Congo Sciences

Journal en ligne de l'ACASTI et du CEDESURK
ACASTI and CEDESURK Online Journal
ISSN: 2410-4299, an International Journal

Malaria treatment with plants remedies in Kinshasa

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Specially dedicated to Professor Jean-Jacques T. MUYEMBE on the occasion of his 75th birthday / Dédié spécialement au 75ème anniversaire du Professeur Jean-Jacques T. MUYEMBE

Paper History
Received:
March 30, 2017
Revised:
July 25, 2017
Accepted:
August 23, 2017
Published:
December 23, 2017

Keywords:
Malaria treatment, Traditinal remedies, Secondary matabolites, Antimalarial activity, phenylpropanoïds, Kinshasa

ABSTRACT

Malaria is the leading cause of morbidity and mortality in sub Saharan Africa. In Kinshasa, the capital of D.R. Congo, malaria is more prevalent in peripheral rural poor communes. A survey carried out in eastern peripheral area of Kinshasa on plant remedies used to treat malaria and fever revealed a total of 55 plants species distributed in 36 families; the most represented are *Euphorbiaceae* and *Rubiaceae*. Eight plants most used, *C. occidentalis, C. papaya, C. citratus, G.kola, L. camara, O. gratissimum, P.niruriand V. amygdalina* were submitted to clinical investigations according to the traditional healer's protocol. The recovery from malaria of voluntary patients range from 97 to 67 %. Biochemical tests on patients under treatment showed no toxicity of the recipes taken according to the dose and the duration proposed by the traditional healer.

***In vitro* antimalarial activity of crude ethanolic extracts of those eight plants showed at 12,5 µg/mL an inhibition of *P. falciparum* ranging from 100 % for *O. gratissimum* to 0 % for *V. amygdalina*.Some secondary metabolites, terpenes, quinones, flavonoids and alkaloids of some of those plants extracts isolated showed an IC50 below 0.5 µg/mL, a clear indication of a significant antimalarial activities of those metabolites.In the butanolic fraction of *O. basiculum*, 4 phenylpropanoids: basicorhammanoside, basicoglycoside, basidicoglycoside A and basicodiglycoside B were isolated and characterized. They were found to have potent antimalarial activity (IC50 2.36 to 9.50).**

Traditional plants recipes used to treat malaria are effective under the conditions recommended by the traditional healer and possess potent antimalarial molecules. This study approach is an important pathway to follow in search of for novel antimalarial molecules

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INTRODUCTION

Malaria is the leading cause of morbidity and mortality in sub-Saharan Africa. Malaria is endemic in Kinshasa, the capital city of Democratic Republic of Congo, with a yearly average morbidity of 900,000 cases with 17,000 cases of death for more than 10 million people [PNLP 2006]

Malaria, which is highly climate dependent, affects mostly tropical regions. As an analysis of meteorological data and malaria between 2004 and 2013 revealed that most cases of malaria in Kinshasa occurred during rainy season and that rain, relative air humidity and temperature influence positively malaria whereas evaporation, atmospheric pressure and large thermal amplitude contribute less to malaria [YINA et al.,2015].

Kinshasa, which has the status of a Province, is divided into four districts which are further divided into 24 municipalities (communes). Malaria prevalence is higher in peripheral and rural districts and lower in Central urban communes [FERRARI et al. 2016]. Peripheral and rural communes are poor and lack basic facilities, running water, electricity and adequate roads. The connection between malaria and poverty is well documented [FOSUS A. *and* MWABU G., 2015]; therefore policies and strategies to alleviate poverty in tropical regions must look for means to control malaria. People living in poor area such as the peripheral and rural communes of Kinshasa are prone to be infected by the malaria parasite, Plasmodium falciparum, and resort to use traditional plants to treat the disease because the cost of recommended standard drugs is beyond their mean.

SURVEY OF MEDICINAL PLANTS USED IN MALARIA MANAGEMENT IN KINSHASA

A survey was conducted from February 1997 to September 1997 on the eastern peripheral and rural area of Kinshasa to identify plants remedies used to treat malaria and fever. It is well stated that collaboration is needed between modern medical practitioners, scientists, traditional healers and users of plant recipes for the development of new drugs. Indeed two modern drugs use to treat malaria, Quinine and Artemisinin, are derived from plants. Safe plant remedies can be considered by health authorities as a promising approach in combating diseases at the primary health care level. We investigated plant remedies uses in traditional treatment of malaria and fever, and looked for concept underlying the preparation of the remedy specifically used to treat patients. We obtained specific information about plants, plant parts used, how the way plant material is collected and processed to remedies and how the remedies are administered and to whom[NGALAMULUME et al., 1995 *et* KASUKU et al., 1999]. Seven hundred and sixty-five (765) informants were recorded; among them 466 are users of the plant recipes. The higher number of users is a clear indication of the common use of plant recipes in case of fever or malaria in that part of town. Such self-medication can sometimes have dangerous consequences on the users if not well standardized.

Knowledge about the efficacy and risks of the use of these herbal treatments could provide useful information for health education and may also provide new leads for a search on new malaria chemotherapy.

A total of 55 species of plants distributed in 36 families used as antimalarial or antipyretic are reported in Table I. Among them Euphorbiaceae and Rubiaceae had each 5 species, Sterculiaceae 3 species, Monosaceae, Acanthaceae, Asteraceae and Verbaceae had each 2 species. The other families are represented with one species each. Predominance of Euphorbiaceae and Rubiaceae families in management of malaria has been reported in surveys of traditional plants to treat malaria in Benin [LAGNIKA et al., 2016], Togo [AGBODEKA et al., 2016] and Mozambique [JURG et al., 1991]. Many of the plants listed in Table Iare used also to cure several other diseases [KUBATA et al., 2005]. It is often how the way plant remedy is administered which defines the use. The Research Institute for Traditional Antimalarial Methods (RITAM) reported in 2004 that there are worldwide 1200 plant species from 160 families used to treat malaria and fever [WILCOX M.L *and* BODEKER G., 2004].

It was found in this survey that leaves were the most used part. Similar founding is reported in several studies in other parts of the world [LAGNIKA et al., 2016]. The other parts used are roots, stern bark, entire plant, fruits and nuts.

