International Journal of Pharmaceutical Research and Development
www.pharmaceuticaljournal.net
Online ISSN: 2664-6870; Print ISSN: 2664-6862
Received: 04-09-2019; Accepted: 05-10-2019
Volume 1; Issue 2; July 2019; Page No. 15-24

Ethnobotanical and clinical knowledge of malaria surveys, antiplasmodial effect and cytotoxicity of plants used in traditional medicine in two departments of Congo

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DOI: https://doi.org/10.33545/26646862.2019.v1.i2a.10

Abstract

For more effective in the treatment of malaria by plants in traditional medicine, a scientific validation on the efficacy and tolerability of the treatments is essential. This study aims firstly to evaluate the knowledge of health tradipractitioners on the pathophysiological manifestations that helped to establish a clinical diagnosis of malaria; secondly to identify the different recipes of the plants used against malaria with a view to select plants to be evaluated for their citotoxicity and antiplasmodial effect. An ethnobotanical and clinical knowledge of malaria surveys have been conducted in Nkayi and Gamboma semi-rural cities. Five plants were selected and submitted to aqueous maceration and sequential maceration using polar croissant solvent system: hexane, dichlorometane (DCM), mix v dichloromethane / v methanol (MeOH) and methanol. Antiplasmodial assay was conducted with D10 sensitive *Plasmodium falciparum* strain. Cytotoxicity was assessed at Vero cells line. *Cassia siamea* bark aqueous, hexane and DCM / MeOH extracts, *Quassia africana* roots aqueous and MeOH extracts, *Nauclea latifolia* leaves DCM/MeOH, MeOH and roots DCM/MeOH extracts, *Rauvolfia vomitoria* leaves DCM/MeOH, MeOH extracts are highly active(IC50 < 6,25 µg / ml). Some of these active extracts are completely devoided of the cytotoxicity, others are weakly cytotoxic (44, 09 ± 1, 47 µg / ml ≤ CC50 ≤ 120, 5 ± 1, 94 µg / ml; 14.22 > SI > 19, 28). Many chemical molecules of these plants are responsible for activity.

Keywords: ethnobotanical, plant, clinical, malaria, antiplasmodial, cytotoxicity

1. Introduction

According to WHO, 212 million morbidity cases and 429,000 mortality cases of malaria were recorded in 2015. 90% of these deaths occurred in Africa, where mortality among children under 5 years of age represents 78% [53]. In Congo, malaria remains the leading cause of clinical consultations with 69.8% of patients [33]. Malaria is a real public health problem. Since it has discovered, many drugs, which were once the reference treatments, solutions and hope in the fight against malaria are no longer recommended today, either because they have lost their effectiveness due to resistance or for their safety [13, 17]. Likewise, today's active drugs could definitely become ineffective in the future. Therefore, there is an urgent need to research and develop new antimalarial drugs. The research and the development of new antimalarial drugs based on ethnobotanical knowledge and medicinal plants is a safe route, as it has already given quinine, atovaquone and artemisinin derivatives to antimalarial therapy [25, 27]. Therefore, the effectiveness of a treatment depends on the correct diagnosis of the disease concerned. It is the duty of public health researchers to keep a constant check on the level of knowledge of nursing staff with a view to guiding policies for strengthening the capacities of health systems. In sub-saharan Africa, traditional medicine plays a crucial role, which attracts about 80% of the population [40]. Unfortunately it is often overlooked by governments and health organizations. Its methods and practices are still archaic. Its dealing staff needs a frame and a technical assistance to improve the quality of their services. Plants used in traditional medicine deserved pharmacological and phytochemical validation to assess their effects, tolerance and to identify their chemical compounds [22, 54]. The scientific valorizaton of plants of therapeutic interest will help to promote the sustainable management of its species. This study aims firstly to evaluate the knowledge of health tradipractitioners on the pathophysiological manifestations that helped to establish a clinical diagnosis of malaria; secondly to identify the different recipes of the plants used against malaria with a view to select five plants to be evaluated for their citotoxicity and antiplasmodial effect.

2. Material and methods

2.1. Plant material

A sample of each plant cited were collected around Nkayi (Department of Bouenza) and Gamboma (Department of Plateaux), Congolese cities where the ethnobotanic survey were conducted, in jully 2011. All Plants were authenticated by the botanists of Centre d'Etudes sur les Ressources Végétales (CERVE), Brazzaville –Congo. A voucher specimen exist at the Herbarium of the botanic laboratory.

2.2. Biological material

The CQ-sensitive strain D10, derived from FCQ-27 from Papua New Guinea was donated by the Division of Pharmacology of University of Cape Town, South Africa. Vero cell line used for cytotoxicity was provided by the Medical Research Council of South Africa.

2.3. Population of study

Surveys were carried out in two localities, one of southern (Nkayi) and one located in the center of the Congo (Gamboma). Nkayi is the main town of the Bouenza region, it is located 250 kilometers from the capital, Brazzaville, at the halfway point of the Congo-Ocean Railway (CFCO), it is an industrial city. Its population is about 71,620 people in the census of 2007. The main languages spoken in Nkayi are the kikongo national, language and some dialects of Kongo ethnic group. Gamboma often called Gam City is created in January 12, 1909. It is located in the department of Plateaux, at 273 km from the capital (Brazzaville). The demography is dominated by the Bangangulu, an ethnic of téké group. It population is 43,200 inhabitants and its area of 662,800 ha (6,628 km2). The main languages spoken in Gamboma are the Gangulu and the Lingala national language. Geographical positions of these two semi-rural cities are indicated on the political map of the republic of Congo (figure 1).

Fig 1: Political map of the republic of Congo

2.4 Evaluation of knowledge on clinical malaria

Using a questionnaire, an interview was carried out among health care tradipractitioners. Malaria clinical signs including fever, digestive disorders, anemia, various pains, vomiting and other have been researched. Information regarding the origin of malaria infection was also verified.

