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Research Article

IN VITRO INHIBITORY ACTIVITY AGAINST PLASMODIUM FALCIPARUM SEXUAL AND ASEXUAL STAGES OF MEDICINAL PLANTS USED IN BURKINA FASO

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ABSTRACT

Air-dried and ground plant leaves and stem barks were extracted with water, ethanol and methanol and assessed for their activities on Plasmodium falciparum asexual and sexual erythrocytic stages. Thus, a screening against late gametocyte stages at the dose of 100 µg/ml was initially performed to select the most active extracts for IC50 determination. A luciferase assay was used for measuring gametocytes stage IV-V viability while the plasmodium lactate deshydrogenase (pLDH) assay was performed for the asexual stages. The ethanolic extract of Lophira lanceolata stem barks showed the best inhibition with IC50 = 8.08 ± 5.14 µg/ml against stage IV-V gametocytes of P. falciparum. Leaves of Terminalia macroptera, Combretum collinum, Argenome mexicana and barks of Zanthoxylum zanthoxoides exhibited a light inhibitor activity against late stage gametocytes with an IC50 < 50 µg/ml. Tested on the asexual stages, leaves of Terminalia macroptera, Terminalia avicennioides and barks of Zanthoxylum zanthoxyloides appeared to be very effective (IC50 ≤ 5 µg/ml) while the leaves of Anogeissus leiocarpus and Argemone mexicana were moderately active (5 < CI50 ≤ 10 µg/ml). This study provides scientific evidence for usage of plants in the traditional medicine to treat malaria. More interesting, the evidence of transmission blocking property of some of them sustaining further investigation for the bioactive compounds isolation with multistage activity.

INTRODUCTION

According to WHO (2016), malaria remains the second cause of death in Africa, after HIV/AIDS and the fifth from infectious diseases worldwide after respiratory infections, HIV/AIDS, diarrhoeal diseases, and tuberculosis. More than 90% deaths occurred in Africa and were due to *Plasmodium falciparum*, despite the use of artemisinin-based combination therapy (ACT) coupled with other preventative measures such as the use of insecticide treated nets (1). Among the current antimalarial drugs targeting the *Plasmodium* parasite asexual blood stages which are responsible for the clinical pathology of malaria, very few are active against the early gametocytes (stage I-III) and nearly all are inactive against the mature gametocyte or sexual mosquito stages that are responsible for the transmission. Thus, a screening of 44 common antimalarial compounds tested showed that only eight (Artesunate, hydroxychloroquine, pentamidine, pyronaridine, NPC1161B, OZ277, Methylene Blue, Thiostrepton) reduced significantly mature gametocyte viability by at least 50%; the remaining 36 compounds include the currently used anti-malarial drugs chloroquine, doxycyclin and quinine, had no activity in their study (Christopher *et al*. 2012). Gametocyte carriage persisted for an estimated period of more than one month after the initiation of treatment that resulted in the apparent clearance of asexual parasites (2). If gametocytes are not cleared in a patient during a standard antimalarial treatment course, such persistent reservoirs of parasites are available for continued re-infection of mosquitoes and thus can sustain malaria transmission well after clinical symptoms have been abated (3).It is now recognized that understanding and attacking the parasites responsible for infection of the mosquitoes is critical to the international efforts striving to eliminate malaria. Considering the current level of knowledge of anti-malarials targeting the asexual parasites in human blood and the constraints of drug development, one ongoing strategy to

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identify transmission-blocking drugs is to screen known blood schizonticides against late stage gametocytes (4). Medicinal plants could be another pathway for transmission blocking drugs discovering as the high blocking activity found previously with meen-azal, Vernolide and verdonalol, three compounds coming from *Azadirachta indica* and *Vernonia amygdalina* (5–7). Very recently, works conducted on eight traditional South African plants and Ghana herbal medicines showed a nice antigametocyte property of some plants which could be candidates for malaria multidrug discovering (8,9).
This study aimed at assessing the schizonticidal activity and screening of a series of commonly used local anti-malarial plants for potential gametocytocidal effect. Here we report the results of different extracts of eighteen medicinal plant materials tested for the activity on sexual and asexual stages of *Plasmodium falciparum*.