The most modes of preparation are decoction or infusion; drugs are administered orally. In the case of fever it is often advised to take a simple bath or a steam bath of the plant decoction, sometimes the plants mixture is used. Administration by purge is often used specially for children. It was found that recipes for malaria were often made of a single plant part (monotherapy) whereas the ones for fever often have been made of a combination of more than one plant. Chewing makes administration of nuts.

We found that 16 recipes were used for prevention, 36 as antipyretics and 27 as curatives for malaria. Morinda morindoides and Garcinia kola are used both for preventive and curative. Morinda morindoides was the most used plant for prevention and as antipyretic. The prevention of malaria for people living in endemic area is not recommended since frequent use of such remedies may lead to development of very resistant parasites to commonly use antimalarial drugs.

Table I | Plant species used as antimalarial or antipyretic in Kinshasa

	Plant species	Family	Purpose	Vernacular names	Method of preparation and administration and part used
1	*Acacia albida*	Mimosaceae	Cur.	Malu-malu (Ki)	Maceration, oral R
2	*Acacia raddiana*	Mimosaceae	Antip.	Mpesikyangolu (Ki)	maceration, oral R
3	*Acanthus montanus*	Acanthaceae	Prev./Cur.	Kikye-Kyango (Kis)	Infusion, oral L
4	*Adansona digitata*	Bombacaceae	Antip.	Nkondo (Ky)	infusion with O.gratissimum, steam bath L
5	*Afromomunal bovialacum*	Zingiberaceae	Antip.	Tondolo (Li)	decoction with O.gratissimum, steam bath L, AP
6	*Alchornea cordifolia*	Euphorbiaceae	Cur.	Kimbasila (Ki)	Decoction, oral R, F
7	*Aloë congensis*	Liliaceae	Cur./Antip.	Dibula (Lb)	steam bath P
8	*Amona senegalensis*	Annonaceae	Cur./Antip.	Kilolo (Ki)	decoction with C. occidentalis, oral L, R
9	*Andrapoga npinguipes*	Poaceae	Cur.	Mbuta (Ki)	maceration, oral A, P
10	*Boerhavia diffusa*	Nyctaginaceae	Cur.	Kabata-bata (Ky)	decoction with M.morindoides, simple bath A P
11	*Borreria chaetocephala*	Rubiaceae	Cur./Antip.	Mbula (Ki)	decoction, oralL, R
12	*Brillantaigia patula*	Acanthaceae	Prev./Cur.	Lembalemba (Ki,Ky)	decoction, oral L
13	*Caetocarpus africanus*	Euphorbiaceae	Antip.	Mpesi-mpesi (Ki)	infusion, oral R
14	*Capsicum fructescens*	Solanaceae	Cur./Antip.	Ndungizitedi (Ki)	decoction with O. gratissimum, purge F
15	*Carica papaya*	Caricaceae	Prev./Antip.	Payi-Payi (Li)	decoction, oral L
16	*Cassia occidentalis*	Caesalpinaceae	Prev./Cur./Antip.	Matsambi-tsambi (Ks)	decoction, oral L, R
17	*Chenopodium ambrosoides*	Chenopadiceae	Cur./Antip.	Manioka-nioka (Ki)	decoction, oral WP
18	*Cinchona succirubra*	Rubiaceae	Antip.	Kinini (Lb)	infusion, oral WP
19	*Cola acuminata*	Sterculiaceae	Cur./Antip.	Dikasu (Ki)	chew the fruit F
20	*Coleus kilimanjari*	Lamiaceae	Cur.	Mutuzo (Sh)	chewleaves L
21	*Combrelum prioides*	Combataceae	Cur.	Emanya (Sg)	Infusion, oral R
22	*Commelina lenghalensis*	Connalinaceae	Cur.	Uretejera (Kr)	Infusion, oral L
23	*Costus afer*	Zugiberaceae	Antip.	Musangamangani (Ki)	maceration in water, purge WP
24	*Croton mibango*	Euphorbiaceae	Antip.	Saku (Ya)	decoction, purge L
25	*Crossopteyx febrifuga*	Rubiaceae	Cur.	Mulolo (Ks)	maceration in palm wine , oral R
26	*Cymbopagon citratus*	Poaceae	Prev.	Sinda (Ks)	Infusion, steam bath L
27	*Cyperus articulatus*	Cyperaceae	Antip.	Tsaku-tsaku (Ks)	crash and anoint the body R
28	*Elaies guinensis*	Arecaceae	Antip.	Mbila (Li)	anoint the body for fever N
29	*Eucalyptus citriadora*	Myrtaceae	Antip.	Mukalbitusi (Sh)	decoction with C. papaya, steam bath L
30	*Euphorbiahirta*	Euphorbiaceae	Antip.	Mogboniangi (Go)	Decoction, oral WP
31	*Garcinia Kola*	Sterculiaceae	Prev./Cur./Antip.	Ngadiadia (Li)	chewseeds F
32	*Heinsia crinata*	Rubiaceae	Cur.	Kilanya (Ki)	Infusion, oral L
33	*Hymenocardia acida*	Euphorbiaceae	Cur.	Mpeti (Ki)	decoction with A. senegalensis, steam bath L
34	*Hyporis sp*	Hyponidaceae	Antip.	Kitundu (Ks)	maceration, oral R
35	*Hyptissna veoleus*	Hamiaceae	Antip.	Mubamvu-bamvu (Kl)	decoction, oral or purge L
36	*Lantana camara*	Verbenaceae	Cur.	ManyaKuku (Ky)	Infusion, oral AP
37	*MangiferaIndica*	Anararadiaceae	Cur./Antip.	Manga (Li)	decoction with O.gratissimum ,steam bath L

Part used: R= root ; L : Leaves ; SB : Stem Barks ; WP : Whole plant ; B : Branch ; F : Fruits ; N : Nuts ; AP : Aerial part ;

Vernacular name: Go = Gombe; Ki = Kikongo; Kl = Kilonzo; Kr = Kinyarwanda; Ks = Kisuku; Ky = Kiyanzi; Lg = Lega; Li = Lingala; Lb = Luba; Na = Nande; Sg = Songye; Sh = Swahili; Ya = yaka;

