



Evaluation of antiplasmodial activity of extracts from endemic medicinal plants used to treat malaria in Côte d'Ivoire

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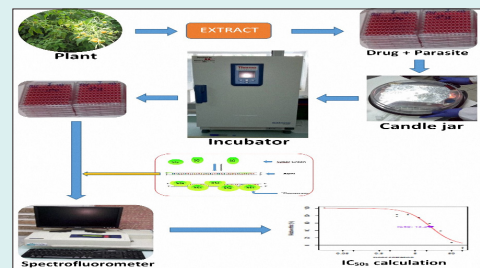
Abstract

Introduction: *Plasmodium falciparum* strains had been increasingly resistant to commonly used molecules including artemisinin. It is therefore urges to find new therapeutic alternatives.

Methods: In this study, the antiplasmodial activity of 21 extracts obtained from seven plants of the *Anthocleista djalonenis*, *Cochlospermum planchonii*, *Harungana madagascariensis*, *Hoslundia opposita*, *Mangifera indica*, *Margaritaria discoidea* and *Pericopsis laxiflora* of the Ivorian pharmacopoeia was evaluated on the chloroquine sensitive (NF54) and multi-resistant (K1) reference strains and on clinical isolates as well. The technique used was the microtiter method based on fluorescence reading with SYBR Green.

Results: The aqueous extract of the bark of *H. madagascariensis* and methanolic extracts of *P. laxiflora* showed the best antiplasmodial activity with IC₅₀ values of 6.16 µg/mL and 7.44 µg/mL, respectively. On the other hand, extracts of *M. indica* showed a very moderate activity with IC₅₀ values between 15 µg/mL and 50 µg/mL (5 < IC₅₀ < 50 µg/mL) on the same strains of *P. falciparum*. Only the aqueous extract of *A. djalonenis* had IC₅₀ values greater than 50 µg/mL. The phytochemical analysis showed a strong presence of polyphenols and alkaloids in extracts with a cumulative rate of 90.47% and 95.23%, respectively.

Conclusion: The results obtained were also justified by the composition of these plants, which have several secondary metabolites involved in the treatment of malaria. The antiplasmodial properties of these plants could partially justify their use in malaria treatment. Further studies on these extracts are needed to manufacture a stable galenic formulation for the development of an improved traditional medicine.



Introduction

Malaria remains a major public health concern, affecting a large part of the population. According to the World Health Organization (WHO), there were approximately 219 million cases of malaria worldwide and 435,000 deaths in 2017.¹

Most cases and deaths occurred in the WHO African region and mainly affected children and pregnant women.^{2,3} In Africa, malaria accounts for more than 10% of the total burden of diseases.⁴ In Côte d'Ivoire, malaria is the main cause of morbidity and mortality of children under 5, and is responsible for 33% of the outpatient visits, 40% of the school and professional absenteeism, and 50% of the agricultural income lost. Ivorian populations spend

about 25% of their income on the prevention and treatment of this disease.^{5,6} In this situation, it is essential to have effective drugs to fight against malaria. However, there is a spread of *Plasmodium falciparum* resistance to many antimalarial drugs, including artemisinin derivatives.^{7,8}

The emergence of this resistance phenomenon seriously compromises the success of currently cared out global operations to reduce the burden of malaria and justifies a continuous effort to find new molecules.⁹ In Africa and most developing countries, 80% of the population uses traditional medicine as first aid, and malaria accounts for about 20% of cases of treated diseases. Indeed, populations prefer preparations made from the leaves and bark of medicinal plants, which are more accessible, less restrictive



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and especially, less expensive than modern antimalarial drugs.¹⁰ Thus, traditional medicine used by populations for thousands of years has proved to be very useful in treating many diseases. At present, the most effective antimalarial drugs, quinine and artemisinin, come from *Cinchona officinalis* plants and *Artemisia annua* from the traditional Peruvian and Chinese pharmacopeia, respectively, which are used by centuries for treating malaria.¹¹