Twenty (20) health tradipractitioners were interviewed. Most of them were between 30 and 50 years old. In the lot there were a half of womens. Thirteen of them had the diploma of elementary education, five (5) had the third level with a certificate of undergraduate study and only two (2) had the university level with a graduate and a bachelor

2.5 Ethnobotanical survey

A semi-structured questionnaire was used to collect information on the common / local names of the plants used alone or in combination in the treatment of malaria or its symptoms: fever, various pains, vomiting, nausea, asthenia, anemia, anorexia, vertigo [36]. The information was collected from health tradipractitioners, medicinal plants vendors and users. A total of forty (40) people, at the rate of twenty (20) by city, were interviewed among them 18 women and 22 men. They were between 25 and 50 years old. Twenty six (26) of them had the diploma of elementary education, twelve (12) had the third level with a certificate of undergraduate study and only two (2) had the university level with a graduate and a bachelor. A literature review was carried out to look for information on the ethnomedical uses and the antimalarial activity of the reported plants.

2.5.1 Plant selections

Plants were selected using following criteria: much cited, available, without serious adverse effects indicated, without antiplasmodial evaluation for the sample of this region and on the D10 strain, without serious toxicity in the literature. Samples of selected plants were then used for extract preparation.

2.5.2 Preparation of extracts

Plant samples were air-dried, at room temperature (25 °) and protected from the light, to a constant weight over a four-week period. The dried material was then powdered using a mechanical grinder. To prepare aqueous extract, 50 g of dried and powdered of each plant sample was macerated 48 h at room temperature with 2 x 500 ml of distilled water. The resulting extracts were decanted, filtered and evaporated to dryness in an oven at 40 ° C. For organic extracts, 50 g of dried and powdered sample was successively macerated 48 h at room temperature with hexane (2x500 mL), then dichloromethane (2 x 500 mL DCM), then mix (1/1) DCM: MeOH (2 x 500 mL) and methanol (2 x 500 ml MeOH). The mixtures were filtered with a Whatman filter to give liquid organic extracts. Then concentrated up to complete drying with Büchi rotary evaporator R-114. The dried extracts were kept at 4 ° C in a refrigerator until use.

2.6. Cultivation of malaria parasites

P. falciparum parasites were cultured as described by Trager and Jesen(1977), with minor modifications.[18,48] The parasites were maintained in RPMI 1640 culture medium supplemented with phenol red, albumax II (25 g/l), HEPES (*N*-[2-hydroxyethyl]-piperazine-*N*'-[2-ethansulphonic acid]) at 6 g/l, 4.25% of sodium bicarbonate and gentamycin (50 mg/l). The reagents were purchased from Sigma-Aldrich, South Africa. Washed O^+ human red blood cells (RBC) and human serum were added to the culture. RBC were washed twice with medium before use. The parasites were cultured in sealed flat bottom flasks and maintained at 37ºC in an atmosphere of 93% N_2, 4% CO_2, and 3% O_2. The haematocrit and parasitaemia were kept between 2-4% by the addition of RBC. Parasites were synchronized at the ring stage by treatment with 5% D-sorbitol. The parasitaemia was determined microscopically using a giemsa stained thin blood smear of culture on the slide [43].

2.7 Antiplasmodial activity assay

The initial stock solution of 2 mg/ml of the crude extracts was prepared in 10% Dimethylsulphoxide (DMSO) then in complete medium to achieve a final stock solution of 200µg/ml. The experiments were carried out under sterile conditions in duplicate in 96-well microtiter plates [41]. Aliquots of 100 µl of complete medium were dispensed to all the wells except in column 3 and 8. In column 3 and 8, aliquots of 200µl of drug stock solutions (200µg/ml) were added and two fold serial dilutions were carried across the plate to achieve 5 dilutions (100, 50, 25, 12.5 and 6.25µg/ml). Parasitized red blood cells (pRBC) in the trophozoite stage were adjusted to a 2% parasiteamia and a 2% haematocrit in complete medium, and 100µl of this suspension was added. The first column served as a blank and contained 100µl of complete medium and 100µl of unparasitised RBC at 2% haematocrit. Column 2 contained no drug and served as a positive control. The plates were covered with a sterile lid, put in a chamber, gassed with 4% CO_2, 3%O_2 and 93%N_2, and then incubated at 37ºC for 48 hours [12].

2.8 Measuring of the PLDH Activity

The parasite lactate dehydrogenase (pLDH) assay was used to measure parasite viability [22, 31]. The pLDH activity was measured using Malstat™ reagent (1ml/L), APAD (0.33g/l) and 0.24 mM phenazine ethosulphate (PES)/1.96 mM nitro blue tetrazolium NBT (Sigma). At the end of a 48 hour incubation period of the test plate, the parasites were re-suspended and 15µl was transferred with a multi-channel dispenser to the corresponding wells in an empty microtiter 96-well plate. To this developing plate, 100µl of Malstat and 25µl of NBT/PES solution were added. The plate was placed in the dark for 5 minutes for colour development. Absorbance values were read at 620 nm using a Multi-purpose plate reader (PHERAstar FS, BMG LABTECH, software V3.10 R6). Reduction of the yellow tetrazoliun salt (NBT/PES) to purple crystals of formazan salt by pLDH enzyme is used primarily as an indicator of parasite survival. Production and accumulation of Purple color is used as indices of parasite viability, whereby the parasite density corresponds to the intensity of the color.