MATERIALS AND METHODS

Plant materials collection and extracts preparation

Eighteen plant materials from twelve investigated medicinal plants were selected according to previous data from an ethno-botanical survey conducted in Banfora, western Burkina Faso. The schizonticidal activity of some of them has been published (10–13). The selected plants were identified and authenticated by a botanist from the Comoé regional department of forestry and confirmed on www.theplantlist.org. A voucher specimen of each plant was deposited in the herbarium of "Centre National de Recherche et de Formation sur le Paludisme in Burkina Faso CNRFP" (Ouagadougou). Plant materials as leaves and stem barks, for a total of 18 parts (table1), were harvested on January 2015 in Banfora and dried under the laboratory conditions (temperature: 25°C, humidity level: 30-85%) for two weeks. After drying, each part was pulverized using a food blender and stored in a clean container.

Crude organic extracts were prepared using plant powder (50 g) by maceration for 24 h in 500 ml of methanol or ethanol. The extracts were filtered and the residue was re-extracted twice under the same conditions to ensure complete extraction. Combined extracts were filtered and evaporated to dryness under reduced pressure at 60°C with a rotary evaporator. Dry extracts were placed in dark bottle, and stored at 4 °C until further analysis. Aqueous extracts were prepared by boiling at 100°C, 50 g of plant powder in 500 ml of distilled water for 30 minutes. After cooling, solutions were filtered on cotton wool and freeze-dried with lyophilisator.

Plasmodium falciparum parasite cultures

Plasmodium falciparum cultures of asexual stage were set up according to the method of Trager and Jensen (14), with minor modifications. The *P. falciparum* strains used in this study are CQ sensitive 3D7 and CQ resistant W2. Parasites were maintained in culture at 5% hematocrit (human type A-positive red blood cells for W2 and O-positive red blood cells for 3D7) in RPMI 1640 medium containing 24 mM sodium bicarbonate (EuroClone; Celbio), with the addition of 0.01% hypoxanthine, 20 mM HEPES, and 2 mM glutamine at 37°C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. W2 strain was cultured in the presence of 1% Albumax II (lipid-rich bovine serum albumin) and the 3D7 strain in the presence of 10% naturally clotted heat-inactivated O^- human serum (Interstate Blood Bank, Inc.) which is needed for gametocyte production. For the drug susceptibility assays, 1% Albumax II was used for asexual parasites of both strains and human serum for gametocytes. For a normal in vitro growth of the parasite, the parasitemia was maintained within 1% and 5% (the number of infected RBC with respect to the total number counted) and evaluated through Giemsa stained smears.

Gametocytogenesis was triggered as previously described (15). The strain 3D7elo1-pfs16-CBG99, genetically modified to express luminescence with luciferase in gametocytes, was used for these experiments (16). In this case, the medium contains 10% naturally clotted heat-inactivated O^- human serum (Interstate Blood Bank, Inc.), which ensures constant and high gametocyte production, instead of Albumax as for the asexual stages culture. Briefly, *P. falciparum* 3D7elo1-pfs16-CBG99 asexual parasite cultures were diluted to 0.5% parasitemia and the medium was changed daily without fresh red blood cells (RBC) addition. When parasites reached a parasitemia of 5% after two or three days of culture, they were stressed by nutrient deprivation. When early gametocytes (stages I and II) and dead asexual forms were found, cultures were treated daily for 72 h with *N*-acetylglucosamine (NAG) (Sigma-Aldrich) at the final concentration of 50 mM to clear residual asexual parasites. NAG was maintained in the culture medium until the day of the experiment and removed before the drug susceptibility assay. Stage IV and V gametocytes were obtained and used for the experiments after 8 to 11 days from NAG addition. Gametocyte stages were routinely checked in the Giemsa-stained smears.

