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In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria

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ABSTRACT

Aim of the study: The aim of the study was to evaluate the *in vitro* antiplasmodial activity of crude extracts of 12 plant species traditionally used in Benin for the treatment of malaria in order to validate their use.
Materials and methods: For each species, dichloromethane, methanol and total aqueous extracts were tested. The antiplasmodial activity of extracts was evaluated using the measurement of the plasmodial lactate dehydrogenase activity on chloroquine-sensitive (3D7) and resistant (W2) strains of *Plasmodium falciparum*. The selectivity of the different extracts was evaluated using the MTT test on J774 macrophage-like murine cells and WI38 human normal fibroblasts.
Results: The best growth inhibition of both strains of *Plasmodium falciparum* was observed with the dichloromethane extracts of *Acanthospermum hispidum* DC. (Asteraceae) (IC_{50} = 7.5 μg/ml on 3D7 and 4.8 μg/ml on W2), *Keetia leucantha* (K. Krause) Bridson (syn. *Plectronia leucantha* Krause) (Rubiaceae) leaves and twigs (IC_{50} = 13.8 and 11.3 μg/ml on 3D7 and IC_{50} = 26.5 and 15.8 μg/ml on W2, respectively), *Carpolobia lutea* G.Don. (Polygalaceae) (IC_{50} = 19.4 μg/ml on 3D7 and 8.1 μg/ml on W2) and *Strychnos spinosa* Lam. (Loganiaceae) leaves (IC_{50} = 15.6 μg/ml on 3D7 and 8.9 μg/ml on W2). All these extracts had a low cytotoxicity.
Conclusion: Our study gives some justifications for the traditional uses of some investigated plants.

1. Introduction

Plasmodium species are protozoan parasites responsible for malaria, an illness killing about 1–2 million people per year (WHO, 2005). Among the four types of human pathogenic parasites, *Plasmodium falciparum* is the most dangerous species. The very high prevalence of this disease and the resistance of parasites to cheap treatments have led to the search for new antimalarial compounds, particularly in plants used in traditional medicine, as a source of new leads with new mechanism of action, such as artemisinin from *Artemisia annua* (Asteraceae) (Klayman, 1985). Thus, we investigated traditional treatment used in Benin to fight malaria. We selected 12 plants which were not or only partially evaluated for their antiplasmodial activity in a list of 88 traditional remedies used to cure malaria by traditional healers in Benin, compiled by the "Direction de la Protection Sanitaire" of the Benin Ministry of Health. Each species was macerated with dichloromethane, methanol and water. Crude extracts were evaluated for their antiplasmodial activity *in vitro* and their selectivity determined on two mammalian cell lines.

2. Materials and methods

2.1. Plant material

Plant materials (leaves, twigs, aerial parts and roots) were collected from the South of Benin, especially from Abomey-Calavi (South-West) to the border area with Nigeria (South-East) between July 2006 and September 2006. Voucher specimens were identified and deposited at the Herbier National of Abomey-Calavi University in Benin and at the Herbarium of the National Botanic Garden of Belgium, at Meise (see Table 1).

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Table 1
Studied plant species.