2.9 Cytotoxicity activity assay

The cytotoxic effects of the active extracts were tested on MTT (3-[4, 5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay as described by Mossman (1983) and evaluated against Vero cell line. The MTT assay is a non-radioactive quantitative colometric assay used for *in vitro* measurement of the cytotoxicity and metabolic activity of cell cultures subjected to different culture conditions. It is based on the ability of viable cells to reduce the yellow water-soluble tetrazolium salt to a water-insoluble purple formazan product. The metabolic activity in the cells and the number of viable cells are directly proportional to the amount of formazan crystals formed. The Vero cells were maintained in complete medium; 45% Dulbeco's Modified Eagle's Medium (DMEM) (13.3 g of DMEM, 3.7 g $NaHCO_3$ at pH 7.1, gentamycin-500 µl/L and 2 L of Millipore water), and 45% HAMS F-12 medium supplemented with 10% of heat inactivated Fetal calf serum and gentamycin (0.04 µg/ml). The cells were incubated at 37 ºC in a humidified incubator with

5% CO2. Once cells have reached 80 % confluency they were sub-cultured. The medium was aspirated and the cells was washed twice with 5 ml phosphate buffered saline containing no Mg^{2+} or Ca^{2+} (PBSA). One milliliter 0.25% (w/v) trypin (Sigma) was added to the plate to detach the cells from the surface. Within a few seconds of allowing the trypsin to cover the surface of the plate, it was aspirated. The culture dish was incubated at 37 ºC for 10 min. The cells were resuspended in 1 ml growth medium. To determine the amount of viable cells, 20 µl of the cell suspension was transferred to an eppendorf tube containing 20 µl of trypan blue solution (HyClone). The cell suspension was counted on a Neubauer heamocytometer. The cell suspension was replated into new culture dishes at a ratio of 1:2 and incubated. They were rinsed with phosphate buffered saline (PBS), detached from the flask by using1% trypsin solution and resuspended in complete medium. Cells were stained by crystal blue nuclear dye for cell viability assessment and cell counting under microscope. The stock culture with a cell density of 10^6 cells/ ml was prepared for the assay. Cells in the exponential growth phase were trypsinized, counted using a Neubauer heamocytometer and diluted to a density of 50 000 cells/ml. The cells were seeded into a 96-well plate at 10 000 cells/well in 200 µl aliquots in RPMI 1640:10% FBS. Plates were incubated at 37 ºC for 24 hours to allow cells to attach. The plant extracts were screened for cytotoxicity using concentrations ranging from 6.25 – 200 µg/ml. Emetine served as a positive control in the same range of concentrations. Following the initial 24 hour incubation period, the growth medium was removed and 200 µl aliquots of the plants extracts were added and the plate were incubated for a further 48 hours. Immediately after the 48 hour incubation period the medium was replaced with 200 µl MTT (0.5 mg/ml in PBS pH 7.4). The MTT was removed after 4 hour incubation at 37 ºC and the purple formazan product dissolved in 100 µl/well DMSO (Sigma). Plates were agitated for 60 seconds and absorbance measured at 540 nm on a multiwall scanning spectrophotometer (PHERAstar). All incubations were carried out at 37 ºC in a humidified incubator with 5 % CO2. The Selectivity index was determined by the ratio between the cytotoxic concentration 50 (CC50)/inhibitory concentration 50 (IC50).

2.10 Statistical analysis

The Inhibitory Concentrations, IC50 were calculated on a dose response curve by non-linear regression analysis using the IC estimator program version1.2 **(**http://www.antimalarial-icestimator.net) [24]. Ms Excel 2010 and XLSTAT version 6 were used to analyze the data.

3. Results

3.1 knowledge of clinical malaria

Health care tradipractitioners believe that in 75% of cases, malaria is always accompanied by fever, in 50% of cases it is manifested by various pains, in 25% of cases it is manifested by digestive disorder in children. Other signs such as convulsions, cold sensation, anorexia, asthenia, anemia and inability to stand up have also been cited (Table 1).

3.2 Plant and recipes used against malaria

Plants, their botanical families, scientific names, local names, organs used, forms of used and diseases or symptoms in connection with the malaria pathophysiology treated are reported in table 2. The possibilities of association of potential antimalarial plants to improve the effectiveness of treatments against persistent or serious malaria a recorded in the table 3. About the origin the same people attributed four (4) kinds of malaria causes: mosquito infection (18; 90%), witchcraft (10; 50%), microbial infection (6, 30%) and malnutrition (3; 15%).

Table 1: Clinical signs of malaria according to Congolese health tradipractitioners interviewed

General signs	General quote (rate)	Number of persons(rate)	Specific signs	Specific quote (rate)
Fever	29 (34, 94%)	15(75%)	Daily Fever	8(9,63%)
			Early fever	4(4,82%)
			Night fever	5(6,02%)
			cold sensation	2(2,41%)
			Thermophobia	5 (6, 02%)
			Burning sensation when urine is evacuated	5(6, 02%)
pain	19 (22, 89%)	10(50%)	Diffuse (aches)	4(4,82%)
			Abdominal	3(3,61%)
			Joints	4(4,82%)
			Muscular	2 (2,41%)
			headaches	6 (7,23%)
Digestive disorders	12 (14, 46%)	5 (25%)	Nausea	2 (2,41%)
			Vomiting	4(4,82%)
			Diarrhea;	6 (7,23%)
Anemia	14 (16, 87%)	5 (25%)	Pale skin	4(4, 82%)
			Pale at the conjunctivae.	4(4, 82%)
			asthenia	6 (7,23%)
Nervous disorder	9 (10, 84%)	4 (20%)	convulsions	3(3,61%)
			Mental confusion;	2(2, 41%)
			Loss of knowledge	2(2, 41%)
			Inability to stand up	2(2, 41%)
Total	83 (100%)	N = 20		83 (100%)

Table 2: Medicinal plants used in the treatment of presumed malaria in traditional medicine in Nkayi (N) and Gamboma (G)

