siamea with petroleum ether;
CSE 2: Cassia siamea bark extract with dichloromethane;
CSE 3: Cassia siamea bark extract with ethanol.

Fig 1: Photo of CCM plate of *Cassia siamea* organic extrac



CSE 1: bark extract of Cassia siamea with petroleum ether;
CSE 2: Cassia siamea bark extract with dichloromethane;
CSE 3: Cassia siamea bark extract with ethanol.

Fig 2: HPLC analysis profiles of *Cassia siamea*

3.4 Study of schizonticidal activity

Table IV shows antiplasmodial effect of the chemical compounds of *Cassia siamea*. Fractions Fc (IC50 = 5 ± 1.0 µg / ml) and Fe (IC50 = 1.5 × 0.98 µg / ml); and P 2 (1.22 × 0.56 µg / ml); P18 (0.4 ± 0.07 µg / ml) and P27 (0.1 ± 0.02 µg / ml) molecules were the most active.

Table 4: *In vitro* antiplasmodial activity of *Cassia siamea* extracts, fractions and molecules

Type of products	Products	IC50 µg/ml on *P. falciparum* isolats	IC50 µg/ml on FcM 29 strain
Extracts	CSE1	7,48±0,73	34 ± 0,050
	CSE2	8,8±0,76	11,5 ±0,09
	CSE3	0,4±0,06	18,2 ± 0,10
	CSE4	18,3±1,1	≥ 50
Reference medicines	Chloroquine	0,280 ±0,02	0,175 ± 0,01
	Quinine	0,300 ±0,07	0,240 ± 0,5
Unidentified molecules	P_a	/	50 ± 2,20
	$P_{5, 17}$	/	12, 5 ± 0,80
	P_2	/	1,22 ± 0, 56
	$P_{18 \text{ et } 27}$	/	0,4 ± 0, 07
	P_{27}	/	0,1± 0, 02
Identified molecules	Lupeol	/	29 ± 5,00
	Betulinic acid	/	1,5± 0,90
	Betulin	/	10± 2,80
	Beta-sitosterol	/	12± 1,70
	Emodin	/	5± 1,55
	Apigenin	/	12± 2,54

	Stigmasterol	/	14± 3,00
	Fisetin	/	8± 1,85
	Coumarin	/	7± 2,40
	Beta-amarin	/	1,8± 0,50
Fractions	F_a	/	40 ± 1,20
	F_b	/	20 ± 1,81
	F_d	/	10 ± 0,96
	F_c	/	5 ± 1,0
	F_e	/	1,5 ± 0,98

n = 5, FcM 29: chloroquine-resistant *Plasmodium falciparum* strain from Cameroon
CSE 1: bark extract of *Cassia siamea* with petroleum ether;
CSE 2: *Cassia siamea* bark extract with dichloromethane;
CSE 3: *Cassia siamea* bark extract with ethanol;
CSE4: *Cassia siamea* bark extract with distilled water
P: molecule and F: fraction.

4. Discussion

This study helps to identify the different pathologies for which traditional preparations of *Cassia siamea* are used in Congo in general, more particularly in Brazzaville. As in many African countries, this plant is used in traditional medicine against malaria, various pains, intestinal parasitosis, inflammations, HIV / AIDS, influenza and genitourinary infection. This work confirms the results of ethnobotanical studies already conducted by many authors and highlights for the first time the use of this plant in the management of HIV / AIDS in traditional medicine. *Cassia siamea* is administered per os, in Congo, Benin, Togo and Sierra Leone to treat malaria, hepatic insufficiency and fever (Mbatchi *et al*., 2006; Nsonde *et al*., 2005) [11, 14]. These uses are not only related to the plasmodium parasite, but also to symptomatic forms not specific to malaria. This justifies the analgesic, anti-inflammatory, antiviral and nutritive properties highlighted in extracts or molecules isolated from *Cassia siamea* (Kamagaté *et al*., 2014; Nsonde Ntandou *et al*., 2016; 2017) [7, 13, 17].

Five main types of secondary metabolites were found in this plant. They are triterpenes, polyphenols (flavonoids and tannins), saponins and alkaloids. In these different chemical groups, we have isolated lupeol, betulin and betulinic acid in the triterpenes family; apigenin and fisetine in the group of flavonoids; stigmasterol and beta sitosterol in the group of phytosterols; emodin in the group of antraquinones and coumarin (Nsonde Ntandou *et al*., 2015; 2017) [14, 17].