Botanical name	Family	Voucher specimen number	Traditional uses
Acanthospermum hispidum DC.	Asteraceae	AA 6315/HNB	To treat vomiting, cephalgias, headaches, abdominal pains, convulsions, cough, eruptive fever, snake bites, jaundice, epilepsy, constipation, blennorrhoea, diarrheas, hepato-biliary disorders and malaria[a,b,c,d]
Anchomanes difformis (Blume) Engl.	Araceae	AA 6316/HNB	As diuretic, to treat diabetes, oral and anal lesions, tuberculosis and malaria[a,b,c,d]
Byrsocarpus coccineus Schumach. & Thonn (syn. *Rourea coccinea* (Schumach. & Thonn.) Hook.f.)	Connaraceae	AA 6321/HNB	To treat male and female infertilities, sexual asthenia, blennorrhoeas, snake bites, furuncles and malaria[a,b]
Carpolobia lutea G.Don	Polygalaceae	AA6317/HNB	As vermifuge, aphrodisiac, to wash feverish and dementia patients, to treat headaches, fever and malaria[a,f]
Dialium guineense Willd.	Leguminosae	AA 6318/HNB	To treat amenorrhoea, female infertilities, anuria, jaundice, gonorrhea, dysmenorrhoea, fever, labor pains, palpitations, diarrheas and malaria[a,b,d,g]
Heliotropium indicum L.	Boraginaceae	AA 6319/HNB	To treat splenomegaly, hypertension, hyperthermias, leucorrhea, candidiasis, dementia patients, colics, eczema, impetigo and malaria[a,b,d]
Keetia leucantha (K. Krause) Bridson (syn. *Plectronia leucantha* Krause)	Rubiaceae	Houngnon 3435	To treat malaria[a,e]
Pupalia lappacea Juss.	Amaranthaceae	AA 6320/HNB	To treat jaundice, abdominal colics, cephalgias, diarrheas, paralysis, erectile dysfunction, vomiting and malaria[a,b]
Sansevieria liberica Hort. ex Gérôme & Labroy	Dracaenaceae	AA 6322/HNB	To treat asthma, sexual weakness, hypertension, diarrheas, abdominal pains, colics, gonorrhea, eczema, piles, snake and dog bites, zona, oedema, jaundice, anuria, palpitations, viral hepatitis and malaria[a,b,c,h]
Schrankia leptocarpa DC.	Mimosaceae	Houngnon 954b	To treat eruptive fever, hypertension, jaundice, abdominal pains, hiccup and malaria[a,b]
Strychnos spinosa Lam.	Loganiaceae	BR S.P. 848106	To treat stomachaches, abdominal pains, colics, sterility, abscess, sleeping sickness and malaria[a,b,d,i,j]
Trichilia emetica Vahl subsp. *suberosa* J.J.F.E. Dewilde	Meliaceae	BR S.P. 848104	As antispasmodic, purgative, antiepileptic, antipyretic, to treat muscle fevers, snake bites, hepatic disorders, sleeping sickness and malaria[a,b,j,k]

[a] List of 88 traditional remedies used to cure malaria by traditional healers in Benin, compiled by the "Direction de la Protection Sanitaire" of the Benin Ministry of Health.
[b] Adjanohoun et al. (1989).
[c] Adjanohoun et al. (1988).
[d] Kerharo and Adam (1974).
[e] Weniger et al. (2004).
[f] Mitaine-Offer et al. (2002).
[g] Odukoya et al. (1996).
[h] Osabohien and Egboh (2008).
[i] Asase et al. (2005).
[j] Hoet et al. (2004).
[k] Germano et al. (2006).

2.2. Preparation of crude plant extracts and alkaloid fraction

Leaves, twigs, roots or aerial parts of plant extracts were prepared by macerating 20–50 g of dried and powdered plant material at room temperature for about 24 h. The material was extracted sequentially with dichloromethane and methanol. A total aqueous extract was also prepared from another sample. The quantity of solvent used for each extraction was at least 10 times the quantity of plant material. Thus, three extracts were obtained for each plant part. The filtrates were evaporated to dryness under reduced pressure with a rotary evaporator at a temperature of 30 °C while the water filtrates were freeze-dried to powder. Yields for each extraction are indicated in Table 2.

The alkaloid fraction of *Acanthospermum hispidum* DC. (Asteraceae) was obtained from 50 g powdered dried plant macerated with water (500 ml) acidified with sulphuric acid (pH 3) during 48 h. The aqueous extract was alkalinized using NH_4OH until pH 8–9 was reached and then extracted with dichloromethane (3× 300 ml) which was evaporated to dryness under reduced pressure with a rotary evaporator.

2.3. Parasites, cells and media

Crude extracts were evaluated for their antiplasmodial activity *in vitro* against a chloroquine-sensitive strain of *Plasmodium falciparum* (3D7) and the most active on this strain were evaluated against a chloroquine-resistant strain (W2).