N°	Familles (herbarium reference)	Scientific names	Local names	Organs used					Forms of use	Illnesses or symptoms treated							C	
				leaves	roots	bark	fruits	Plant		Fever	aches	Headache	pain	diarrhea	anemia	malaria	N	G
1	Acantaceae (1155)	*Brillantaisia Patula*	Mpoulien(Gangoulou)	yes	no	no	no	no	juice, p.o	no	no	no	yes	no	no	no	0	02
2	Apocynaceae (1810)	*Rauvolfia obscura* Afzel	Ongoué-ngoué ou opiépié (gangoulou)	yes	no	no	no	no	Maceration, p.o	no	no	no	no	no	no	yes	0	06
3	Apocynaceae *(P.Sita 121)*	*Rauvolfia vomitoria* Afzel	Mounoungou bakouyou (kongo)	no	yes	no	no	no	Maceration, p.o.	no	no	no	no	no	no	yes	3	0
4	Anonaceae (11948)	*Annona senegalensis* subsp. Aulatricha le T	*Moulolo ntséké (Dondokongo)*	yes	yes	no	no	no	Juice, decoction, p.o	yes	yes	no	yes	yes	no	no	1	0
5	Arecaceae **(4535)**	*Elaeis guineensis* Jacq	Ebâ (Tékéou Gangoulou)	no	no	no	yes	no	Oil friction	no	yes	no	no	no	no	no	0	01
6	Astéraceae (Néré5529)	*Aspilia kotschyi* (sch.Bip) Oliv	Nganouon(gangoulou, téké)	yes	no	no	no	no	Juice nasal instillation	no	no	yes	no	no	no	no	0	01
7	Asteraceae (7716)	*Mikania cordata Brum*	*Loumbousibousi (Bembé)*	yes	no	no	no	yes	juice /decoction, p.o	yes	yes	no	yes	no	no	yes	2	0
8	Rubiaceae (7002)	*Morinda lucida Benth*	Ossiô (Gangouou)	no	yes	yes	no	no	Decoction	yes	yes	no	no	no	no	yes	5	2
9	*Caricaceae (NN03)*	*Carica papaya*	Papayer	yes	yes	no	no	no	Decoction p.o.	no	no	no	no	no	no	no	2	02
10	Lamiaceae (NN04)	*Hyptis suaveolens* Poit	*Douté diabadondo (kikongo)*	yes	no	no	no	no	Infusion, decoction, p.o.	yes	yes	no	no	no	no	no	1	0
11	Cucurbitaceae (307; 738)	*Momordica Charanchia*	Loumboumbougi (lari)	yes	no	no	no	no	Diluted juice, p.o.	yes	no	no	no	no	no	yes	1	0/
12	Dilleniaceae (5606)	*Tetracera alnifolia* Wild.var	Lékouéletsio(téké)	yes	no	no	no	yes	Decoction p.o.	no	no	no	yes	no	no	yes	0	03
13	Euphorbiaceae (1643)	*Hymenocardia acida*	Engôon (Gangoulou)	no	no	yes	no	no	Decoction p.o.	no	yes	yes	yes	no	no	yes	1	02
14	Euphorbiacea (763)	*Jatropha curcas*	*Puluka (lari)*	yes	no	no	no	no	Juice /decoction, p.o.	no	no	no	yes	no	no	yes	1	/0
15	Euphorbiaceae (12.829)	*Phyllantus amarus*	Ondouantsiè (Gangoulou)	yes	no	no	no	no	Juice, p.o.	no	yes	no	no	no	no	no	0	01
16	Fabaceae (NN07)	*Cassia occidentalis* L	*Kinkeliba,Nsounsoumbi (kikongo)*	yes	yes	no	yes	no	Decoction, p.o.	yes	no	yes	no	no	no	yes	5	0
17	Fabaceae(NN08)	*Cassia siamea* Lam	Accassia, ntifofolo (lari)	no	yes	yes	no	no	Decoction, p.o.	no	yes	yes	no	no	no	yes	4	0
18	*Flacourtaceae (50.2688)*	*CaloncobaWelwitschü* (Oliv) Glig	Onkâon (Gangoulou)	no	no	yes	no	no	Decoction, p.o.	no	no	no	no	no	no	yes	0	1
19	Malvaceae(8.885)	*Urena lobota* L	Eouo (Gangoulou)	yes	no	no	no	no	Diluted juice p.o.	no	no	no	no	yes	no	no	0	1
20	Myrtaceae(NN09)	*Psidium guyavia* L.	Goyavier	yes	no	no	no	no	Decoction p.o.	no	no	no	no	yes	no	no	0	1
21	Olacaceae(9837)	*Olax wildemannii* E.	Ompripri (Gangoulou)	no	no	yes	no	no	Decoction p.o.	yes	no	no	no	no	no	no	0	3
22	Pentadiplandraceae (4457)	*Pentadiplandra brazzeana Baill.*	*Ngounza (lari)*	no	yes	no	no	no	Pate friction	no	yes	yes	no	no	no	no	2	0
23	Rubiaceae (7895)	*Crossoptex febrifuga*	*Moumpalambaki (lari), mouwala(kikongo)*	yes	yes	no	no	no	Juice nasal instillation	yes	no	yes	yes	no	no	no	1	1
24	Rubiaceae(NN10	*Hallea stipulosa* (DC) Leroy	Opoapô (Gangoulou,téké)	yes	no	no	no	no	Maceration, p.o.	no	no	no	no	no	no	yes	0	1
25	Rubiaceae(9267)	*Morinda morindoïdes Back) Milne- Redh*	Moussiki (Dondo, kikongo)	yes	yes	no	no	no	Decoction, p.o.	yes	no	no	yes	no	no	yes	2	0
26	Rubiaceae (2023)	*Naucléa latifolia* Smith	Ombouémbouo (Gangoulou)	no	yes	yes	no	no	Maceration, p.o.	yes	yes	yes	yes	no	no	yes	7	4
27	Rubiaceae (NN11)	*Ocimum gratissinum*	Dzama-dzan (Gangoulou)	yes	yes	no	no	no	Juice nasal instillation/friction	yes	no	yes	no	no	no	yes	0	1
28	Simaroubaceae (84)	*Quassia Africana* Baill	*Moumpessi (Dondo, Lari)*	no	yes	yes	no	no	P.o. Maceration/ decoction/ infusion	yes	no	yes	no	yes	no	yes	3	0
29	Sterculiaceae (10127)	*Cola acuminata* S.	Obèle (Gangoulou)	No	no	yes	no	no	Dection p.o.	no	no	no	no	no	yes	no	0	01
30	Verbenaceae (NN12)	*Vitex madiensis*	M'filou (Dondo, lari)	yes	no	no	no	no	Decoction p.o.	yes	yes	no	no	no	no	no	1	0