In vitro P. falciparum drug susceptibility assay

Methanolic and ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO), the aqueous extracts in distilled water and then all were diluted with medium to achieve the required concentrations (final DMSO or ethanol concentration ≤ 1%, which is nontoxic to the parasites). Chloroquine (CQ) was used as reference control for the asexual stage and methylene blue (MB) was used for gametocytes assay. The prepared extracts were placed in 96- well plates (EuroClone) and serial dilutions were done performed in the culture medium (final volume of 100 µl/well). Asexual asynchronous parasite stages or gametocyte cultures with parasitemia of 0.5 to 1% were distributed into the plates (volume 100 µl/well). The final volume and hematocrit were 200 µl/well and 1% respectively. For the gametocyte assay, a primary screening was first done at a single dose of 100 µg/ml of extract in quadruplicate wells in order to identify the promising extracts. Extracts inhibiting the viability of gametocytes by more than 50% were selected for the IC_{50} determination. Plates were incubated for 72 h at 37°C in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. Asexual parasite growth was determined spectrophotometrically by measuring the activity of the parasite lactate dehydrogenase (pLDH), according to a modified version of Makler's method (17).

For the determination of gametocytes viability, the luciferase assay was used. Briefly, at the end of the incubation, 100 µl of supernatant were carefully removed from each well of experimental plate and discarded. The remaining was then re-suspended and 70 µl of each well were transferred into a new black plate. 70 µl of the substrate D-luciferin (1 mM in 0.1 M citrate buffer, pH 5.5) were added in each well and the plate was kept in the dark for 10 min. The luminescence signal was read using a luminescence reader Synergy4 (BioTek) with integration time 500 ms.

Table 1 List of Investigating Plants for the assessment on malaria parasite

Plant part ID	Plant full Name	Familly	Collected part	Extraction solvent
1	Terminalia avicennioides Guill. & Perr.	Combretaceae	Stem barks	Methanol, Ethanol, Water
2	Terminalia avicennioides Guill. & Perr.	Combretaceae	Leaves	Methanol, Ethanol, Water
3	Terminalia macroptera Guill. & Perr.	Combretaceae	Stem barks	Methanol, Ethanol, Water
4	Terminalia macroptera Guill. & Perr.	Combretaceae	Leaves	Methanol, Ethanol, Water
5	Combretum collinum Fresen.	Combretaceae	Stem barks	Methanol, Ethanol, Water
6	Combretum collinum Fresen.	Combretaceae	Leaves	Methanol, Ethanol, Water
7	Mitragyna inermis (Willd.) Kuntze	Rubiaceae	Stem barks	Methanol, Ethanol, Water
8	Mitragyna inermis (Willd) Kuntze	Rubiaceae	Leaves	Methanol, Ethanol, Water
9	Anogeissus leiocarpa (DC.) Guill & Perr.	Combretaceae	Leaves	Methanol, Ethanol, Water
10	Argemone mexicana L.	Papaveraceae	Leaves	Methanol, Ethanol, Water
11	Pavetta crassipes K. Schum.	Rubiaceae	Leaves	Methanol, Ethanol, Water
12	Zanthoxylum zanthoxyloides (Lam.) Zepern. & Timler	Rubiaceae	Stem barks	Methanol, Ethanol, Water
13	Combretum adenogonium Steud. ex A.Rich.	Combretaceae	Leaves	Ethanol, Water
14	Lophira lanceolata Van Tiegh. ex Keay	Ochnaceae	Stem barks	Ethanol, Water
15	Lophira lanceolata Van Tiegh. ex Keay	Ochnaceae	Leaves	Ethanol, Water
16	Vitex doniana Sweet	Lamiaceae	Stem barks	Ethanol, Water
17	Vitex doniana Sweet	Lamiaceae	Root barks	Ethanol, Water
18	Cassia sieberiana DC	Leguminosae	Leaves	Ethanol, Water

The results of the chemo-sensitivity assays were expressed as the percent viability compared to the untreated controls, calculated with the following formula:

100 X ([OD of treated sample- blank]/ [OD of untreated sample- blank])

OD is the Optical Density.

As blank, uninfected RBC and MB high concentration (300ng/ml) were used for the gametocyte assay and only uninfected RBC were used for the asexual assay.