In Côte d'Ivoire, several traditional pharmacopeia plants have been used by various traditional practitioners in malaria treatment. Among them are *Anthocleista djalonenis* (Loganiaceae), *Cochlospermum planchonii* (Cochlopermaceae), *Harungana madagascariensis* (Hippuriaceae), *Hoslundia opposita* (Lamiaceae), *Mangifera indica* (Anacardiaceae), *Margaritaria discoidea* (Euphorbiaceae), and *Pericopsis laxiflora* (Fabaceae).¹²⁻¹⁴ This work is in line with research on the development of traditional pharmacopeia in order to find new molecules to strengthen the therapeutic arsenal against malaria. These plants have been poorly studied from the viewpoint of their antimalarial effect, particularly on field isolates. Thus, the general objective of this work was to evaluate *in vitro* antiplasmodial effect of endemic medicinal plants.

Materials and Methods

Plant material

Plant material consisted of bark of *M. indica*, *H. madagascariensis* (Hippuriaceae), with *P. laxiflora*, and leaves of *A. djalonenis*, *C. planchonii*, *M. discoidea*

(Euphorbiaceae), and *H. opposita* (Fig. 1). Plant material was collected from the locality of Moronou located 20 km from Toumodi in the Center of Côte d'Ivoire, GPS coordinates 6°19'N, 4°58'W. It is located 198 km from Abidjan (economic capital) and 30 km from Yamoussoukro (political capital). Its population is estimated at 4000 inhabitants, is a sub-group of Baoulé (Akan people) called N'gban. The climate is of the equatorial type of transition between the Sudanese and Guinean climates called the Baouleian climate. It is characterized by two rainy seasons (March to June and September to October) and two dry seasons (July to August and November to February) with the presence of harmattan.¹³ Samples were collected in September 2014, early in the morning (8:00 AM) at the site at various locations. After identification at the National Floristic Center (University of Félix Houphouët Boigny, Côte d'Ivoire) by ASSI Jean and a deposit of samples to the herbarium, the remaining plant material was dried in the open air away from the sun during two weeks, then reduced to a fine powder using a blender.

Materials

We needed RPMI 1640 (Gibco®, Life Technology, UK), sodium bicarbonate (NaHCO₃) (Sigma Chemical Co., USA), NaCl (Sigma Chemical Co., USA), HEPES (Sigma Chemical Co., USA), tritiated hypoxanthine (Sigma Chemical Co, USA), glycerol (Sigma Chemical Co, USA), D-sorbitol (Sigma Chemical Co., USA), and Albumax® (Gibco®, Life Technology, UK). These products were