N: Nkayi; G: Gamboma; C: number of citation

Table 3: different combinations of plant extracts used in the treatment of malaria in Nkayi and Gamboma in traditional medicine

N°	Scientific name	families	Organs	Form of used	C	dosages
1	*Nauclea latifolia*	Rubiaceae	Roots, leaves	maceration	1 N	Adult:50 ml x 3/day for one week child: 25 ml x 3/day for one week infant:5-10 ml 3/day for one week
	Cassia siamea	Fabaceae	Roots, bark	Maceration/decoction		
	Cassia occidentalis	Fabaceae	Roots, leaves	decoction		
	Carica papaya	Caricaceae	Roots, leaves	decoction		
2	*Nauclea latifolia*	Rubiaceae	Roots, leaves	maceration	1 N	
	Cassia siamea	Fabaceae	Roots, bark	Maceration/ decoction		
	Cassia occidentalis	Fabaceae	Roots, leaves	Decoction		
	Mikania cordata	Asteraceae	Roots	Decoction/ maceration		
3	*Nauclea latifolia*	Rubiaceae	Roots, leaves	Decoction/ maceration	3N	
	Morida lucida	Asteraceae	Roots, leaves	Maceration/decoction		
4	*Morinda lucida*	Asteraceae	Rots, leaves	Maceration/decoction	2 N	
	Nauclea latifolia	Rubiaceae	Roots, leaves	Maceration		
	Cassia siamea	Fabaceae	Roots, bark	Maceration/decoction		
	Momordica charantia	Cucurbitaceae	All the plant	Decoction/ juice		
	Hyptis suaveolens	Lamiaceae	Roots, leaves	maceration		
5	*Morinda lucida*	Asteraceae	Roots, leaves	Maceration/décoction	2 N	

	Nauclea latifolia	Rubiaceae	Roots, leaves	Maceration/décoction		
	Mikania cordata	Asteraceae	All the plant	Decoction, juice		
6	*Nauclea latifolia*	Rubiaceae	Roots, leaves	Maceration	2 N	
	Hyptis suaveolens	Lamiaceae	Roots, leaves	Maceration		
	Cassia occidentalis	Fabaceae	Roots, leaves	Decoction		
7	*Psidium guyavia L.*	Myrtaceae	Leaves	Decoction	1G	
	Citrus limonen	(rutaceae)	Leaves	Decoction		
8	*Tetracera alnifoli*	Dilleniaceae	Leaves	Decoction	1 G	
	Rovolfia obscura	Apocynaceae	Leaves	Decoction		
9	*Ocimum gratissinum*	Rubiaceae	leaves	Decoction	1 G	
	Crinum sp		Bulb	Juice		
10	*Elaeis guineensis Jacq*	Arecaceae	Heart of tree	Decoction	1G	
	Cassia occidentalis	Fabaceae	Leaves	Decoction		
11	*Nauclea latifolia*	Rubiaceae	Stem bark	Maceration /decoction	1G	
	Cogniauxia Pololaena	Cucurbitaceae	roots			

C: number of citations

3.3 Antiplasmodial effect

A total of 39 extracts from five (5) plants were prepared using water, methanol (MeOH), dichloromethane (DCM), hexane and mixture DCM/MeOH V/V and screened against *P. falciparum* D10 strain. 22 of these extracts are found active (IC50 ≤50 µg/mL). Ten (10) are very active IC50 values < 6, 25 µg/mL. Two (2) are moderately active 10 µg/mL≤ IC50 ≤ 25 µg/mL and five are low active (25 µg/mL< IC50 ≤ 50 µg/mL). Most of active extracts are note cytotoxic and cytotoxic indices is good as shown in Table 4.

Table 4: Antiplasmodial effect of selected plant extracts on *Plasmodium falciparum* D10 strain from Papouasi and cytotoxicity on vero