Statistical Analysis

The percentage of viability was plotted as a function of drug concentrations and the curve fitting was obtained by nonlinear regression analysis using a four-parameter logistic method (software Gen5 1.10 provided with the Synergy4 plate reader [Biotek]). The $IC_{50,}$ which is the dose capable of inducing 50% inhibition of parasite viability, was calculated from the sigmoidal dose-response curve. At least, three experiments in duplicate were performed with each investigated parasite stages and/or strains.

RESULTS

Primary screening of plant extracts on P. falciparum gametocytes

Eighteen plant materials extracted with three different solvents at increasing polarity (water, methanol and ethanol) were used in this study to assess the gametocytocidal activity. In order to select the promising extracts for IC_{50} determination, gametocytes cultures were incubated first with a high dose of each extract for 72 h and then the percentage of live gametocytes was evaluated compared to the negative control using the luciferase method (figure 1). At the test concentration of 100 µg/ml, the methanolic and ethanolic extracts of *Terminalia macroptera* leaves inhibited completely late gametocytes viability while the aqueous appeared to be inactive by less than 30% inhibition, after 72h of incubation. At That concentration, the ethanolic extract of barks of *Lophira lanceolata* showed a good inhibitor activity against late gametocytes by 93.00±2.06%. A moderated activity was observed with the methanolic extracts of *Combretum collinum* leaves, *Argemone mexicana* leaves and *Zanthoxulum zanthoxyloïdes* barks mature gametocyte inhibition with around 50% mature gametocyte inhibition (figure1). The remaining extracts were considered inactive against late gametocytes viability with less than 50% inhibition. Extracts with less than 50% inhibition against mature gametocytes were discarded for the IC_{50} determination based on the dose-response curve fitting. Thus, the five active plant materials with more than 50% of late gametocytes inhibition were considered for determining IC_{50} values.

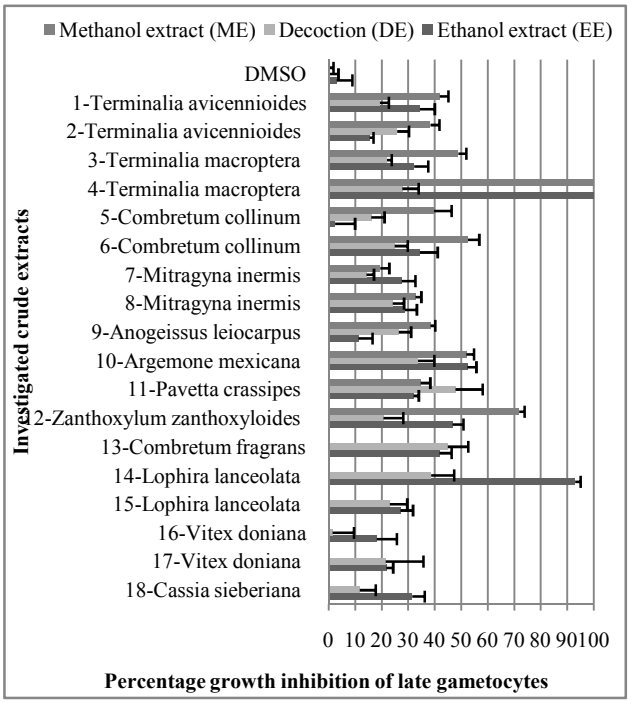

Figure 1 Primary screening of crude extracts at the dose 100µg/ml against late gametocytes of 3D7 *Plasmodium faciparum* using the luciferase assay after 72h of incubation. Methylene blue at 300 nM and uninfected RBC were used as blank, untreated gametocytes culture in DMSO was used as negative control and a serial dilution of methylene blue ranging from 100 nM to 1 nM was used as positive control drug. Results are the mean ± SEM of three independent biological repeat seach done in triplicate; Percentage of inhibition. NB: The methonalic extracts of N°s 13 to N°18 was not tested in this study.