Plasmodium falciparum (chloroquine-sensitive strain 3D7, originally isolated from a patient living near Schipol airport, The Netherlands and chloroquine-resistant strain W2 from Indochina) asexual erythrocytic stages were cultivated continuously *in vitro* according to the procedure described by Trager and Jensen (1976) at 37 °C and under an atmosphere of 5% CO_2, 5% O_2 and 90% N_2. The host cells were human red blood cells (A or O Rh+). The culture medium was RPMI 1640 (Gibco) containing 32 mM $NaHCO_3$, 25 mM HEPES and L-glutamine. The medium was supplemented with 1.76 g/l glucose (Sigma–Aldrich), 44 mg/ml hypoxanthin (Sigma–Aldrich), 100 mg/l gentamycin (Gibco) and 10% human pooled serum (A or O Rh+). Parasites were subcultured every 3–4 days with initial conditions of 0.5% parasitaemia and 1% haematocrit.

The macrophage-like cell line, J774, derived from BALB/c murine reticulum cell sarcoma, was cultivated *in vitro* in RPMI 1640 medium (Gibco) containing 2 mM L-glutamine supplemented with 10% heat-inactivated foetal bovine serum (Gibco) and penicillin–streptomycin (100 UI/ml to 100 μg/ml).

The human normal fibroblast cell line, WI38, was cultivated *in vitro* in DMEM medium (Gibco) containing 4 mM L-glutamine, 1 mM sodium pyruvate supplemented with 10% heat-inactivated foetal bovine serum (Gibco) and penicillin–streptomycin (100 UI/ml to 100 μg/ml).

Table 2
In vitro antiplasmodial activity, cytotoxicity and selectivity index of the selected plant extracts.

Plant species	Part studied[a]	Extract	Yield (%)	Cytotoxicity (IC_{50}, μg/ml) average ± standard deviation		Antiplasmodial activity *Plasmodium falciparum* (IC_{50}, μg/ml) average ± standard deviation		Selectivity index[b] WI38/3D7
				J774	WI38	3D7	W2	
Acanthospermum hispidum	AP	CH_2Cl_2	5.0	43.2 ± 15.7	34.9 ± 6.4	7.5 ± 1.2	4.8 ± 1.6	4.7
		CH_3OH	9.0	>100	>100	47.1 ± 3.5	nd	>2.1
		H_2O	5.7	>100	>100	55.6 ± 28.7	nd	>1.8
Anchomanes difformis	R	CH_2Cl_2	4.4	22.0 ± 1.5	26.0 ± 4.2	>100	nd	0.3
		CH_3OH	5.7	2.2 ± 0.1	14.6 ± 3.5	>100	nd	0.1
		H_2O	7.1	8.2 ± 1.7	12.7 ± 1.0	>100	nd	0.1
Byrsocarpus coccineus	AP	CH_2Cl_2	6.4	>100	>100	41.6 ± 22.1	nd	>2.4
		CH_3OH	17.0	>100	>100	54.7 ± 21.9	nd	>1.8
		H_2O	5.2	>100	>100	>100	nd	1.0
Carpolobia lutea	AP	CH_2Cl_2	8.9	38.7 ± 1.6	65.4 ± 15.1	19.4 ± 6.5	8.1 ± 4.5	3.4
		CH_3OH	13.0	>100	>100	85.4 ± 2.2	nd	>1.2
		H_2O	4.1	66.1 ± 9.7	>100	>100	nd	1.0
Dialium guineense	AP	CH_2Cl_2	10.4	>100	77.3 ± 0.2	42.1 ± 17.7	nd	2.3
		CH_3OH	13.5	>100	>100	>100	nd	1.0
		H_2O	7.9	>100	>100	65.5 ± 3.9	nd	>1.5
Heliotropium indicum	AP	CH_2Cl_2	5.4	>100	>100	>100	nd	1.0
		CH_3OH	11.9	>100	>100	>100	nd	1.0
		H_2O	4.3	>100	>100	>100	nd	
Keetia leucantha	LF	CH_2Cl_2	5.6	91.5 ± 3.1	65.6 ± 1.3	13.8 ± 8.3	26.5 ± 9.5	>4.8
		CH_3OH	9.7	>100	>100	>100	nd	1.0
		H_2O	9.0	>100	>100	>100	nd	1.0
	TW	CH_2Cl_2	0.5	50.5 ± 4.2	>100	11.3 ± 3.8	15.8 ± 2.3	>8.8
		CH_3OH	5.3	>100	>100	>100	nd	1.0
		H_2O	2.9	>100	>100	>100	nd	1.0
Pupalia lappacea	AP	CH_2Cl_2	13.7	66.5 ± 13.3	68.3 ± 4.9	50.29 ± 16.1	nd	1.4
		CH_3OH	6.6	>100	>100	>100	nd	1.0
		H_2O	7.2	>100	>100	>100	nd	1.0
Sansevieria liberica	AP	CH_2Cl_2	1.7	59.0 ± 3.1	>100	44.5 ± 3.7	nd	1.3
		CH_3OH	7.1	>100	>100	>100	nd	1.0
		H_2O	11.6	>100	>100	>100	nd	1.0
Schrankia leptocarpa	LF	CH_2Cl_2	12.0	>100	>100	34.3 ± 13.5	nd	>2.9
		CH_3OH	17.5	>100	>100	>100	nd	1.0
		H_2O	17.7	>100	>100	>100	nd	1.0
	TW	CH_2Cl_2	1.5	>100	>100	29.6 ± 5.1	nd	>3.4
		CH_3OH	7.4	>100	>100	>100	nd	1.0
		H_2O	5.5	>100	>100	>100	nd	1.0
Strychnos spinosa	LF	CH_2Cl_2	1.9	>100	>100	15.6 ± 3.8	8.9 ± 2.1	>6.4
		CH_3OH	11.1	>100	>100	>100	nd	1.0
		H_2O	25.7	>100	>100	>100	nd	1.0
Trichilia emetica	LF	CH_2Cl_2	7.5	83.7 ± 4.5	88.6 ± 8.9	59.2 ± 11.3	nd	1.5
		CH_3OH	20.2	>100	>100	>100	nd	1.0
		H_2O	21.7	>100	>100	>100	nd	1.0
Campthotecin				0.03 ± 0.003	0.4 ± 0.2	nd	nd	nd
Chloroquine				nd	nd	0.02 ± 0.01	0.49 ± 0.15	nd
Artemisinin				nd	nd	0.01 ± 0.001	0.005 ± 0.001	nd