Table I | Plant species used as antimalarial or antipyretic in Kinshasa

	Plant species	Family	Purpose	Vernacular names	Method of preparation and administration and part used
38	*Markhame atomentosa*	Bignonuaceae	Cur./Antip.	Musengi (Ks)	maceration in water, purge R
39	*Moremi adissecta*	Convalulaceae	Antip.	Kangalupangu (Li)	Decoction, oral L
40	*Milletia setuelmean*	Fabaceae	Cur./Antip.	Mutambulangilu (Ks)	Decoction, oral R
41	*Morindamorindoides*	Rubiaceae	Prev./Cur.	Kongo bololo (Li)	Decoction, oral WP
42	*Nauclea latifolia*	Rubiaceae	Prev.Antip.	Kilolokiakwango (Ks)	crush in palm wine, oral L
43	*Ocimun gratissimun*	Lamiaceae	Prev./Antip.	Lumba-Lumba(Ki, Li, Lb)	Decoction, oral L
44	*Ocimun trichodon*	Lamiaceae	Prev.	Umwenya (Na)	Decoction, oral L, R
45	*Pentaclethra macrophylla*	Mimosaceae	Cur.	Bonja	decoction, oral L, F
46	*Phylanthus niruroides*	Euphorbiaceae	Prev./Cur.		chewleaves WP
47	*Rauwolfia vomitoria*	Apocynaceae	Antip.	Mulade (Lg)	Decoction, oral R
48	*Sesamun radiatum*	Pedaliaceae	Antip.	Buangila (Ki)	Decoction, oral L
49	*Sida acuta*	Malvaceae	Cur./Antip.	Kombo-kembo (Li)	maceration in water, purge L
50	*Solanum sp*	Solanaceae	Prev./Cur.	Kiludi (Ki)	decoction with M.morindoides, oral L
51	*Stirculia setigera*	Sterculiaceae	Cur.	umwete (Ki)	Decoction, oral SB
52	*Tephrosia vogelii*	Fabaceae	Cur.	Mbaaka (Ya)	decoction, purge L

Part used: R= root ; L : Leaves ; SB : Stem Barks ; WP : Whole plant ; B : Branch ; F : Fruits ; N : Nuts ; AP : Aerial part ;

Vernacular name: Go = Gombe; Ki = Kikongo; Kl = Kilonzo; Kr = Kinyarwanda; Ks = Kisuku; Ky = Kiyanzi; Lg = Lega; Li = Lingala; Lb = Luba; Na = Nande; Sg = Songye; Sh = Swahili; Ya = yaka;

The large number of plants and plant recipes found in this survey compared to studies done in other parts of the country is related to the fact that Kinshasa, being a heterogeneous community from different ethnics background belonging to different parts of the country, regroups also traditional knowledge from those different ethnic groups [MANSIANGI et al., 2002]. Result of the survey led us to carry out clinical investigations to assert the antimalarial efficacy of the most used plant recipes.

CLINICAL INVESTIGATION

Among the 58 plant recipes recorded, the eight plants most used to treat malaria (Cassia Occidentalis (CO), Carica papaya (CP), Cymbopogon citratus (CS), Garcinia kola (GK), Lantana camara (LC), Ocimum gratissimum (OG), Phyllanthus niruri (PN) and Vernoniaamygalina (VA)) were subjected to clinical investigation. These plants usually grown as herbs or shrubs in home gardens are therefore easily accessible hence their frequent use to treat malaria. Some of these plants are also reported to treat malaria in other countries in Africa [ODUGBENI et al., 2007; GESSLER et al., 1995].

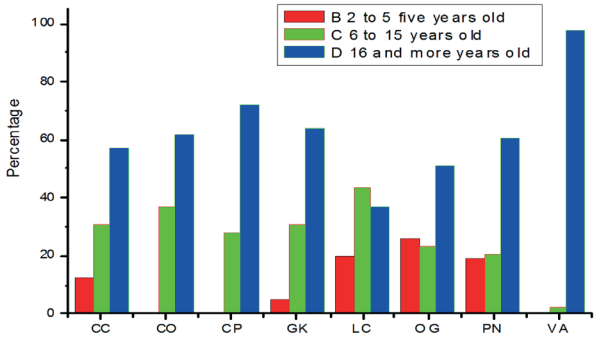


Figure 1: Repartition of patients for each plant recipes

Patient's selection was based on the following criteria (1) indication of malaria and parasites P. falciparum trophozoites in (see Table II)).

Table II | Traditional healer decoctions preparation and administered dosages

Plants	Part	Quantity used for decoction in (g/1 liter)[a]	Daily dosage	Duration of treatment (days)
C. citratus	leaves	10	1 x 2 Cad 1 x 1 Cch	4
C. occidentalis	Aerial part without seed	100	1 x 1 Cad 1 X 0.5 Cch	5
C. papaya	leaves	200	3 x 1 Cad 1st day 2 x 1 Cad 2 to 5 day 3 x 0.5 Cch,1st day 2 x 0.5 Cch 2to 5 day	5
G. kola	seeds		2 x 1 Sad to chew	5
L. camara	leaves	100	1 x 1 Cad 1 x 0.5 Cch	5
O. gratissimum	leaves	250	1 x 2 Cad 1 x 1 Cch	6
P. niruri	Aerial part	100	1 x 1 Cad 1 x 0.5 Cch	5
V. amygdalina	leaves	10	2 x 1 Cad 1 x 0.5 Cch	5

C= 145 ml; S= seed; 1 x or 2 x= one or two times a day; a= 30 minutes boiling; ad= adult; ch= child.

Patients with malaria were treated with each traditional remedy according to traditional healer's protocol at modern health center (Centre de MedecineMixteetd'Anemie SS de Kinshasa, Kalamu Yolo Giemsa stained film prepared from capillary blood;(2) the absence of anti-malarial drug use prior treatment; (3) age between 1 and 60 years old (pregnant women and severe sick patient were excluded

[JURG A. et al., 1991]. Four hundred and twenty nine patients participated in this study; repartition of patient for each plant recipes is shown in **Figure 1**.

The study was carried out from July 1998 to November 2000. According to age, 11% patients were between 2 to 5 years, 25 % between 6 to 15 years and 64 % were above 16 years old. Several patients knew some of the plant recipes and had been using them.