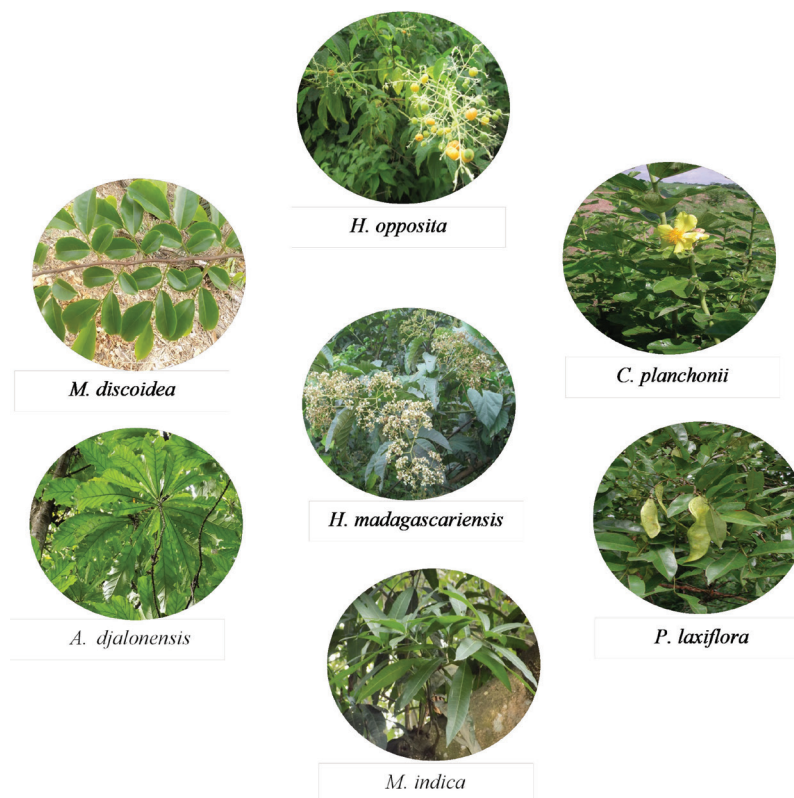


Figure 1. Study plants.

necessary for the preparation of the culture medium: Sodium bicarbonate and HEPES [N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)]. The antibiotic used to limit microbial contamination was neomycin (Sigma Chemical Co, USA).

The technical equipment used mainly were 96-well microplates (Costar™ 96), Leica® DM500 microscope, laminar flow hood (ESCO Airstream® Class II), incubator (Heratherm®, Thermo Scientific) with a spectrofluorometer (Spectra max GEMINI XPS, Molecular Devices).

Preparation of plant extracts

For better extraction of bioactive substances, water, ethanol, and methanol were used because of their increasing polarity. For the aqueous extraction, 100 g of vegetable powder was homogenized in 1 L of water using a blender. After 2 homogenization cycles, the mixture was collected in a square of clean fabric and pressed by hand by applying strong pressures and then filtered twice on hydrophilic cotton and once on Whatman 3 mm filter paper. The filtrate was dried in a HERAEUS® type study at a temperature of 50°C for 3 days. The ethanolic extract was prepared by the same method but instead of water a hydro-alcoholic solvent composed of 30% distilled water and 70% pure ethanol was used.¹⁵ For the methanolic extract, 50 g of dry powder was introduced into 1.5 L of pure methanol by homogenization using a blender. The following steps are identical to those of aqueous extraction.¹⁶ After weighing and yield calculation, the extracts were stored at 4°C until being used.

Phytochemical screening

The major families of secondary metabolites were investigated in plants using conventional characterization methods. Tannins and polyphenols were identified by the FeCl₃ test and Stiasny's reagent; flavonoids by the cyanidin reaction; saponins by the foam test; quinones by the Bornträger test; triterpenes and steroids by the Liebermann-Burchard test, and finally alkaloids by the Mayer and Dragendorf.¹⁷

Antiplasmodial activity

Continuous culture of reference strains

The chloroquine-resistant K1 strain and NF54 chloroquine-sensitive strain of *P. falciparum* were supplied by the Cytology Laboratory of the Swiss Center Research Scientific (CSRS) in Côte d'Ivoire. They were maintained in continuous cultivation using the technique of Trager and Jensen 1976.¹⁸

Field isolates

Patients who came for consultation at the Urban and Community Health Centre of Yopougon Wassakara (Côte d'Ivoire), suffered from a mono-infection with *P. falciparum*, then confirmed by malaria RDTs (Care Start

TM type, HRP2/PLDH COMBO (Pf/VOM)). All were informed about the study objectives. Study approval was issued by the National Ethical Committee and Research of Côte d'Ivoire.

After obtaining the patients' informed and written consent, blood samples were collected by laboratory technicians in previously identified EDTA tubes. Donor patients should not have taken an antimalarial medicine two weeks before the onset of the disease. Then, the blood samples were sent to the Chemosensitivity Laboratory of the Centre Suisse de Recherche Scientifique (CSRS) in a cold box.

Chemosensitivity test

In vitro activity tests were performed according to the Rieckmann micro-test recommended by WHO.