Evaluating the antimalarial activity of a plant is complex and requires many analytical tools. In the case of this study, the activity of the plant was demonstrated by the in vitro test of extracts, bioguide fractions and isolated molecules against *P. falciparum*. It is true that many plants do not respond positively to this type of test, but still have activity against malaria (plants treating the symptomatology of the disease or potentiating other drugs). Nevertheless, the simplicity, the reproducibility and the objectivity of the in vitro tests allow a rational selection of the active products. In this work, the standard test for *P.falciparum* in vitro only concerns the exoerythrocytic cycle of the parasite. This test cannot also allow the demonstration of a pharmacological activity, the expression of which requires the intervention of parameters directly dependent on the vertebrate host such as, biotransformation (for prodrugs), bioavailability and genetics.

In vitro bioguidance proved to be essential during this work. This technique, which integrates phytochemistry and biology in a complementary way, not only led to separation, purification and isolation, but also to the selection of fractions and active chemical compounds against the chloroquine-resistant strain of *Plasmodium falciparum* (FcM29). However, it is important to point out that bioguidance has certain limitations that need to be clarified here in order to better interpret the results obtained. For molecules that have synergistic effects (activities against the parasite on the same metabolic pathway) or potentiation effects (activities against the parasite on different and complementary metabolic pathways), the purification of such compounds does not lead to a linear concentration of pharmacological activity. This purification sometimes involves manipulations denaturing the active principles under the effects of heat, the pH of the medium and the volatility of certain chemical compounds.

During this study, the ethanolic extract of the bark of this plant was most effective on *P.falciparum* isolates collected from patients (IC50 = 0.04 µg / ml); the Fe fraction (IC50 = 1.5 µg / ml) as well as the P2 molecules (IC50 = 1.2 µg / ml), P18 (IC50 = 0.04 µg / ml) and P27 (IC50 = 0.01) are very active on the FCM 29 reference strain of *Plasmodium falciparum*. These results confirm the preliminary studies conducted on this plant (Mbatchi *et al*., 2006, Morita *et al*., 2007) [11, 12]. This effect is explained by the presence of alkaloids. Indeed, in this chemical group the team of Morita (2008) [14] isolated and elucidated the chemical structure of a *Cassia siamea* leaf alkaloid, cassiarine A. This molecule has an IC50 = 0.005 µg / ml on *Plasmodium falciparum*. The antiplasmodial effect can also be explained by the presence of

the triterpenes which are part of the majority chemical groups in *C.siamea* bark extracts and include, among others, betulin (IC50 = 12 µg / ml), betulinic acid (IC50 = 1.5), beta-amarin (IC50 = 1.8 µg / ml) and P18, P2 and P27 molecules for which chemical structures have not been determined. Finally, this effect could also be related to the presence of anthraquinones. Emodin, which is one of the anthraquinones of *Cassia siamea*, has an IC50 = 5µ / ml on FcM29 and D6 of *Plasmodium falciparum*, which are two reference strains respectively from Cameroon and Nigeria (Ajaiyeoba *et al*., 2008) [1].

5. Conclusion

This work made it possible to know the different uses of *Cassia siamea* and demonstrated a schizonticidal effect of *Cassia siamea* on *Plasmodium falciparum*. This effect is thought to be due to many metabolites that act alone or in synergy. We also understood that this plant is also used against non-specific symptoms of malaria which could justify its use in several pathologies.