At the health center all external signs of malaria including fever, sweating, vomiting, nausea, headache, muscle and joint pains were recorded before and during treatment by qualified medical personnel. Blood samples were also taken at the starting and at days 5,7,9 and 11 of treatment. Asexual parasitaemia was quantified by counting white blood cells (number of asexual parasites/500 leucocytes X 8000 leucocytes/µl). The results of clinical investigation are reported in Table III. Patients diagnosed with malaria were treated for a period of 7 days. By the fifth day, all symptoms of malaria disappeared in all cases. The level of malaria parasites in the blood, however, did not completely disappear. Although the number of participants in the clinical investigation is small, the results obtained give a clear trend on the efficacy of the recipes. The percentage of recovery calculated based on both the amount of residual parasite in the blood and the elimination of symptoms is above 60 %. It ranged from 97 % for C. occidentalis Linn to 67 % for V. amygdalina Delile. The observed high level of recovery of more than 90 % showed that plant remedies are potential candidates in the search of new molecules with potent biological activities [TABA et al., 2012]. All the eight plants recipes contain active effective antimalarial compounds. The dose and the duration of treatment proposed by the traditional healers are appropriate since there was reduction of side effects (headache, fever and parasiteamia).

To evaluate recipes toxicity, ten patients were selected randomly for each recipes and submitted to the following biochemical tests: bilirubine (total and direct) transaminases (SGOT, SGPT), urea, and creatine for kidney, and glycemia (Carried out at Fondation Lurhuma, Kinshasa)

Table III| Results of clinical investigation

Plants	N° of patients	% of recovery
C.citratus	56	93
C. occidentalis	52	97
C. papaya	46	94
G. kola	39	94
L. camara	50	90
O. gratissimum	69	86
P. niruri	83	93
V. amygdalina	40	67

No toxicity was found with the dosage recommended by the traditional healer, however minor side effect was reported in the case of Garcinia kola and Phyllanthus niruri. They were found to have disturbing effect on creatine and direct Billirubine. P. niruri was also found to have an effect on transaminase fraction GOT whereas O. gratissimum on GPT fraction. The antimalarial effects of P. niruri and G.kola on creatine and Billi rubineis are in contradiction to the hepato protecting effect of these two plants reported in the literature [WEGWU *AND*DIDIA, 2007].

It was also found also that O. gratissimum had hypoglycemia effect. Several studies have reported similar observation [MOHAMMED *et al.*, 2007]. In view of this finding, patients taking these recipes were advised to take a soft drink in order to increase sugar level in blood. It is reported that some traditional healers use this plant to treat diabetes.

Clinical study has shown that the recipes can be used for uncomplicated case of malaria because they are effective, safe, and no toxic in the dosage recommended by the traditional healer. A small booklet was then published with some standardized recipes for dissemination mostly in the peripheral parts of Kinshasa [KASUKU et al., 1998]. We then set Forth for the next step to evaluate in vitro antimalarial activity of the recipes.

IN VITRO ANTIMALARIAL ACTIVITY

Crude ethanol extracts were prepared for primary in vitro antimalarial assay. The dried and grinded leaves or seeds (200 g) was taken up in 1 liter ethanol and refluxed for 4 hours. Upon cooling, ethanol was removed under reduced pressure.

Table IV| Anti malarial activities of alcohol plant extracts

Plants	Concentration µg/mL	% inhibition
Cymbopogon citratus	25	93
	12.5	33
Cassia occidentalis	25	83
	12.5	24
Carica papaya	25	91
	12.5	20
Garcinia kola	n.d.	n.d.
Lantana camara	25	93
	12.5	0
Ocimum gratissimum	12.5	100
Phyllanthus niruri	12.5	100
Vernonia amygdalina	25	0
Chloroquin	0.1	100

The in vitro assays were conducted by using the micro dilution technique of Desjardin [DESJARDIN et al., 1997]. The P. falciparum parasites were derived by direct visualization and micromanipulation from fresh patient isolates. The test compounds were initially dissolved in ethanol: water mixture (1:3) or in dimethylsulfoxide and diluted 100 fold in Roswell Park Memorial Institute 1640 RPMI (Sigma Aldrich) culture medium, supplemented with 25 mM Hepes and 32 mM $NaHCO_3$. These solutions were diluted in 10 different concentrations. The parasites were exposed to different dilutions of each compound for 48 hrs and incubated at 37 °C. Direct estimation of parasite growth inhibition was used and it was based on direct reading of smears made in 24 well, flat-bottomed plates to estimate growth and evolution stages of parasites. Parasitaemia and parasite stage were determined after 48hrs of contact between extracts and parasites. Concentration-response data was analyzed by nonlinear regression logistic dose response model and IC50 values for each compound were determined graphically in terms of concentration versus inhibition percentage [KAYEMBE S.J., 2010].

Inhibition percentage at 12.5 µg range from 100 % for O. gratissimum and P. niruri to 0 % for V. amygdaline; at 25 µg the inhibition percentage was above 80 % for all plant recipes except for V. amygdaline. Our Results for V. amygdaline are contrarily to those reported by Agbodeka et al.[2016] who found an IC50 of 11.2ug for similar extract. The difference could be due to the geographical location of the cultivated plant.

We carried then color based chemical screening of the different recipes according to the procedure described by [BRUNETON J., 1999] to identify major's secondary metabolites most likely responsible of the antimalarial activity. The results obtained are shown in Table V.

The amounts and the type of secondary metabolites varied from each plant. C. occidentalis alcoholic extract had a large amount of flavonoids and quinones than alkaloids. G. kola had more flavonoids

Table V| Results of chemical screening of plants

	C. citratus	*C. occidentalis*	*C. papaya*	*G. kola*	*L. camara*	*O. gratissimum*	*P. niruri*	*V. amygdalina*
Saponins	+	++	++	+	+	+	-	+
Flavonoids	+	+++	+	++	+	++	+++	++
Quinones	+	+++	-	+	-	-	-	-
Tannins	+	+++	+	+	-	++	++	+
Alkaloids	-	++	++	++	+	+	++	+
Anthocynanins	+	+	-	+	+	+	-	-
Steroids	+	++	+	-	+	+	-	-