To carry out the antiplasmodial tests, the parasitized red blood cells were washed three times in RPMI medium (Roswell Park Memorial Institute) 1640. The red blood cells were suspended in RPMI medium containing 10% AB human serum, 25 mM d HEPES and 25 mM NaHCO₃ at a hematocrit of 5% and an initial parasitemia of 0.1 to 0.3%. When the parasitemia was greater than 0.3%, the parasitized erythrocytes were diluted with erythrocytes from healthy donors of group O and positive Rhesus, provided by the National Center for Blood Transfusion of Abidjan (CNTS), to achieve a parasitemia of 0.3%.

The plasmodial strains were brought into contact with decreasing concentrations (100 µg/mL - 1.56 µg/mL) of the drugs studied in 96-well microplates for 72 hours (Fig. S1, Supplementary file 1). Each extract has been tested in duplicate. This assay was executed twice. Chloroquine dihydrophosphate (CQ), used as a reference molecule provided by Medicines for Malaria Venture (MMV), was dissolved in deionized water, and dilutions were made from 200 to 3.12 nM.

SYBR Green assay (parasite viability)

After 72 hours of incubation of the parasites in the presence of the test drug, red blood cells were lysed by addition of a lysis solution prepared with SYBR Green then added to the culture. The lysis buffer was prepared by dissolving 1.21 g of Tris-HCl in 350 mL of distilled water. Then 5 mL of EDTA (0.5M), 40 mg of saponin and 400 µL of Triton X-100 were added thereto. The volume was then adjusted to 500 mL with distilled water. The solution obtained was filtered with a 0.22 µm millipore filter (Stericup Durapore®) and was finally stored at room temperature.

SYBR Green is an intercalator that binds between the bases of DNA and emits a fluorescence whose intensity is proportional to the DNA of the medium which is itself proportional to the number of parasites. Fluorescence was then evaluated using a spectrofluorometer spectra Max Gemini-XPS GEMINI® XPS-05153 microplate reader (Fig. S2, Supplementary file 1) (Molecular Devices,

Sunnyvale, CA, USA) to monitor parasitic growth.

Statistical analysis

The results were expressed as 50% inhibitory concentrations (IC_{50}). The IC_{50} and corresponding correlation coefficients were determined graphically, using *In Vitro* Analysis and Reporting Tool (IVART) software using sigmoidal no linear regression based on the concentration-inhibition model applied to the data.¹⁹

Results

Phytochemical screening

The preliminary phytochemical screening of studied plants carried out on aqueous, ethanolic 70% and methanolic extracts from plants, gave the results recorded in Table 1. These results showed a strong presence of polyphenols and alkaloids in extracts with cumulative rates of 90.47% and 95.23%, respectively. Flavonoids and sterols/polyterpenes followed with the rates of 87.71% and 61.9%, respectively. Quinone substances were present at a rate of 50%. Tannins and saponins were the poorest extracts with a cumulative rate of 14.28% for each chemical group. Most plant extracts contained polyphenols, polyterpenes, and alkaloids. Only the aqueous extracts of *H. madagascariensis* and methanolic extracts of *P. laxiflora* contained both quinones and alkaloids. Flavonoids were also present in almost all extracts except for the aqueous

extract of *P. laxiflora* and *A. djalonenensis* and the ethanolic extract of *H. opposita*.

Antiplasmodial activity

The antiplasmodial activity of plant extracts was expressed as a 50% inhibitory concentration (IC_{50}). All the results are reported in Tables 2 and 3.

Raw plant extracts had variable activities on the strains of *P. falciparum* tested. The IC_{50} values classified from 4.7 $\mu\text{g/mL}$ to 48.9 $\mu\text{g/mL}$ for active extracts. Only the aqueous extract of *A. djalonenensis* had IC_{50} values greater than 50 $\mu\text{g/mL}$. Of the four isolates, only one (Is W6622) was resistant to chloroquine.