		Inhibition % depending on the extracts concentrations, inhibitory(IC50) and cytotoxic 50 (CC50) concentrations								
Plant (family)	**organs**	**Solvent**	**100 µg/ml**	**50 µg/ml**	**25 µg/ml**	**12,5 µg/ml**	**6,25 µg/ml**	**IC50 (µg/ml)**	**CC50 (µg/ml)**	**SI**
Cassia siamea (Fabaceae)	Stem bark	Aqueous	89,14	92,25	91,8	88,39	91,35	< 6,25	NT	ND
		Hexane	98,97	95,78	96,13	89,07	91,14	< 6,25	88,88± 0,14	>14,22
		DCM	75,04	65,71	53,64	30,86	17,95	22,38	NT	ND
		DCM:MeOH	96,59	92,86	92,57	85,89	77,35	< 6,25	> 200	>32
		MeOH	38,58	14,22	11,65	0	0	>100	NT	ND
	Leaves	Aqueous	69,62	11,81	0	0	0	78,5	NT	ND
		Hexane	55,38	19,53	0	0	0	89,13	NT	ND
		DCM	55,05	35,29	0	0	0	83,18	NT	ND
		DCM:MeOH	57,88	53,68	32,44	29,05	19,3	44,66	NT	ND
		MeOH	61,28	50,27	24,77	9,72	8,44	50	NT	ND
Quassia africana (Simaroubaceae)	Roots	Aqueous	98,11	98,32	99,84	98,05	98,25	< 6,25	112,44± 5,63	
		Hexane	71,08	64,16	57,82	26,21	32,53	17,78	NT	ND
		DCM	-	-	19,76	36,53	41,92	50	NT	ND
		DCM:MeOH	38,35	45,57	31,49	43	39,68	>100	NT	ND
		MeOH	72,55	73,06	63,68	66,57	60,66	< 6,25	44,09±1,47	> 17,99
Rauvolfia vomitoria (Apocynaceae)	Leaves	Hexane	78,48	44,24	20,79	23,03	2,36	56,23	NT	ND
		DCM	76,39	46,89	36,6	20,29	13,85	58,88	NT	ND
		DCM:MeOH	90,07	93,2	87,06	84,19	78,51	< 6,25	116,41±2,22	>18,62
		MeOH	90,53	94,91	92,17	85,49	82,75	< 6,25	120,5± 1,95	>19,28
Nauclea latifolia (Rubiaceae)	Leaves	Aqueous	50,86	27,1	13,47	12,85	0,78	> 100	NT	ND
		Hexane	56,55	35,35	18,91	10,29	1,69	> 100	NT	ND
		DCM	92,17	68,99	68,09	50,43	35,95	12,5	NT	ND
		DCM:MeOH	94,4	86,89	85,53	71,71	73,54	< 6,25	110± 4,01	>17,6
		MeOH	88,43	66,12	72,58	64,19	56,13	< 6,25	98±2,5	>15,68
	Roots	Aqueous	86,47	58,9	37,91	0	1,23	38,02	NT	ND
		Hexane	70,25	46,71	23,88	11,76	0,2	56,25	NT	ND
		DCM	20,42	0,86	0	0	0	> 100	NT	ND
		DCM:MeOH	89,78	81,38	81,12	78,36	78,4	< 6,25	106± 3,2	> 16,96
		MeOH	50,2	39,7	38,29	19,7	10,6	100	NT	ND
Tetracera alnifolia (Dilleniaceae)	Whole plant	Aqueous	86,47	58,9	37,91	30,22	12,3	38,02	NT	ND
		DCM	70,25	46,71	23,88	11,76	0,2	56,26	NT	ND
		MeOH	20,42	0,86	0	0	0	<100	NT	ND
	Control	CQ	62,6	59,45	62,18	58,18	55,54	< 6,25	NT	ND
	Emetine								7,87±0,88	ND

NT: non-toxic; ND: not determined; N= 5

4. Discussion

4.1. Evaluation of knowledge on clinical malaria

Unlike conventional medicine, traditional medicine does not have the technical tools for biological diagnosis of malaria. To overcome this failure, traditional healers use divination and their clinical knowledge to diagnose and treat suspected malaria cases. Although the clinical signs of malaria are not specific, in malaria endemic areas, as Congo, most of cases of thick positive drop in

Plasmodium falciparum are accompanied by a fever [34, 46]. Ensuring the traditional practitioner's level of knowledge about a disease is already an essential element of the adequacy between the treatment administered and the pathology treated which should increase the confidence and security of ethnobotanical data. This survey was also necessary in so far as it gives an idea of the state of health practitioners' level of knowledge about malaria and will help to define the basis for training in capacity strengthening or in capacity building for malaria needs. All respondents are aware that malaria is transmitted through the infection of a mosquito, which migrates and multiplies in clear stagnant waters, mosquitoes often stings in the evening and at night. Malaria is not a contagious disease. These informations are in accordance with those known scientifically and popularized by the ministry of health and population in our country [33].

Most traditional health practitioners are partially aware of the clinical signs of malaria [14]. However, despite this knowledge, 50% of traditional healers do not use clinical diagnosis and are only satisfied with divination to diagnose malaria in trational medicine [11].

4.2. Ethnobotanic survey

In total thirty plants were cited in the two rural communes. Fourteen (14) of them quoted in Gamboma were not quoted in Nkayi. Twelve of them quoted in Nkayi were not quoted in Gamboma. However, five (5) plants were both cited in two communes. Among the plants most cited in Nkayi we retain: *Morinda lucida, Cassia occidentalis, Cassia siamea, Nauclea latifolia.* The most cited in Gamboma are *Rauvolfia obscura and Nauclea latifolia.* Next come *Olax wildemannii* Engl and *Tetracera alnifolia* in Gamboma, Quassia *Africana* and *Rauvolfia vomitoria* in nkayi. The survey was conducted in two semi-rural towns where ethnically distinct populations have some cultural differences. The department of Bouenza, which is home the town of Nkayi, has a savanna-like vegetation growing on clay soil, whereas in the plateaux department, where the town of Gamboma is located, the same type of soil and vegetation is found on the plateaux, on the valleys, the soil is sandy with a distinct savanna of that of the Bouenza from the point of view of its flora. All these differences could explain the differences in both frequency and use of medicinal plants. Moreover, with the exception of *Olax wildemannii* Engl, all the other most cited plants have already been identified in a similar survey carried out in Brazzaville.[36] Brazzaville is the political capital and the biggest city of the country where we find a representative of all the Congolese ethnic groups at the origin of a Cultural Brewing. This may explain the use of all these plants in the control of malaria in traditional medicine in Brazzaville. In addition plants used in Brazzaville come sometimes from distant departments. They are not necessarily collected around Brazzaville as is the case with plants used in Nkayi or Gamboma [35]. The most common preparation methods are decoction, followed by maceration. These two modes of extraction, widely used in traditional medicine, use as extraction solvent for the most, a water or a local alcoholic drink [21]. This makes it possible to obtain a large quantity of diluted extract. Unlike maceration, the decoction has the advantage of being faster in production, more efficient in extraction, heat-sterilized and possible to be administered hot. This could explain the preponderance in the use of this method of preparation. These results are in agreement with those of our predecessors [26, 36]. The most used organ is the leaf, which is very advantageous because the leaf harvesting of a plant does not threaten its life, in addition the leaves regenerate more easily and rapidly, unlike roots and bark for which the massive harvest, leads inevitably to the death of the plant and indirectly threatens the survival of the species and contributes to the loss of biodiversity. Leaves are also more accessible than barks or roots because they are located on the aerial part of the plant, their removal requires no more effort than that of the barks or roots which first need to dig hole on the ground and use mechanical instruments like ax, machete or knife to harvest.