Table VI | IC_{50} values of isolated compounds

Isolated compounds	Plant species	Eluents	Rf	IC_{50} value, µg/ml
Terpenes	Cassia alata	EtOAc : PE 1 : 4	0.35 0.48 0.55 0.65	0.94 0.23 0.44 0.52
	Ocimum gratissimum	EtOAc : PE 1 : 4	0.06 0.14 0.21 0.37 0.47 0.59 0.87	0.32 0.27 1.41 3.96 0.44 0.65 0.52
Quinones	Cassia alata	Pyridin : amyl alcohol : H_2O 4 : 6 : 3	0.61 0.70 0.86 0.92	<0.1 5.4 0.54 <0.25
	Cassia occidentalis	$CHCl_3$: EtOAc 5 : 1	0.17 0.28 0.33 0.53 0.64 0.84	0.25 0.25 <1 0.25 <0.1 <0.1
	Garcinia kola	EtOAc : PE 1 : 1.2	0.17 0.25 0.42 0.54 0.85	1.02 2.0 12.9 15.75 n.d
	Ocimum basilicum	EtOAc : PE 1 : 1.2	0.12 0.23 0.50 0.63 0.88	0.52 <0.35 1.42 <0.35 18
Flavonoids	Cassia alata	EtOAc : Formic acid : Acetic acid : H_2O 10 : 1 : 1 : 3	0.24 0.35 0.44 0.58 0.65 0.78 0.94	0.75 n.d 26.5 0.5 1.25 19 12.5
Flavonoids	Cassia occidentalis	EtOAc : Formic acid : Acetic acid : H_2O 10 : 1 : 1 : 3	0.26 0.35 0.43 0.58 0.89 0.97	12.5 5 0.25 0.1 0.1 0.1
	Garcinia kola	CCl_4 : EtOH 1.8 : 1	0.15 0.45 0.52 0.64	<1.5 3 n.d 10
	Ocimum basilicum	EtOAc : Formic acid : Acetic acid : H_2O 10 : 1 : 1 : 3	0.21 0.51 0.75 0.87	0.18 0.30 9.8 0.44

EtOAC:Ethylacetate; PE: Petroleum Ether; EtOH: Ethanol

Table VI | IC_{50} values of isolated compounds

Isolated compounds	Plant species	Eluents	Rf	IC_{50} value, µg/ml
Alkaloids	Carica papaya	Total		185
		EtOAc : *n*-hexane 1 : 1	0.50	50
	O. gratissimum	Total		100
		EtOAc : *n*-hexane 1 : 1	0.20	<65
	Phyllantus niruri	Total		100
		EtOAc : *n*-hexane 1 : 1	0.23	<8

EtOAC:Ethylacetate; PE: Petroleum Ether; EtOH: Ethanol

and alkaloids and less amount of quinone. All eight plants extracts had the most likely known antimalarials metabolites: alkaloids, flavonoids, quinones and terpenes.

ANTIMALARIAL ACTIVITIES OF SECONDARY METABOLITES

The most likely antimalarial secondary metabolites, alkaloids, quinones, terpenes and flavonoids of some of the plant recipes were isolated according to the classical procedure described by Bruneton[1993] by thin layer chromatography analysis and preparative on pre-coated Silica gel 60 F254 Merck plates. The spots were observed under UV light (254 and 366 nm). Ocimum basilicum was added because of its use and it belongs to the same family with O. gratissimum. The IC50 values of isolated compounds are reported in Table VI.

Among 11 isolated terpenes, 5 (T1 and T2 of O. gratissimum, T2 and T3 of C. alata) have a IC50 value < 0.5 µg/ml and two only have their IC50 between 1 and 4µg/ml [KAYEMBE et al., 2012]. Terpenes present interesting antimalarial activities, which explains the unceasingly crescent interest granted to this class of metabolites since the discovery of Artemisinine[BRICKIN, 2000; KALAUNI et al.2006 ; MA et al. 2006].

The IC50 values reported for many terpenes described as very active are between 0.1 and 3.5µg/ml, the interval in which all the values determined for various terpenes isolated by our team. Indeed, 44 Cassane and Norcassane types terpenes isolated from Caesalpinia crista in Indonesia appeared very active against plasmodiums with IC50 of 0.29 µM; whereas the 3 triterpenes isolated from Zizyphuscam bodiana have IC50 of 3.7, 0.9, and 3.0 µg/ml respectively.It is the same for terpenes isolated from Grewiabila melleta [KALAUNI et al. 2006; MA et al. 2006]. Terpenes are major components of essential oils of the plants and have various therapeutic virtues justifying their use in traditional medicine for the treatment of many affections.

Among the 20 isolated quinones, 11 have IC50 lower or equal to 0,6µg/ml. They are Q1, Q3 and Q4 quinones of C.alata, Q1, Q2, Q4, Q5, and Q6 of C.occidentalis, Q1, Q2 and Q4 of O. basilicum. Three quinones only had an IC50 ranging between 10 and 20µg/ml; they are Q3 and Q4 quinones of Garcinia kola and Q5 of O. basilicum.The others have values of IC50 ranging between 1 and 6µg/ml [KAYEMBE et al., 2010]. Growing interest to quinones arose since the discovery of in vitro antimalarial activity of Atovaquone[BASCO et al. 1995]. Seven quinones isolated from Salacia krausii by Figueiredo et al. [1998]presented potent activity against Plasmodium falciparum. Naphtoquinones from Kigeli apinnata were reported to possess a very good in vitro antimalarial activity (IC50≈0.002µg/ml) [WEISS C.R.et al. 2000].

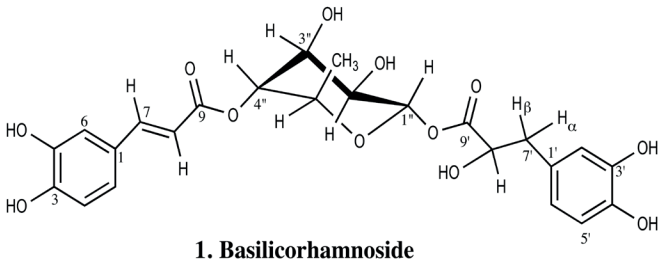
Moreover a quinone named Xestoquinone isolated from a marine sponge (Xestospongia sp.) in Vanuatu showed a promising growth inhibiting activity on FCB1 of P. falciparum with a IC50 of 3 µM [LAURENT et al. 2006]. These results added to ours, show that quinones are a class of compounds with potent antimalarial activity and could be used as starting point for the synthesis of antimalarial leads molecules.