Discussion

Phytochemical triage tests were carried out in tubes and mainly targeted alkaloids, sterols/polyterpenes, quinonic substances, flavonoids, tannins, and saponins because of their great importance for health. Thus, the presence of polyphenols, alkaloids, flavonoids, sterols/polyterpenes, quinonic substances, tannins, and saponins in extracts was determined. The data obtained confirm the results of previous work on the phytochemical composition, the presence of tannins, saponins and flavonoids in the leaves of *M. indica*, and in addition, that of quinones in the bark of *H. madagascariensis*.^{20,21} The presence of alkaloids in extract from *M. discoidea* and *C. planchonii* has already

Table 1. Phytochemical compounds of plant extracts

Plants	Extract	Sterols		Poly-phénols	Flavonoids	Tannins		Quinones	Alkaloids		Saponins
		Polyterpenes				Gal	Cat		D	B	
B. Indima	EthOH	-	+	+	-	-	+	+	+	-	
	MeOH	+	+	+	+	-	+	+	+	-	
	Aq	-	+	+	+	-	-	+	+	-	
B. Laper	EthOH	+	+	+	-	-	-	+	-	-	
	MeOH	+	+	+	-	-	+	+	-	-	
	Aq	-	+	-	+	-	-	+	-	+	
L. Dismar	EthOH	+	+	+	-	-	+	+	+	-	
	MeOH	+	+	+	-	+	+	+	+	-	
	Aq	+	+	+	-	+	+	+	+	+	
B. Madhar	EthOH	+	+	+	-	-	-	+	+	-	
	MeOH	+	+	+	-	-	+	+	+	-	
	Aq	-	+	+	-	-	-	+	+	-	
L. Placo	EthOH	+	+	+	-	-	-	+	+	-	
	MeOH	+	+	+	-	-	-	+	+	-	
	Aq	-	+	+	-	-	-	+	+	-	
L. Djantho	EthOH	+	+	+	-	-	-	-	-	-	
	MeOH	+	+	+	-	-	+	+	+	-	
	Aq	-	-	-	-	-	+	+	+	-	
L. H. Opposita	EthOH	+	+	-	-	-	+	+	+	-	
	MeOH	-	-	+	-	-	-	+	+	-	
	Aq	-	+	+	-	-	-	+	+	+	

+: Presence; -: Absence; Aq: Aqueous extract; EthOH: Ethanolic extract; MeOH: Methanolic extract; B. Indima: Bark of *M. indica*; B. Laper: Bark of *P. laxiflora*; L. Dismar: Leaves of *M. discoidea*; B. Madhar: Bark of *H. madagascariensis*; L. Placo: Leaves of *C. planchonii*; L. Djantho: Leaves of *A. djalonenensis*; D: Dragendorff; B: Bouchardat, Gal : Gallique, Cat : Catechique.

Table 2. IC₅₀ values of extracts (µg/mL) and Chloroquine (nM) on NF54 and K1 strains

Plants	Extracts	IC ₅₀ (µg / mL) strains	
		NF54	K1
<i>F. A. djalensis</i>	Aq	10.23±0.40	8.36±2.07
	EthOH	38.30±1.27	36.07±0.25
	MeOH	>50	>50
<i>L. C. planchonii</i>	Aq	40.76±1.35	42.81±1.10
	EthOH	18.15±2.39	20.36±1.15
	MeOH	13.15±0.42	12.15±1.11
<i>B. H. madagascariensis</i>	Aq	6.16±0.40	7.3±1.75
	EthOH	20.32±0.70	22.21±1.40
	MeOH	23.91±0.47	22.78±0.27
<i>L. H. Opposita</i>	Aq	43.25±1.37	27.41±2.01
	EthOH	10.45±0.10	17.69±1.60
	MeOH	42.70±1.56	40.50±0.14
<i>B. M. Indica</i>	Aq	39.40±1.12	33±0.60
	EthOH	21.27±1.15	19.08±0.23
	MeOH	20.34±2.10	22.87±0.82
<i>L. M. discoidea</i>	Aq	39.56±2.13	43.61±0.92
	EthOH	31.23±0.50	13,60±1.80
	MeOH	23.67±0.44	27.41±1.75
<i>B. P. Laxiflora</i>	Aq	25.46±1.90	26.56±2.10
	EthOH	33.28±0.34	40.41±0.97
	MeOH	11.53±0.68	7.44±1.02
Chloroquine (nM)		12.5±0.42	124±0.74