When the disease is severe, health care tradipractitioners resort to the combination of several extracts of the presumed antimalarial plants, with a view to increasing the effectiveness of treatments. In combinations of different plant species, each extract used is believed to have pharmacological property related to the treatment of malaria [28]. The purpose of associations of different plant extracts is either to potentiate an activity or to simultaneously heal different aspects of manifestation of a pathology or to improve tolerance while preserving efficacy [37]. Knowledge of plants used in combination in the treatment of malaria could lead to the discovery and isolation of co-active or synergistic active chemical new compounds against malaria. This practice may be very necessary because a development of *Plasmodium falciparum* resistance is become very important, in conventional medicine, we resort to therapeutic combinations associating several antimalarial molecules to carry out effective treatments. Artemisinin-combined therapies (ACTs) are first-line treatment of uncomplicated malaria in many sub-Saharan African countries [29]. Most of these plants are also used for the same pathologies in other localities in Congo and other African countries such as Nigeria [50], Zimbabwe, DR-Congo [49], Cote d'Ivoire, as reported by our predecessors [32, 47]. There are: *Ageratum conyzoides* Linn [6], *Carica papaya* Linn, *Alium sativum* [39], *Cassia occidentalis* Linn, *Cassia siamea* Lam [7, 19], *Crossopteryx febrifuga* (Afzel, ex G. Don) Benth [4, 51]. *Elaeis guineensis* Jacq [19], *Morinda lucida* Benth. Tor, *Morinda moridoides* [26], *Nauclea latifolia* Sm [6], *Phyllanthus amarus* Schum. & Thonn [8, 9]. *Psidium guajava* Linn. (Myrtaceae) [6], *Rauvolfia vomitoria* Afzel. (Odugbemi *et al*., 2007) [39], *Tithonia diversifolia* (Hemsl.) A. Gray [6], in Nigeria Decoctions and infusions or plasters of root, bark and leaves of *Morinda lucida* are recognized remedies against various types of fever, including yellow fever, malaria and feverish condition during labor [23]. The stem bark and the leaves (1 mg / kg) extracts had the most promising result with 96.4% suppression of parasitaemia *in vivo* [3]. *Cassia occidentalis*, which is one of the most widely used plants, is widely used in sub-Saharan Africa and has already been the subject of pharmacological investigation on malaria.

4.3. Antiplasmodia activity and cytotoxicity

Casssia siamea is the very active plant. At the bark level, activity was greater than at the leaves. Three (3) bark extracts prepared with water (IC50 < 6,25 ± 0.16 µg / ml), hexane (IC50 < 6,25 ± 0.06 µg / ml) and DCM / MeOH (IC50 < 6,25 ± 0.38µg / ml) are very active and a DCM extract (IC50 = 22,38 ± 2.33 µg / ml) is active. On the leaves the antiplasmodial activity is less important, only the DCM / MeOH extract (IC50 = 11.26 ± 0.92 µg / ml). With the exception of the hexane extract of bark which is weakly toxic (SI = 9.23), all extracts are not cytotoxic. These findings

confirm those of Nsonde Ntandou *et al*. (2005) who have already demonstrated an antimalarial effect of this plant *in vivo*, in clinical malaria, and *in vitro*, on *Plasmodium falciparum* strain, with the bark [36, 37]. These effects are due to the presence of alkaloids, terpenoides and quinones present variably in the different extracts. However, with other African or Asian samples, antiplasmodial activity was demonstrated *in vitro* and *in vivo* and the molecules responsible for this activity were also isolated and identified among the alkaloids (Cassiarin A = 0.020 µg / ml), triterpenes (lupeol = 5,000 with K1strain µg / ml) and flavonoids (emodin IC50 = 5,000 with K1 µg / ml). In Nigeria on another plant of the fabaceae family, *Cassia singueana*, *in vivo* antimalarial activity has been demonstrated with 200 mg / kg of organic extract on *plasmoduim berghei* [1, 30]. However, it should be noted that, in this study, whatever extract, antiplasmodial activity is more important in bark that in the leaves. While other authors reported important antiplasmodial activity and isolated the active ingredients in the leaves [7, 2, 45]. Another important aspect to retain here is that the aqueous extract used in traditional medicine is very active and is not cytotoxic. Our previous investigations have already demonstrated no cytotoxicity, no acute and no subacute toxicities of *Cassia siamea* sterm bark extract [37].