Observation of Table VI reveals that of 21 isolated flavonoïdes, 8 have an IC50 value lower or equal to 0,5µg/ml (flavonoïdes F1, F2 and F4 of O. basilicum; F4 of C. alata; F3, F4, F5, and F6 of C. occidentalis). Only one flavonoide (F3 of C. alata) has a IC50 higher than 25 µg/ml while the rest of flavonoids have IC50 ranging from 1 to 15µg/ml. Many studies were undertaken on the antimalarial activity of flavonoïdes. Flavonoïdes named Exiguaflavones A and B isolated from Artemisia indica presented IC50 value of 4,6 and 7,05µg/ml respectively on Plasmodiums in vitro[CHANPHEN et al. 1998]. Isoflavones from Andirainernis as well as Biflavonoïdes from Ochnain terregerrinma presented very interesting activities on chloroquine resistant Plasmodiums[ICHINO et al. 2006]. Let us note that the activities described as promising by various authors for many flavonoïdes isolated from plants are from 4 to 10µg/ml [VAN BAREN et al. 2006; WIRASATHIEN et al. 2006]. These values are higher than the majority of those consigned confined in Table VI. The interesting activity of this class pushed Picot and its colleagues to affirm that flavonoïdes can, in an immediate future, be associated with the existing drugs used in the treatment of malaria to delay the appearance of resistance of Plasmodium [MONBRISON et al. 2006].

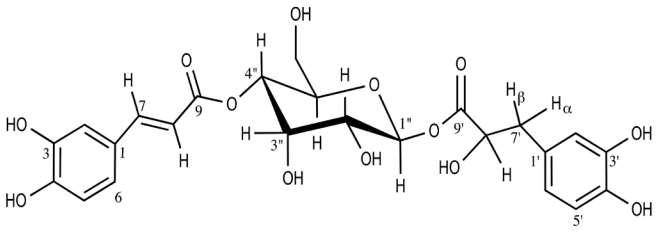
Antimalarial activities of four phenylpropanoids isolated from butanolic fraction of O. basilicum

Among usual common antimalarials, we find amino quinolines (quinine, chloroquine, amodiaquine etc....) and terpenes whose basic skeleton is the artemisinine [WANG *and* XU 1985]. Rare are those which have a quinone structure like Lapinone and Lapachol [THE MERCK, INDEX, 2005]; and very little have flavonoid structure [LI *X*, RIECKMANN K., 1992]. However, there are no works that mention antimalarial activity ascribable to compounds resulting from the n-butanolic extract that contains primarily saponins, glycosides and derivatives of the caffeic acid. However, all these compounds possess many other biological activities [SHIAO et al. 2002; NAGAI et al..1996; MAZUNDER et al.. 1997; FUNG K.P. et al..2005]. Thus, we aim to isolate, and to try to elucidate structures of some compounds isolated from this extract in order to test assess their antimalarial activities in vitro[KAYEMBE, 2010].

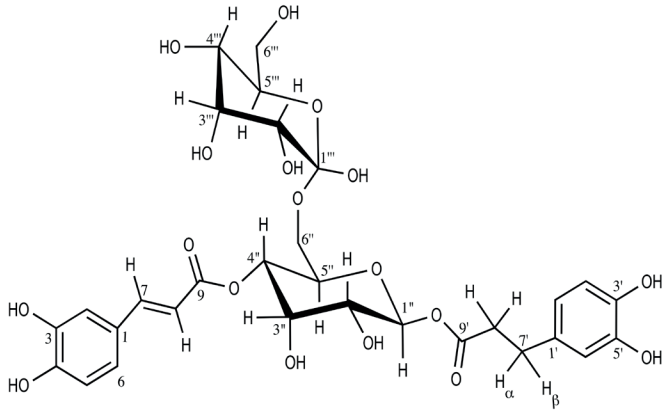
The crude methanolic extract of O. basilicum leaves was suspended in MeOH – H_2O 8:2 mixture and successively extracted with Petroleum ether (60 - 80°C fraction), $CHCl_3$, EtOAC, and n-BuOH. Vacuum liquid chromatography, column chromatography (CC) on silica gel, polyamide and Sephadex LH 20 of the n-BuOH phase led to the isolation of four phenylpropanoid glycosides esters 1 – 4 (**Figure 2**). Structures of these compounds were elucidated by spectroscopic techniques (MS; ^{1}H-NMR, ^{13}C-NMR, HMBC, IR) and they were found to be (1)Basilicorhamnoside ((4 – O – Caffeoyl, 1 – O – 2 Hydroxy (1), 3 – (3',4' Dihydroxyphenyl) PropanoylRhamnopyranoside); (2)Basilicoglycoside ((4 – O – Caffeoyl, 1 – O – 2 Hydroxy, 3 – (3',4' Dihydroxyphenyl) PropanoylGlycopyranoside); (3)Basilicodiglycoside A ((4 – O – Caffeoyl, 1 – O – 2 Hydroxy, 3 – (3',4' Dihydroxyphenyl) Propanoylα – D - glycopyranosyl(1 → 6)α – D - Glycopyranoside); and (4) Basilicodiglycoside B ((4 – O – Caffeoyl, 1 – O – 2 Hydroxy, 3 – (3',4'



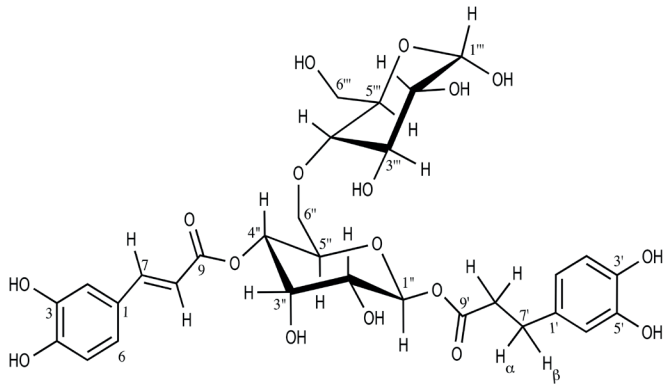
1. Basilicorhamnoside



2. Basilicoglycoside



3. Basilicoglycoside A



4. Basilicoglycoside B

Figure 2. Structures of phenylpropanoids isolated from *O. basilicum*

Dihydroxyphenyl) Propanoylβ – D - glycopyranosyl(4 → 6)α – D - Glycopyranoside) (4).