Table 2 Inhibitor activities of five selected plant materials against the late stages of 3D7-CBG99 Plasmodium falciparum gametocytes using luciferase assay

Extract ID	Plants	Plant materials	Type of Extracts	Inhibition of mature gametocytes by plant extracts 100µg/ml (Primary screening)	Gametocytocidal activity of plant extracts IC_{50} (µg/ml)[a]
4	Terminalia macroptera GUILL. et PERR.	Leaves	Ethanol	100±4.4	32.74±11.9
			Methanol	101±0.8	36.34±11.6
6	Combretum collinum Fresen.	Leaves	Ethanol	34.50±18.7	54.69±27.9
		Leaves	Methanol	52.50±12.3	28.35±3.3
10	Argenome mexicana L.	Leaves	Ethanol	52.33±9.7	35.95±11.1
		Leaves	Methanol	52.00±8.1	42.95±16.1
12	Zanthoxylum zanthoxyloïdes LAM.	Stem barks	Ethanol	46.71±4.1	31.96±7.9
		Stem barks	Methanol	71.88±5.8	20.63±2.3
14	Lophira lanceolata Van Tiegh. ex Keay	Stem barks	Ethanol	93.00±5.8	11.35±3.2
			Methylene blue		0.015±0.001

[a] the gametocytocidal activity was tested against stage IV-V gametocytes of P. falciparum 3D7-CBG99 strain using the luciferase assay. Methylene blue was used as positive control. The IC_{50} values were extrapolated from the dose-response curve after plate reading using the software Gen5 1.10. Data in the table come from three or four different experiments performed in duplicate and are the means±SD.

Table 3 In vitro antimalarial activities of crude extracts against schizonte stages of chloroquine-sensitive strain 3D7 P. falciparum using the pLDH method.

Extracts ID	Plants	Part	Inhibition activity of extract on asexual stage IC50 in µg/ml 3D7 strain		
			Ethanolic	Aqueous	Methanolic
1	Terminalia avicennioides GUILL. et PERR.	Stem barks	13.2±3.7	20.88±3.2	15.20±4.4
2	Terminalia avicennioides GUILL. et PERR.	Leaves	4.18±0.8[a]	9.23±1.5[b]	5.95±2.5[a]
3	Terminalia macroptera GUILL. et PERR.	Stem barks	13.57±3.5	32.22±8.2	21.5±3.2
4	Terminalia macroptera GUILL. et PERR.	Leaves	2.86±0.5[a]	10.07±0.5[b]	4.40±1.6[a]
5	Combretum collinum Fresen.	Stem barks	22.86±6.0	>50	47.14±16.0
6	Combretum collinum Fresen.	Leaves	27.89±6.6	50.26±14.4	62.33±15.7
7	Mitragyna inermis (WILLD) O. KTZE	Stem barks	30.19±8.5	>50	57.44±7.4
8	Mitragyna inermis (WILLD) O. KTZE	Leaves	25.9±11.7	21.60±6.1	42.54±11.4
9	Anogeissus leiocarpus (DC) Guill et Perr.	Leaves	8.76±2.4[b]	13.16±1.2	12.26±1
10	Argenome mexicana L.	Leaves	7.21±2.4[b]	63.38±23.2	12.76±4.6
11	Pavetta crassipes K. SCHUM	Leaves	38.97±11.8	>50	52.31±19.7
12	Zanthoxylum zanthoxyloïdes LAM.	Stem barks	4.87±0.83[a]	48.33±11.72	4.55±0.6[a]
13	Combretum fragrans F. Hoffm.	Leaves	27±4.6	>50	NT
14	Lophira lanceolata Van Tiegh. ex Keay	Stem barks	38.18±5.6	>50	NT
15	Lophira lanceolata Van Tiegh. ex Keay	Leaves	35.11±5.9	>50	NT
16	Vitex doniana Sweet	Stem barks	44.73±7.6	>50	NT
17	Vitex doniana Sweet	Root barks	56.81±0	>50	NT
18	Cassia sieberiana DC	Leaves	46.25±8.1	66.37±26.5	NT
	Cloroquine control			0.0076±0.0017	

Chloroquine was used as positive control. The IC_{50} values are calculated by extrapolation of the dose response curve after plate reading using the software Gen5 1.10. Data in the table come from three or four different experiments performed in duplicate and are the means ± SD. a- Good inhibitor activity against asexual parasites (IC_{50}≤5µg/ml); b- Moderate inhibitor activity against asexual parasites (5µg/ml<IC_{50}≤10µg/ml).