Quassia Africana, which is one of the most frequently cited plants, showed a very important antiplasmodial activity with roots aqueous and DCM/MeOH v/v extracts (IC50 < 6,25 ± 0.23 µg / mL) and moderate with DCM extract (IC50 = 22,38 ± 4.6 µg / mL). About cytotoxicity, the aqueous extract showed no cytotoxicity, only the DCM extract showed a low cytotoxicity (CC50= 44, 09 ± 1, 17; SI > 17, 99). These results are in agreement with those of Mbatchi *et al*. (2006) [26], who have previously shown an important antiplasmidal on FcM 29, a resistant strain of *Plasmodium falciparum* from Cameroon with the sample from the Pool department, an another region of Congo [5, 26]. This activity is attributed to quassinoid. This effect has also been demonstrated with *Quassia amara* and *Quassia indica*, which are two other plants of the Simaroubaceaee family that have allowed the isolation of Simalikalactone and Samaderines X, which are quassinoides with a strong antiplasmodial effect [2]. It should be noted that the aqueous extract used in traditional medicine is the most active and not cytotoxic at least on the vero strain.

For the extracts of *Nauclea latifolia* (Rubiaceae), a very pronounced antipasmodial activity with leaves DCM: MeOH and MeOH extracts (IC50 < 6, 25 µg / ml) and modered with DCM extract (IC50 = 12, 5 ± 0.63 µg / ml). At a level of the roots, DCM: MeOH extract showed very interesting antiplasmodial activity (IC50 < 6, 25 µg / ml) and aqueous extract moderate activity ((IC50 < 38, 02 ±0.63 µg / ml). All these very active extracts are not really cytotoxic (98 ± 4, 01 µg / ml ≤ CC50 ≤ 110 ± 2, 50 µg / ml; SI > 15, 68). However, it should be noticed that the most antiplasmodial activity was found with organic extracts of leaves and roots. Only the aqoueous roots extract showed modered antiplasmodial activity. In addition all of these organic extracts are not known, or available or used in traditional medicine. It's perhaps why they use only aqueous roots extracts which expressed a moderate antiplasmodial effect. These results are in agreement with those of the previous studies which also showed an interesting antiplasmodial effect *in vitro* and *in vivo* on the Plasmodium strains for the same plant extracts [10, 15]. *Nauclea latifolia* has also shown an interesting effect against cerebral malaria [42]. This effect is related to the presence of alkaloids, saponins and flavonoids found in this plant [15]. Indeed of the following molecules: Two novel tetrahydro-β-carboline monoterpene alkaloid glycosides, naucleaorin, epimethoxy-naucleaorine, strictosidine lactam, and oleanolic acid isolated in *N. latifolia*, shown moderate *in vitro* activities against *Plasmodium falciparum* [44]. The first antimalarial drug used in the Occident was extracted from the bark of the Cinchona (Rubiaceae) species, the alkaloid quinine, still largely used. *Nauclea latifolia* is also a rubiaceae with alkaloid group as antimalarial product [52].

Concerning leaves of *Rauvolfia vomitoria* DCM: MeOH and MeOH extracts showed a very interesting antiplasmodial activity with a very low cytotoxicity (IC50 < 6, 25 µg / mL; CC50= 120 ± 0.23; IS > 19, 28). This work confirmed the results obtained by Zirihi *et al*. (2005) [54], who have already demonstrate the antiplasmodial effect of leaves organic extracts of *Rauvolfia vomitoria* on a sample of West Africa.

Tetracera alnifolia did not showed an important antiplasmodial effect, this result is similar to that obtained by Mbatchi *et al*. (2006) [26] on FcM29 strain from Cameroon. Evaluation of the therapeutic effect in patients suffering of uncomplicated malaria does not showed a decrease in parasitemia in patient group treated by *Tetracera alnifolia* aqueous extract [36]. This main that, *Tetracera alnifolia* extract treatment do not intend to reduce the parasitemia, but only symptoms of malaria as pain. A recent study conducted by our team showed interesting analgesic and anti-inflammatory effects of *Tetracera alnifolia* aqueous extract [35]. We know that in conventional medicine we used the analgesic and anti-inflammatory drug in combination of antiplasmodial drug to treat malaria some time. Antiplasmodial effect is variable in function of species of plant or families. We notice that, in the same species and with the same solvent of extraction, the antiplasmodial effect is variable when we change organ. In the same organ the antiplasmodial effect is modified on changing the solvent when we prepare the extract. This is explained by the fact that each species and each organ of plant have a variability of phytochemical composition in nature and in quantity. Each solvent is specific to extract types of chemical compounds variables in quantity and quality. The antiplasmodial effect changes because our *Plasmodium falciparum* strain is not every time exposed to the same chemical compounds composition. We also noted a difference by comparing our IC50 with those obtained in anterior studies; this is explained on the one hand by the difference of sensitivity between strains of *Plasmodium falciparum* and on the other hand by variability of the composition between samples of even plant species when they are collected in the different ecological places or season. Because the changes of genotype within the same species of Plasmodium implies of change in phenotype in the origin of the variation of the sensitivity overlooked of drugs.

Conclusion

Congolese health care tradipractitioners use several plants to treat malaria. Some of *Cassia siamea* extracts, *Quassia Africana* extracts, *Nauclea latifolia* extracts and *Rauvolfia vomitoria* extracts are highly active against *Plasmodium falciparum* D10.

All very active extracts are no significant cytotoxicity on vero cells line. Many chemical molecules of these plants are responsible of this activity.

Acknowledgments
The authors are indebted to a National Research Foundation (NRF) of Cape Town, South Africa, for the financial assistance in equipment and reagents acquisition and travels support in the realization of this work. We are grateful to Marien NGOUABI University of republic of Congo whish host the surveys and extracts preparation studies. We are grateful to Medical Research Council (MRC) of Cape Town in South Africa to host and accept the realization of antiplasmodial and cytotoxicity studies in it Laboratory. The authors are also grateful to botanists of Laboratoire de Botanique du Centre d'Etudes sur les Ressources Végétales, Brazzaville-Congo for authenticating the plant materiel and health care tradipratitioners to accept the participation in the surveys and to give its traditional knowledge.

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