All these compounds were found to have possess a potent antimalarial activity as shown in Table VII.

Table VII | IC50 values of compounds 1, 2, 3 and 4

Compound	IC50 values (µM)
Basilicorhamnoside	2.37
Basilicoglycoside	4.21
Basilicodiglycoside A	9.50
Basilicodiglycoside B	2.36

These results reveal that 3 out of the four isolated and identified phenyl propanoids have IC50 value < 2.5 µg/ml and thus are very active; the fourth compound has also a considerable activity (9.5 µg/ml). It should be noted that in spite of many works devoted to the butanolic extracts of plants [HUANG Y. *and* ZHANG 1992; SPORN et al., 1994; ZOU et al..1993], no other report is found to date, to our knowledge, on the antimalarial activity of phenylpropanoid glycosides.

Traditional plant remedies to treat malaria were found to be effective and no toxic under the conditions recommended by the traditional healer. Secondary metabolites isolated from some of the remedies were found to possess high antimalarial activities.

From the butanolic fraction of Cassia alata, 4 phenyl propanoids glycosides were isolated and characterized. Those glycosides have a potent antimalarial activity.

RÉSUMÉ

La malaria est la principale cause de la morbidité et de la mortalité en Afrique Sub-Saharienne. À Kinshasa, capitale de la RD Congo, la malaria est plus répandue dans des communes rurales périphériques pauvres. Une enquête effectuée dans la partie périphérique Est de Kinshasa sur des remèdes à base des plantes utilisés par des tradipraticiens a relevé un total de 55 plantes réparties en 36 familles ; les familles les plus représentées sont les Euphorbiaceae et les Rubiaceae. Les huit plantes les plus utilisées : *C. occidentalis, C. papaya, C. citratus, L. camara, G. kola, O. gratissimum, V. amygdalina*, et *P. niruri* ont été soumises aux investigations cliniques selon le protocole du tradipraticien. Le pourcentage de guérison des patients volontaires s'étend de 97 à 67 %. Les tests biochimiques sur des patients sous traitement n'a montré aucune toxicité des recettes prises selon la dose et la durée proposées par le traditipraticien. La détermination de l'activité antimalarienne *in vitro* des extraits éthanoliques bruts de ces huit plantes a montré que l'inhibition de *P. falciparum* pour une concentration de 12.5 µg/mL va de 100 % pour l'*O. gratissimum* à 0 % pour le *V. amygdalina*. Quelques métabolites secondaires, les terpènes, les quinones, les flavonoïdes et les alcaloïdes isolés de certains de ces extraits de plantes ont montré un CI50 en-dessous de 0.5 µg/mL, ce qui est une indication claire de l'activité antimalarienne significative de ces métabolites. Dans la fraction butanolique de l'*O.basiculum*, 4 phénylpropanoïdes : le basicorhammanoside, le basicoglycoside, le basidicoglycoside A et le basicodiglycoside B ont été isolés et caractérisés. Ils possèdent une importante activité antimalarienne *in vitro* (CI50 de 2.36 à 9.500.5 µg/mL). Les recettes traditionnelles de plantes employées pour traiter la malaria sont efficaces dans les conditions recommandées par le guérisseur traditionnel et renferment des molécules à activité antimalarienne importante. L'approche de cette étude est une voie intéressante à suivre dans la recherche de nouvelles molécules à activité antimalarienne

Mots-clés : *Traitement de la Malaria, Remède Traditionnel, Métabolites secondaires, Activité Anti malarienne, phenylpropanoïdes, Kinshasa*

REFERENCES AND NOTES

AGBODEKA K., GBEKLEY H.E.,KAROU S.D., ANANI K., AGBORON A.,TCHACONDO T., BATAWILA K.,SIMPORE J., *and* GBEASSOR M.[**2016**]. Ethobotanical study of medicinal plants used for treatment of malaria in the Plateau region, Togo. *Pharmacognosy Research*, *8* (suppl. 1) 12-18.

BASCO L.K., RAMILIARISOA O.,LE BRAS J.[**1995**]. *In vitro* activity of atovaquone against the African isolates and clones of *Plasmodium falciparum* *Am. J. Trop. Med. Hyg.* *53*(**4**) :388-391.

BRICKIN J., NJIFUTIE, YAFORFOYER J.A., BOSCO K. *and* RINGUARD

P. [**2000**]. *In vitro* Antimalarial activity of limonoides from *Khaya grandifolia*. *J. Ethnopharmacol.* 68(**2**), 27 – 33..

BRUNETON [**1999**] *J.Pharmacognosie, phytochimie des plantes médicinales.* 3e Edition, Revue *et* Augmentée, Tec & Doc, Paris.

CHANPHEN R., THEBTARANONTH Y., WANAUPPATHAMKUL S., YUTHAVONG Y. [**1998**]. Antimalarial principles from *Artemisia indica* J. *Nat.Prod.*61(**9**) : 1146-1147.

DE MONBRISON F, MAITREJEAN M, LATOUR C, BUGNAZET F, PEYRON F, BARRON D, PICOT S. [**2006**]. *In vitro* antimalarial activity of flavonoid derivatives dehydrosilybin and 8-(1;1)-DMA-kaempferide. Acta Trop. 97(**1**) :102-107.

FERRARI G., NTUKU H.M., SCHMIDLIN S., DIBOULO E. *and* TSHEFU A.K., [**2016**]. A malaria risk map of Kinshasa, Democratic Republic of Congo, *Malaria journal, 15* :27.

FIGUEIREDO J.N., RAZ B., SEQUIN U.[**1998**]. Novel quinonemethides from *Salacia kraussii* with *in vitro* antimalarial activity. *J. Na.t Prod.* 61(**6**):718-723.

FOSU A., MWABU G. MALARIA *and* POVERTY IN AFRICA, [**2015**] Africa Books Collection, Ghana .

GESSLER M.C., MSUYA D.E., NKUNYA M.H.H., MWASUMBI L.B., SCHAER A., HEINRICH M. *and* TANNER M. [**1995**]. *Journal of ethnopharmacology* 48 : 131-144.