J774 = macrophage-like murine cells, WI38 = human normal fibroblast cell, 3D7 = Chloroquine-sensitive strain of *Plasmodium falciparum*, W2 = Chloroquine-resistant strain of *Plasmodium falciparum*, nd = not determined

[a] Plant part used: LF = leaves, TW = twigs, R = roots, AP = aerial parts.

[b] Selectivity index = $IC_{50(WI38)}/IC_{50(3D7)}$.

The cells were incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

2.4. *In vitro* test for antiplasmodial activity

Parasite viability was measured using parasite lactate dehydrogenase (pLDH) activity according to the methods described by Makler et al. (1993). The *in vitro* test was performed as described by Murebwayire et al. (2008). Chloroquine (Sigma) or artemisinin (Sigma) were used as positive controls in all experiments with an initial concentration of 100 ng/ml. First stock solutions of crude extracts were prepared in DMSO or ethanol at 20 mg/ml. The solutions were further diluted in medium to give 2 mg/ml stock solutions. The highest concentration of solvent to which the parasites were exposed was 1%, which was shown to have no measurable effect on parasite viability. Extracts were tested in nine serial twofold dilutions (final concentration range: 200–0.03 μg/ml) in 96-well microtiter plates. The parasitaemia and the haematocrit were 2% and 1%, respectively. All tests were performed in triplicate.

2.5. *Cytotoxicity assay*

The cytotoxicity of the extracts on J774 and WI38 cells was evaluated as described by Stevigny et al. (2002), using the tetrazolium salt

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma)) colorimetric method based on the cleavage of the reagent by mitochondrial dehydrogenase in viable cells (Mosmann, 1983). Camptothecin (Sigma) was used as positive cytotoxic reference compound. Stock solutions of crude extracts were prepared in DMSO or ethanol at 20 mg/ml. The solutions were further diluted in medium with a final concentration range of 200–6.25 μg/ml. The highest concentration of solvent to which the cells were exposed was 1%, which was shown to be non-toxic. Each extract was tested in six serial fourfold dilutions in 96-well microtiter plates. All experiments were made at least in duplicate.