Table 4 In vitro antimalarial activities of 5 selected crude extracts against the asexual stages of chloroquine-resistant W2 P. falciparum strain, using the pLDH method.

Extract ID	Plants	Part	Antimalarial activity of plant extracts on CQ-resistant, W2 strain		
			Ethanolic	Aqueous	Methanolic
2	Terminalia avicennioides GUILL. et PERR.	Leaves	4.58±1.2	5.43±1.6	6.58±0.1
4	Terminalia macroptera GUILL. et PERR.	Leaves	3.07±0.4	5.7±0.8	2.89±0.4
6	Combretum collinum Fresen.	Leaves	29.27±4.2	NT	NT
9	Anogeissus leiocarpus (DC) Guill et Perr.	Leaves	8.32±1.7	NT	14.89±1.6
10	Argenome mexicana L.	Leaves	15.63±3.6	NT	16.92±6.1
12	Zanthoxylum zanthoxyloïdes LAM.	Stem barks	10.14±1.3	NT	4.47±0.2
14	Lophira lanceolata Van Tiegh. ex Keay	Stem barks	30.33±1.7	NT	NT
	Cloroquine control			0.295±0.069	

Chloroquine was used as standard control. The IC_{50} values are calculated by extrapolation after plate reading using the software Gen5 1.10. Data in the table come from three or four different experiments performed in duplicate and are the means ± SD. NT: Not Tested

IC_{50} determination of the most active extracts against late gametocytes development

Based on the primary screening results, methanolic or ethanolic extracts of *Terminalia macoptera* leaves, *Combretum collinum* leaves, *Argenome mexicana* leaves*, Zanthoxylum zanthoxyloïdes* barks and *Lophira lanceolata* barks were selected for the IC_{50} determination (figure 1).

Only, the ethanolic extract of *Lophira lanceolata* barks exhibited a good inhibitor activity against late gametocytes with an IC_{50}= 11.35 ± 3.2 µg/ml. It was followed by the methanolic extract of *Zanthoxylm zanthoxyloides* barks which showed a moderate activity with an IC_{50} 20.63 ± 2.3 µg/ml. The ethanolic extracts of *Terminalia macroptera* leaves, *Combretum collinum* leaves, *Argemone mexicana* leaves and *Zanthoxylum zanthoxyloides* barks exhibited a similar inhibitor activity ($p>0.05$) against late gametocytes with IC_{50} values around 30µg/ml (table 2). The leaves extract of *Terminalia*

macroptera had an IC_{50} of 32.74±11.9 µg/ml, significantly higher than the results obtained in the primary screening in which almost 100% inhibition of gametocytes viability was seen at 100 µg/ml after 72h of treatment.

Antiplasmodial activity of plant extracts against Plasmodium falciparum chloroquine-sensitive asexual stages

Aqueous and ethanol extract of all the eighteen materials of plant and the methanolic extracts of the plant from number 1 to 12 were tested on parasite asexual stage. *Terminalia avicennioides* leaves, *Terminalia macroptera* leaves and *Zanthoxylum zanthoxyloides* barks extracts showed a good inhibitor effect against the chloroquine-sensitive strain 3D7 with an IC_{50} ranging from 2.86±0.5 to 4.87±0.83 µg/ml, while *Anogeissus leiocarpus* leaves and *Argemone mexicana* leaves showed a moderate activity, 5< IC_{50}≤10 µg/ml (table 3). All the plants exhibited an inhibitory activity with an IC_{50} at least 50 µg/ml supporting their traditional use in malaria treatment.

Schizonticidal activity of some extracts on Plasmodium falciparum chloroquine-resistant W2 strain

The extracts found to be active or moderately active against chloroquine-sensitive strain 3D7 were assessed for their effect

against chloroquine-resistant W2 strain. The best activities were found with the methanolic and ethanolic extracts of *Terminalia macroptera* leaves and the ethanolic extract of *Terminalia avicennioides* leaves. The remaining extracts, with the exception the leaf extracts of *Argemone mexicana*, showed a moderated activity against the chloroquine-resistant strain (table4). There is not a significant difference between of the inhibitory activities of the methanol and ethanol extract (sig=0).There is a very weak correlation between the inhibitory activities of the extracts against sexual and sexual stage of *Plasmodium falcipaum* parasite after 72 h of incubation (r = -0.42). While the leaves extracts of *Terminalia macroptera, Argemone mexicana* and the barks of *Zantholoxym zanthoxyloides* showed a good inhibitory activity on the erythrocytic stages, they were moderately active on the mature gametocytes. On the other hand, *Lophira lanceolata* barks extract which displayed a high inhibitory effect against late gametocytes was found to be slightly active against parasites asexual stages (figure 3).