HUANG Y., ZHANG J.[**1992**]: Peroxidation inhibition of rat microsomes by cafeic acid derivatives, *Acta Pharm. Sin. 27*; 96 – 100.

ICHINO C., KIYOHARA H., SOONTHORNCHAREONNON N., CHUAKUL W., ISHAYAMA A., SEKIGUSHI H., NAMATAME M., OTOGURO K., OMURA S. *and* YAMADA H.[**2006**].Antimalarial activity of Biflavonoids from *Ochnainte gerrina*, *Planta med.* 72(**7**) :611-614.

JURG A., TOMAS T. *and* PIVIDAL J. [**1991**].Antimarial activity of some plant remedies in use in Marracuene, Southern Mozambique, *J. Ethnopharmacol., 33* : 79-83.

KALAUNI S.K., AWALE S., TEZUKA Y., BANSKOTA A.H., LINN T.Z., ASIH P.B., SYAFRUDDIN D., KADOTA S.[**2006**]. Antimalarial activity of cassane- and norcassane-type diterpenes from *Caesalpinia crista* and their structure-activity relationship, *Biol Pharm Bull. 29*(**5**) :1050-1052.

KASUKU W. LULA F. PAULUS J. KABELE N. *et* KALWILA K. [**1999**]. Contribution à l'inventaire des plantes utilisées pour le traitement du paludisme à Kinshasa, *Rev. Pharm. Afr. 13*(**1**), 95-105.

KASUKU W., LULA F. *and* PAULUS J. [**1998**]. Cinq plantes pour combattre la malaria, Collection se prendre en charge, Libraries Paulines 4e Edition, Dépôt légal N° 0762.9502, Kinshasa-Limete .

KAYEMBE S.J. [**2010**]. Etude phytochimique et de l'activité antipaludique in vitro de quatre composés isolés de l'extrait butanolique des feuilles de l'*Ocimum basilicum* L. Thèse de doctorat, Université de Kinshasa.

KAYEMBE S.J., TABA K.M.,NTUMBA K.J. *and* KAZADI K.T. [**2012**]. *In vitro* antimalarial of eleven terpenes isolated from *Ocimum gratissimum* and *Cassia alata* leaves. Screening of their binding affinity with haemin, *Journal of plant studies, 1* (**2**) : 168-172.

KAYEMBE S.J., TABA K.M., NTUMBA K.J. TSHIONGO M.T.C. *and* KAZADI K.T. [**2010**]. *In vitro* anti-malarial activity of twenty quinones isolated from four plants used by traditional healers in the Democratic Republic of Congo, *Journal of Medicinal Plants Research, 4* (11), 991-994.

KUBATA B.K., NAGAMUNE K., MURAKAMI N., MERKEL P., KABUTUTU Z., MARTIN S.K., TABA K.M.,MUSTAKUK H., YOSHIDA M., OHNISHI-KAMEYAMA M., KINOSHITA T., DUSZENKO M. *and* URADE Y. [**2005**]. *Kola acuminate* proanthocyanidins: a class of anti-trypanosomal compounds effective against *Trypanosoma brucei*, *Inter. J. for Parasitology, 35* : 91-103.

LAGNIKA L., DJEHOUE R., YEDIMONHAN H., *and* SANNI A. [**2016**]. Ethnobotanical survey of medicinal used in malaria management in South Benin, *Journal of Medicinal Plant research, 10* (41) : 748-756.

LAURENT D, JULLIAN V, PARENTY A, KNIBIEHLER M, DORIN D, SCHMITT S, LOZACH O, LEBOUVIER N, FROSTIN M, ALBY F, MAUREL S, DOERIG C, MEIJER L, SAUVAIN M. [**2006**]. Antimalarial potential of xestoquinone, a protein kinase inhibitor isolated from a Vanuatu marine sponge *Xestospongia sp,*| *Bioorg. Med. Chem.*14(13) :4477-82.

MA C., ZHANG H.J., TAN G.T., HUNG N.V., CUONG N.M., SOEJARTO D.D., FONG H.H.[**2006**]. Antimalarial compounds from *Grewiabila mellata*, *J. Nat.Prod. 69*(**3**) :346-350.

MANSIANGI P., TABA K.M. *et* PAULUS J. [**2002**]. Les plantes anti malariennes dans les provinces de Bandundu et du Bas Congo, *Ann.Fac. Sciences, 2* : 43-46.

MOHAMMED A., TANKO Y., OKASHA M.A., MAGUJI R.A. *and* YARO A.H. [**2007**]. Effects of aqueous leaves extract of *Ocimum gratissimum* on blood glucose levels of streptozocin-induced diabetic wistar rats, *African Journal of Biotechnology, 6*(18) : 2087-2090.

NGALAMULUME T., PAULUS J., NLANDU L. *et* KIZEKA K. [**1995**]. Plantes medicinales à usage domestique cultivées dans deux quarties de Kinshasa, *Rev. Pharm. Afr. 9* (**1**) : 9-14.

ODUGBENI T.O., AKINSUBIRE O.R., AIBINU I.E. *and* FABEKU P.O. [**2007**]. *Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, Southern west Nigeria, African Journal of Traditional Complementary and Alternative Medecines, 4* (**2**) : 191-198.

PNLP [**2013**] Programme National de lute contre le paludisme en RDC.

Sporn M.B., Roberts A.B. (1994): Goodman D.S., The retinoids: Biology, Chemistry *and* Biochemistry, 2nd ed. Raven Press: New York.

TABA K.M., PAULUS J. *and* KAYEMBE J.S. [**2012**].Malaria : novel plant remedies show great promise in treating the deadly disease, *Global Journal of research on medicinal plants &Indigeneous medicine,1*(**3**) : 62-68.

VAN BAREN C., ANAO I., LEO DI LIRA P., DEBENEDETTI S., HOUGHTON P., CROFT S., MARTINO V. [**2006**]. Triterpenic acids and flavonoids from Saturjaparvifolia.Evaluation of their antiprotozoal activity, *Z. Naturfosch C.*61(3-4) :189-192.

ZOU Z., XU L.N., TIAN J.Y. [**1993**]: Inhibitory activity of cafeic derivatives on venous thrombosis, *Acta Pharm. Sin. 28*; 241 – 245.