Aq: Aqueous extract; EthOH: Ethanolic extract; MeOH: Methanolic extract; B.: Barks; L.: Leaves; IC₅₀ = inhibition concentration at 50.

been shown.^{22,23}

According to several pharmacological studies, triterpenoids, flavonoids, and alkaloids present in our extracts had antiplasmodic properties.^{24,25} Indeed, alkaloids are known for their antiplasmodic properties by blocking protein synthesis in *P. falciparum*.²⁶⁻²⁸

The results obtained were also justified by the composition of these plants, which have several secondary metabolites involved in the treatment of malaria.

The results of the chemosensitivity tests revealed that the crude extracts from the seven plants had activity on *P. falciparum* strains according to the classification scale proposed by Jansen et al.²⁹ According to these authors, a plant extract is considered to have a moderate effect if its IC₅₀ value is between 15 µg/mL and 50 µg/mL (5<IC₅₀<50 µg/mL). However, these activities were different from one extract to another for the same plant and from one plant to another. This could be explained by the difference in the content of active compounds. In addition, aqueous extracts from *H. madagascariensis* with *A. djalensis*, and methanolic extracts from *C. planchonii* and *P. laxiflora* were the most active ones. These activities below 15 µg/mL are proponent according to the previous studies.^{30,31} In addition, the promising effect of these four extracts could be due to the cumulative presence of compounds such as polyphenols, triterpenes, and alkaloids in the extracts which could have a synergistic effect on *P. falciparum*.^{32,33}

By comparing the IC₅₀ of the hydroethanolic extract

Table 3. IC₅₀ values of extracts (µg/mL) and Chloroquine (nM) on field isolates

Plants	Extracts	IC ₅₀ (µg/mL) field isolates				Medianes
		Is. W6622	Is. W6708	Is. W6743	Is. W7177	
<i>F. Djantho</i>	Aq	8.29	26.46	31.71	21.04	26.46
	EthOH	43.06	47.91	37.65	40.53	40.53
	MeOH	>50	>50	>50	>50	>50
<i>F. Placo</i>	Aq	46.61	42.54	42.24	43.32	42.45
	EthOH	17.88	17.59	18.5	16.35	17.56
	MeOH	13.39	11.77	13.20	12.25	12.25
<i>E. Madhar</i>	Aq	04.7	5.85	6.11	6.7	6.11
	EthOH	19.53	24.03	27.42	31.82	27.42
	MeOH	21.68	24.26	25.67	32.82	25.67
<i>F. Oppo</i>	Aq	12.17	41.32	43.69	41.35	41.35
	EthOH	6	41.45	42.91	41.77	41.37
	MeOH	21.10	42.45	42.87	42.08	42.45
<i>E. Indima</i>	Aq	22.70	43.84	35.49	41.88	41.88
	EthOH	23.97	20.88	26.95	24.85	24.85
	MeOH	20.62	25.72	27.89	20.12	25.72
<i>E. Dismar</i>	Aq	32.25	42.20	35.62	36.23	36.23
	EthOH	4.9	28.02	35.75	37.65	35.75
	MeOH	22.45	25.75	24.76	26.65	25.75
<i>E. Laper</i>	Aq	24.06	22.72	18.82	24.46	24.72
	EthOH	34.99	30.69	35.5	39.51	35.50
	MeOH	13.87	10.57	12.69	14.04	12.69
Chloroquine (nM)		106.39	5.92	16.22	12.04	14.13