3. Results

The 12 plants traditionally used were extracted to give 42 extracts. *In vitro* antiplasmodial and cytotoxic activities of these extracts are summarized in Table 2.

Concerning the antiplasmodial activity on 3D7, we observed that 5 extracts could be considered as good with IC_{50} values ≤20 μg/ml, 10 had a moderate activity with IC_{50} values between 21 and 60 μg/ml, 2 showed a low activity with IC_{50} values between 60 and 100 μg/ml and 25 may be considered as inactive with IC_{50} >100 μg/ml. The effects of the promising extracts were confirmed on the chloroquine-resistant strain. There are no significant differences between the two strains. The dichloromethane extracts were generally more active than the methanol and water extracts. The best growth inhibition of both strains of *Plasmodium falciparum* was observed with the dichloromethane extracts of *Acanthospermum hispidum*, *Keetia leucantha* (K. Krause) Bridson (syn. *Plectronia leucantha* Krause) (Rubiaceae) leaves and twigs, *Carpolobia lutea* G.Don. (Polygalaceae) and *Strychnos spinosa* Lam. (Loganiaceae) leaves. All these extracts had a low cytotoxicity.

Table 2 shows that most extracts could be considered as not cytotoxic on J774 and WI38 cells (IC_{50} > 50 μg/ml). The only exceptions were *Anchomanes difformis* (Blume) Engl. (Araceae) extracts which were toxic on both cell lines, the dichloromethane extracts of *Acanthospermum hispidum* and *Carpolobia lutea* which were moderately toxic on both cell lines and the dichloromethane extract of the twigs of *Keetia leucantha* on J774.

4. Discussion

4.1. Acanthospermum hispidum

The present study showed an antiplasmodial activity of the lipophilic extract, on 3D7 strain (IC_{50} = 7.5 μg/ml) and W2 strain (IC_{50} = 4.8 μg/ml). This activity is in agreement with those obtained by Sanon et al. (2003a) (IC_{50} ~ 10 μg/ml of a chloroformic extract tested on W2 strain). In addition this extract has only a moderate cytotoxicity (IC_{50} > 30 μg/ml) on the two cell lines tested (WI38 and J774). Sanon et al. (2003b) showed that this activity was concentrated in the alkaloid fraction (IC_{50} ~ 5 μg/ml) but the type of alkaloids responsible for this activity was not identified. We obtained the same results with the alkaloid extract of our sample on both strains (IC_{50} = 3.6 ± 1.9 μg/ml for 3D7 and IC_{50} = 1.9 ± 0.2 μg/ml for W2) (data not presented). It also has to be noted that this plant was previously shown to contain sesquiterpenic lactones of the germacranolide group (Cartagena et al., 2000). The antiplasmodial activity for these molecules was not determined yet. However other molecules of the germacranolides group coming from other Asteraceae were already shown to have a very strong antiplasmodial activity (Francois et al., 1996) but their strong cytotoxicity on tumor cell lines did not support their use as antimalarial agents.

4.2. Anchomanes difformis

Despite its inactivity against the plasmodial strains, we noticed the strong cytoxicity (2.2 μg/ml < IC_{50} < 26 μg/ml) of all extracts of *Anchomanes difformis* roots against WI38 and J774. This should be further analysed because aqueous extract corresponds best to the use by the traditional healers. Tchiakpe et al. (1980) also showed that rhizomes of this plant appeared toxic for the guinea-pigs by oral administration (300 mg/kg). However, molecules responsible for this toxicity have not yet been described but the phytochemical studies also carried out by Tchiakpe et al. (1980) showed the presence of phenolic compounds (catechins, épicatechins and tannins) and two unidentified compounds they named anchominines A and B.

4.3. Byrsocarpus coccineus

No extract of *Byrsocarpus coccineus* Schumach. & Thonn (syn. *Rourea coccinea* (Schumach. & Thonn.) Hook.f.) (Connaraceae) showed a good antiplasmodial activity, IC_{50} are higher than 60 μg/ml except the dichloromethane and methanol extracts which showed a moderate activity (IC_{50} = 41.6 and 54.7 μg/ml, respectively). The phytochemical screening according to Akindele and Adeyemi (2007) revealed the presence of alkaloids, tannins (phlobatannins), saponins, anthraquinones, glycosides and simple sugars. These authors also showed its anti-inflammatory properties which can perhaps explain its use in the treatment of the malaria's symptoms.