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7. References

1. Ajaiyeoba EO, Ashidi JS, Okpako LC, Houghton PJ, Wright CW. Antiplasmodial compounds from *Cassia siamea* stem bark extract, Phytotherapy Research. 2008; 22:254-255.
2. Basco LK, Ringwald P. Molecular epidemiology of malaria in Yaoundé, Cameroon Analysis of the omega repetitive region of the *Plasmodium falciparum* Cg2 gene and chloroquine resistance. American. Journal of Tropical Medicine and Hygiene. 1999; 61:807-813.
3. Benoit-Vical F, Valentin A, Pellesier Y, Mallier M, Bastide J M. Journal of Ethnopharmacology. 1998; 61:173-178.
4. Budzikiewicz H, Djerassi C, Williams DH. Interpretation of mass spectra of organic compounds. Holden Day, London. 1998, 14.
5. Clark C, Key SW. WHO releases revised facts on malaria, Malaria Weekly. 1996; 15(8):7-9.
6. Frappier F, Jossang A, Soudon J, Calvo F, Rasoanaivo P, Rastimamanga-Urveg S *et al*. Bisbenzylisoquinoline as modulators of chloroquine resistance in *Plasmodium falciparum* and multidrug resistance in tumor cells. Antimicrobial Agents and Chemotherapy. 1996; 6:1476-1481.
7. Kamagaté M, Koffi C, Kouamé NM, Akoubet N' guessan A, Yao AR, Die Kakou HM. Ethnobotany, phytochemistry, pharmacology and toxicology profiles of *Cassia siamea* Lam. The Journal of Phytopharmacology. 2014; 3(1):57-76.
8. Khan M, Shingare MS, Siddiqui AR, Angadi SS, Maske PV, Yahya M *et al*. TLC-colourimetric estimation of free and combined forms of chrysophanol, emodin, and physcione in some *Cassia* species. Indian Journal of Pharmaceutical Sciences. 2005; 67(6):767-770.
9. Koukouikila-Koussounda F, Abena AA, Nzoungani A, Mombouli JV, Ouamba J-M, Kun J *et al*. In vitro evaluation of antiplasodial activity of extracts of *Acanthospermum hispidium D.C. (*Asteraceae) and *Ficus thonninga* Blume (Moraceae), two plants used in traditional medicine in the republic of Congo. Afr J Tradit Complement Altern Med. 2013; 10(2):270-276.
10. Lekana-Douki JB, Bongui JB, Oyegue Liabagui SL, Zang Edou SE, Zatra R, Bisvigou U *et al*. In vitro antiplasmodial activity and cytotoxicity of nine plants traditionally used in Gabon. Journal of Ethnopharmacology. 2011; 133:1103-1108.
11. Mbatchi SF, Mbatchi B, Banzouzi JT, Bansimba T, Nsonde-Ntandou GF, Ouamba J-M *et al*. In vitro antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. Journal of Ethnopharmacology. 2006; 104(1-2):168-174.
12. Morita H, Oshimi S, Hirasawa Y, Koyama K, Honda T, Ekasari W *et al*. Cassiarins A and B, novel antiplasmodial alkaloids from *Cassia siamea*. Org Lett. 2007; 9(18):3691-3693.
13. Ndounga M, Mayengue PI, Tahar R, Casimiro PN, Matondo MDW, Miakassissa-Mpassi V *et al*. Efficacy of sulfadoxine-pyrimethamine, amodiaquine, and sulfadoxine-pyrimethamine-amodiaquine combination for the treatment of uncomplicated *Plasmodium falciparum* malaria in the urban and suburban areas of Brazzaville (Congo). Acta tropica. 2007; 103(3):163-166.
14. Nsonde-Ntandou GF, Ndounga M, Ouamba JM, Gbeassor M, Etou-Ossibi A, Ntoumi F *et al*. Enquête ethnobotanique: screening chimique et efficacité thérapeutique de quelques plantes utilisées en médecine traditionnelle à Brazzaville. Phytotherapia. 2005; 1:13-18.
15. Nsonde Ntandou GF, Bassoueka DJ, Banzouzi JT, EtouOssibi AW, Elion Itou RDG, Makambila MC *et al*. Assessment of *Cassia siamea* stem bark extracts toxicity and stability in time of aqueous extract analgesic activity. African Journal of Pharmacy and Pharmacology. 2015; 9(41):988-994.
16. Nsonde Ntandou GF, Bassoueka DJ, Banzouzi JT *et al*. *Cassia siamea* lam extracts analgesic mechanism of action and pharmacodynamic interaction with paracetamol (acetaminophen) European J of Res in Medical Sci. 2016; 4:1-13.

17. Nsonde Ntandou GF, Makambila MC, Banzouzi JT, Ouamba J-M, Abena AA. Central Mechanism of Analgesic Effect of The Aqueous Extracts of *Schwenckia Americana* and Lupeol Its Main Isolated Active Molecule. Sci Fed Pharmaceutics Journal. 2017; 1(1):1-12.
18. Pradines B, Vial H, Olliaro P. Prophylaxie et traitement du paludisme : problèmes, récents développements et perspectives. Med. Trop. 2003; 63:79-98.
19. Rai CAN, Sivaraman CA. Simple techniques for in vitro culture of *Plasmodium falciparum* by the candle jar method. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1884; 78:137-141.
20. Robert ES. Gametocytes and sexual develpement in: Malaria; parasitology, Pathogenis, and Protection. Edited ASMPress, Washington U.S.A, D.C. 1998, 25-44.
21. Sachs J, Malaney P. *Nature* (London). 2002; 415:680-685.
22. Sriwilaijaroen N, Kelly JX, Riscode M, Wilairat P. Cyquant cell proliferation assay as a fluorescence-based method for in vitro screening of antimalarial activity. Southeast Asian Journal of Tropical Medicine of Public Health. 2004; 35:840-844.
23. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976; 193:673-675.
24. WHO. Report on infectious diseases, removing obstacles to healthy development, World Health Organization, Geneva, Switzerland. 2002,10.