Aq: Aqueous extract; EthOH: Ethanolic extract; MeOH: Methanolic extract; B. Indima: Bark of *M. indica*; B. Laper: Bark of *P. laxiflora*; L. Dismar: Leaves of *M. discoidea*; B. Madhar: Bark of *H. madagascariensis*; L. Placo: Leaves of *C. planchonii*; L. Djantho: Leaves of *A. djalensis*; IC₅₀ = inhibition concentration at 50; Is: Isolate

from *A. djalonenensis* obtained in this study with those reported in the literature, it was found that these results are close to those reported by Attemene et al.²⁸ Indeed, these authors obtained an IC₅₀ of 15.94 µg/mL on strain K1 for the hydroethanolic extract from the same plant. In 2007, work by Muthaura et al showed that the aqueous extract from *H. madagascariensis* contained active ingredients on the chloroquine-resistant K1 strain of *P. falciparum* with an IC₅₀ of 3.1 µg/mL. On the other hand, other studies have reported results that are contrary to ours.^{34,35} In their studies, the activity of *P. laxiflora* extract on W2 and *M. indica* ethanolic extract on K1 was greater than 50 µg/mL. This difference could be due to the origin of the plant, its collection period, the experimental technique, and the profile of the strains used in each of these studies. It is also important to note that the drugs used in our series were effective on chloroquine-resistant strains. Two tested extracts, namely the aqueous extracts from *A. djalonenensis* and methanolic extracts from *P. laxiflora*, were more effective on CQ-R than CQ-S. This could be explained by the fact that these extracts have a different mechanism of action than chloroquine. This result is rather encouraging and gives hope for the possibility of selecting substances to overcome the problem of chloroquine-resistance.

In our work, we used two strains of NF54 and K1 for which the IC₅₀s for chloroquine were identical to those found for the chemosensitivity tests described by different authors on plasmodium growth.^{36, 37}

The effect of crude extracts on field isolates was practically equal to that obtained on reference strains. Then isolates (freshly collected) such as laboratory strains could be used to determine the schizonticidal effect of any antimalarial drug. Thus, the use of clinical isolates would be an advantage for research laboratories in African countries, as the storage and maintenance of laboratory strains require very expensive equipment.

Conclusion

To conclude, we note that the aqueous extracts from *H. madagascariensis* and *A. djalonenensis* and methanolic extract from *P. laxiflora* and *C. planchonii* showed better antiplasmodial activity. The other extracts had moderate activity. The results of this study therefore indicated that the tested extracts possess antiplasmodial properties and could partially justify the use of these plants as traditional remedies against malaria attacks. It would be advisable to carry out further studies on these extracts, in order to envisage the manufacture of a stable galenic formulation for the development of an improved traditional medicine which could contribute to the transition from the control phase to the phase of pre-elimination of malaria in Côte d'Ivoire.

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Research Highlights

What is the current knowledge?

✓ The results of this study indicate that the tested extracts possess antiplasmodial properties and could partially justify the use of these plants as traditional remedies against malaria.

What is new here?

✓ Twenty-one extracts obtained from endemic medicinal plants of the Ivorian pharmacopoeia was evaluated on the chloroquinosensitive (NF54) and multi-resistant (K1) reference strains and on clinical isolates as well.

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Ethical statement

This study approval was issued by the National Ethical Committee and Research of Côte d'Ivoire (Code of Ethics: 038/MSLS/CNER-dkn).

Competing interests

The authors do not declare any conflict of interest.

Authors' contribution

JAK contributed to the multiplication of reference strains, the chemosensitivity test, the conception and design, analysis and interpretation of data, drafting the article and final version approval. DKT and TMD contributed to the multiplication of reference strains. DKS participated in the data analyses and IC₅₀ value determination. YW contributed to the correction of the draft and final version approval.

Supplementary Materials

Supplementary file 1 contains Figs. S1-S2.

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