4.4. Carpolobia lutea

The lipophilic extract showed a more significant activity on the W2 strain (IC_{50} = 8.1 μg/ml) than on 3D7 strain (IC_{50} = 19.4 μg/ml). An anti-diarrheal and anti-ulcer activity was found for the ethanolic extract of the leaves (Nwafor and Bassey, 2007). Concerning the type of compounds already described in this plant, only triterpenic saponins (Mitaine-Offer et al., 2002) were isolated in the roots, however the phytochemical screening of the leaves realized by Nwafor and Bassey (2007) revealed the presence of alkaloids, tannins, flavonoids, anthraquinones and also cardiotonic glycosides. It is the first time that the antiplasmodial activity of this plant is shown. This fact increases its interest especially because the oral LD_{50} in mice estimated by those authors was 2450 mg/kg which makes it safe for a traditional use.

4.5. Dialium guineense

Dialium guineense Willd. (Leguminosae) has no significant antimalarial activity in our study except the dichloromethane extract with a moderate activity (IC_{50} = 42.1 μg/ml) and the total aqueous extract with a low activity (IC_{50} = 65.5 μg/ml). Odukoya et al. (1996) have already reported the molluscicidal activity of the fruits and leaves due to triterpenoid glycosides.

4.6. Heliotropium indicum

Extracts of *Heliotropium indicum* L. (Boraginaceae) did not reveal any antiplasmodial activity in our study. As this plant is used for hyperthermias or colics which are two symptoms of malaria, this could explain its use as adjuvant in mixture remedies (Adjanohoun et al., 1989). Previous studies of *Heliotropium indicum* showed antitumor, antileukemic and ganglion blocking activities (Kugelman et al., 1976; Pandey et al., 1982).

4.7. Keetia leucantha

In our study, the dichloromethane extract of the leaves and twigs showed a good antiplasmodial activity, particularly on the chloroquine-sensitive strain (IC_{50} = 13.8 and 11.3 μg/ml, respectively on 3D7 and IC_{50} = 26.5 and 15.8 μg/ml, respectively on W2). The selectivity index of leaves and twigs were 4.8 and 8.8, respectively. Antiplasmodial activities have previously been described for aerial parts of a sample of *Canthium setosum* from the same area of Benin which may correspond now to *Keetia leucantha* or *Keetia hispida* and were comparable to our results (Weniger et al., 2004). In addition, we can note that other *Canthium* species (Rubiaceae) are also used as traditional antimalarials: the stem barks of *Canthium phyllanthoideum* Baill. (now renamed *Pyrostria phyllanthoidea* (Baill.) Bridson Kew Bull.) and the roots of *Canthium zanzibaricum* Klotzsch. Now named *Keetia zanzibarica* (Klotzsch) Bridson in Tanzania (Chhabra et al., 1991; Gakunju et al., 1995).

4.8. Pupalia lappacea

Pupalia lappacea Juss. (Amaranthaceae) did not display any antiplasmodial activity except its dichloromethane extract which has a moderate activity (IC_{50} = 50.29 μg/ml). Its traditional use against abdominal colics, cephalgias and vomiting could explain its use against malaria symptoms (Adjanohoun et al., 1989).

4.9. Sansevieria liberica

The dichloromethane extract of the leaves of *Sansevieria liberica* Hort. ex Gérôme & Labroy (Dracaenaceae) showed moderate activity (IC_{50} = 44.5 μg/ml) with a low selectivity index. This is the first study on the *in vitro* antiplasmodial effects of *Sansevieria liberica* but we can note that leaves and twigs extracts of *Sansevieria guineensis* (L.) Willd. from Guatemala showed antiplasmodial activity (Franssen et al., 1997).