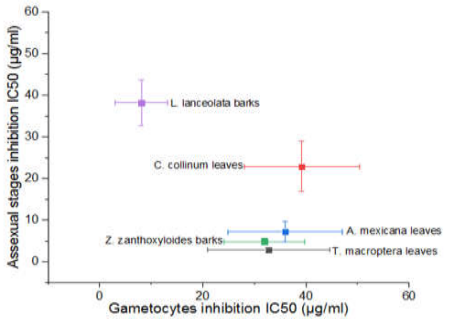

Figure 2 Comparative In vitro inhibitory effects against sexual and asexual development stages of Plasmodium falciparum 3D7 strain of the ethanol extract of the five plant materials. Data expressed as the IC_{50} values and are the mean ± SD of at least three independent experiments per development stage each done in duplicate.

DISCUSSION

This study was undertaken to assess the *in vitro* inhibitor effect of crude extracts of some common medicinal plants used in Burkina Faso to cure malaria, against *Plasmodium falciparum* CQ-R and CQ-S strains, and mature gametocyte stages. Some of these extracts were already known to be active the asexual stages and therefore, by testing them against gametocytes, we could identify molecules with the ability of combating the clinical symptoms and with the additional capacity of blocking malaria transmission. Here, in this study, we demonstrated for the first time, the antigametocyte and schyzonticidal properties of some medicinal plants commonly used traditionally to treat malaria in Burkina Faso western area.

Thus eighteen medicinal plant materials used in western Burkina were selected to assess their transmission property through the antigametocyte activity. Only *Lophira lanceolata* barks showed a great inhibitor activity against *Plasmodium falciparum* mature gametocyte with an IC_{50}= 11.35±3.16µg/ml, while no activity was observed with this plant on the parasite asexual stage. However a moderate activity has been found previously with the dichloromethane extract of the barks of this plant (10). *Lophira lanceolata* is widely distributed in the sudano-guinean savanna zone from Senegal through the Central African Republic. It is used traditionally to treat a lot of illness in west and Central Africa like fevers, gastro-intestinal problems, yellow fever, headache, hypertension, syphilis intestinal worms, dysentery, diarrhoea in children, respiratory tract infections, erection dysfunction and dysentery (18). The *in vitro* anthelmintic activity of the ethanolic extracts of trunk bark has been demonstrated against Onchocerca ochengi with LC50 values of 6µg/ml after 72 h (19).

The *in vivo* oral administration of the ethanolic extracts of leaves, barks of the trunk and root bark of *L. lanceolata* revealed that no abnormal behavior, no mortality during the treatment and observation periods in animals treated at the doses 1500 mg/kg, 3000 mg/kg and 5000 mg/kg. Adverse reactions like increased motor activity, blinking eyes, tremors, convulsion, stimulation, muscle weakness, sedation, urination, salivation, lethargy, sleep, arching and rolling and coma up to a dose of 5000 mg/kg were not noticed within 14 days (19). Phytochemical analysis of *Lophira lanceolata* stem bark extract reported the presence of tannins, alkaloids, resin, saponin and flavonoid (19). Studies conducted previously by some authors on the stem bark extract of *Lophira lanceolata,* permitted to isolate and characterize several groups of flavonoids. That includes a group of related biflavonoids called lophirones form A up to J, the biflavonoid isombamichalcone and the tetraflavonoid lanceochalcone. The wood contains the nitrile glycoside esters lanceolin A and B. The research was extended to the leaves of this plant from which two new bioflavonoids called Lanceolatin A and B were isolated and identified (20–24). The antigametocyte property of *L. lanceolata* may be due to the high number of flavonoids which chemical groups are well known for their antibacterial, antiparasite and antiviral activities (25).Of the eighteen plant materials, investigated *in vitro* on *Plasmodium* intra-erytrocytic stages in this study, *Terminalia avicennioides* leaves, *Terminalia macroptera* leaves and *Zanthoxylum zanthoxyloides* barks were found to be active with IC_{50} ranging from 2.86±0.54 to 4.87±0.83 according to the classification of Deharo *et al*., 2001 (26) (IC_{50} value ≤5 µg/ml). The *in vitro* antiplasmodial activity against asexual stages of those plants has been retorted previously with similar results on the parasite strain K1 by some authors (11–13,27,28). While some fractions from *Zanthoxylum zanthoxyloides* exhibited good inhibition activity against asexual forms ranging from 1.91 to