4.10. Schrankia leptocarpa

In our study, no extract showed promising activity except a moderate activity for the dichloromethane extracts of the leaves and twigs (IC_{50} = 34.3 and 29.6 μg/ml, respectively), but *Schrankia leptocarpa* DC. (Mimosaceae) is often used in association with *Acanthospermum hispidum* possessing antimalarial activity (Adjanohoun et al., 1989). A better inhibitory activity on *Plasmodium falciparum* was observed by Weniger et al. (2004) but on samples obtained by another extraction method.

4.11. Strychnos spinosa

The dichloromethane leaves extract showed high antiplasmodial activity with a better effect on the chloroquine-resistant strain (IC_{50} = 15.6 and 8.9 on 3D7 and W2, respectively) and a selectivity index higher than 6.4. Studies on bark, stem and bough of samples from other origins showed low or no activity (Frederich et al., 1999; Philippe et al., 2005; Zirihi et al., 2005) but it is the first time that the *in vitro* activity of the leaves of *Strychnos spinosa* is demonstrated. Many antiplasmodial alkaloids were found in various *Strychnos* species (Frederich et al., 2002) but phytochemical investigations showed that alkaloids were not present in detectable quantities in our extracts. Fractionation of the dichloromethane and essential oil leaf extract from *Strychnos spinosa* led to the isolation of sterols and terpenoids which were shown to possess *in vitro* antitrypanosomal activity (Hoet et al., 2006, 2007).

4.12. Trichilia emetica

In our study, the leaves extracts of *Trichilia emetica* Vahl subsp. *suberosa* J.J.F.E. Dewilde (Meliaceae) which were collected in Benin showed no activity on *Plasmodium falciparum* except the dichloromethane extract which has a very moderate effect (IC_{50} = 59.2 μg/ml). These results confirmed the results obtained by Traore et al. (2007). In Mali, another study on this subspecies found an antiplasmodial activity for the dichloromethane extract with an IC_{50} of 11.9 μg/ml (Bah et al., 2007). This activity could be due to variations in the chemical content of samples from different localities. The other subspecies, *Trichilia emetica* subsp. *emetica* was active in various studies (El Tahir et al., 1999; Prozesky et al., 2001; Clarkson et al., 2004) but the taxonomic differentiation is the proof of different biological properties linked to different chemical compositions.

5. Conclusions

Our study presents elements to justify the traditional use of some investigated plants to treat malaria in Benin. The dichloromethane extracts of *Acanthospermum hispidum* aerial parts, *Keetia leucantha* (leaves and twigs), *Carpolobia lutea* aerial parts and *Strychnos spinosa* leaves showed promising antiplasmodial activities towards both strains and have selectivity indices of 3.4 to >8.8 which are the highest found among all extracts. The dichloromethane extracts of *Schrankia leptocarpa* leaves and twigs, the aerial parts of *Sansevieria liberica*, *Dialium guineense*, *Byrsocarpus coccineus* and *Pupalia lappacea* and roots of *Anchomanes difformis* showed moderate antiplasmodial activities with IC_{50} between 21 and 60 μg/ml in addition to the methanol extracts of the aerial parts of *Byrsocarpus coccineus* and *Acanthospermum hispidum* and the aqueous extract of the aerial parts of *Acanthospermum hispidum*. Despite the permanent use of all these plants in traditional medicine for malaria treatment, the lack of activity of several extracts in our study can be explained by the fact that some metabolites are more active *in vivo* than *in vitro*, because they are active against malaria symptoms (fever, vomiting, abdominal pains, cephalgias, etc.) or because their action takes place on another stage of the plasmodium cycle (liver stage for example) than the erythrocyte stage tested here. Moreover, this is the first report on the analysis of the antiplasmodial and cytotoxic properties of *Sansevieria liberica*, *Byrsocarpus coccineus*, *Dialium guineense*, *Heliotropium indicum*, *Pupalia lappacea* and *Carpolobia lutea*. According to the results obtained, the antiplasmodial properties of *Acanthospermum hispidum*, *Keetia leucantha*, *Carpolobia lutea* and *Strychnos spinosa* need to be further investigated while we would suggest caution for the use of *Anchomanes difformis* extracts in traditional medicine. The next step will be the isolation and identification by bioguided fractionation of the constituents responsible for the observed *in vitro* activities in the active species.

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