4.32 µg/ml, the IC_{50} of its methanolic extract has been reported to be too high (12,29). That difference could be due to many parameters including the local environment, the collection times and periods and the laboratory techniques used for extracts preparation and drying. Seasonal factors may contribute to the variation of plant chemical components as shown previously with the study conducted by Aires *et al*., 2011 (30) on seasonal effects on bioactive compounds. The most active part against asexual stages, *Terminalia macroptera* leaves, found to have an antibacterial effect against *Neisseria gonorrhoea* strains (31), confirming their use in traditional medicine against the infectious diseases as gastritis, tuberculosis, fever, headache, vaginal infections, malaria, conjunctivitis, skin diseases. Chebulagic acid, chebulinic acid, ellagic acid, gallic acid, punicalagin and isoorientin were identified in the leaves of this plant demonstrated antimalarial and antiviral properties (31,32). A moderate antiplasmodial activity was found with *Anogeissus leiocarpus* leaves and *Argemone mexicana* leaves extracts ranging from 7.21±2.42 µg/ml to 12.76±4.6 µg/ml. The activity of *Anogeissus leiocarpus* leaves confirms its use in traditional medicine in many areas in Burkina Faso (10). Concerning *Argemone mexicana*, that plant coming from Latino-America, is widely used in Mali as an anti-malarial phytomedicine according to the results found through an experimental clinical trial conducted in the country using a decoction of *Argemone mexicana* comparing to artesunate amodiaquine for the treatment of malaria (33–35). Propopine, allocryptotine and sanguinarine are some isolated compounds from the active fraction of *Argemone mexicana* decoction with exhibited antiplasmodial effect with the IC_{50} 0.32, 1.46 and 7.02 µg/mL respectively (36). In this study, extracts from *Vitex doniana* and *Cassia sieberiana* displayed a very poor antiplasmodial activity against both of the asexual and sexual stages with IC_{50}>50 µg/ml.

The results showed that there is not systematically a correlation between schyzonticidal and gametocytocidal properties of a compound. That tendency may be due to the structural of functional differences between the different development stages of *Plasmodium*. These finding support the hypothesis that it may be more relevant to investigate on medicinal plants or any compounds for their antigametocyte property, without considering their erythrocytic activity. A compound could be effective on Plasmodium asexual stages and completely inactive against gametocyte stages and vice versa.

CONCLUSION

While the literature provide a lot of finding on the medicinal plants screening against *Plasmodium falciparum* intra-erythrocytic property of medicinal plants, there is very little knowledge on their transmission blocking properties. Nevertheless, the ideal antimalarial product, in case of malaria eradication and elimination, must target both of the sexual and asexual stages of the *Plasmodium* parasite. The present study has given the inhibitor property against the sexual and asexual stages of malaria parasite of medicinal plants widely used traditionally in the western Burkina Faso. *L. lanceolata* stem barks which showed the highest activity against late gametocytes viability and was moderately active against asexual stage could be a best candidate for effective natural products discovering by bioguided fractionation. However, a bioguided investigation could be also promising on *Z. zanthoxyloides* barks, *T. macroptera* leaves, *A. mexicana* leaves, *C. collinum* barks, *T. avicennioides* leaves and *T. macroptera* leaves regarding either their asexual or sexual effectiveness.

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