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Antiplasmodial, inhibitor of hemozoin synthesis and antioxidant activities of some plants used as antimalarial drugs in Bagira (DR Congo)

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Abstract
This study was carried out to evaluate antiplasmodial, inhibitor of hemozoin synthesis and antioxidant activities of 10 plants used in traditional medicine in Bagira in the treatment of malaria. Antiplasmodial activity was carried out by pLDH assay and inhibitory activity of the synthesis of hemozoin, by b-hematin formation assay. The antioxidant activity was evaluated by the DPPH assay. Six plants showed a good activity on W2 strain among them 3 plants showed an inhibitory activity on synthesis of hemozoin and all an antioxidant activity. *Dialium angolense*, *Dalbergia katangensis* and *Ochna schweinfurthiana* showed all tree interest activities with $IC_{50} \leq 14.7 \pm 0.7$ µg / mL. This result establishes a scientific basis for the use of these plants in traditional medicine and highlights a mechanism of action of 3 of them. It prompts the continuation of preferential work to isolate the actives compounds or to develop an improved traditional medicine.

Keywords: plasmodium, 3d7 et w2 strain, malaria, b-hematine, bagira, DPPH.

Introduction

Malaria is the leading parasitic disease in the world and the leading cause of death in sub-Saharan Africa [1, 2]. Each year, more than 200 million people are affected by the disease and nearly 600 miles die from it [3]. Despite the multiple efforts undertaken both globally and locally to address them, no significant progress has been made in the past decade [4, 5]. We note a poor accessibility to healthcare for the most exposed population, an emergence of resistance [6, 7] and an absence of effective preventive means [3, 8]; however, the African population which supports 93% of cases of the disease [5], first-line use of traditional medicine [9, 10]; moreover, this medicine seems to be accepted by this population and presents evidence of efficacy, in particular traditional phytomedicine improved drugs in use such as Kilma ® [11] and antimalarials used in biomedicine such as quinine [12] and artemisinin [13] derived from traditional medicine. This traditional medicine therefore presents itself as a credible alternative in the fight against malaria. We would then understand the craze for screening plant extracts for compounds with antimalarial activity, as shown by several works carried out at the continental level [14, 15]. In DRC antimalarial activity of several plant was previously evaluated [16–18]. In Bukavu-DR Congo, several plants are used in traditional medicine in the treatment of malaria [19, 20] and during our investigation work we identified those which are particularly used in Bagira, among them we count *Aframomum laurentii* (De Wild & T. Durand), *Chenopodium opulifolium* Schrad. Ex WDJ Koch & Ziz, *Dalbergia katangensis* Lechenaud, *Dialium angolense* Welw ex Oliv, *Ekebergia benguellensis* Welw ex CDC, *Julbernardia paniculata* (Benth.) Troupin, *Ochna schweinfurthiana* F Hoffm, *Psorospermum corymbiferum* Spachy Kochia cineraria (DC), as plants for which no literature is available about antimalarial activity. It should also be noted that antimalarials currently admitted in clinics act by several mechanisms of action, including the inhibition of the synthesis of hemozoin [21, 22]. It is therefore possible to carry out a screening of anti-malarial substances by exploiting this property. On the other hand, during malarial infection, an oxidative stress is observed which, unbalanced, is likely to progress towards cerebral malaria or to cause anemia mainly in small children [23,24]. Considering that plants generally have several properties, it is therefore possible in this momentum for screening plant species with antimalarial activity to systematically associate antioxidant activity with a view to seeking the principles with properties both antiplasmodial and

antioxidant which would give these plants add value compared to the antimalarials currently used in the clinic. In this study, we are evaluating antiplasmodial activity *in vitro* on chloroquinosensitive 3D7 and chloroquine resistant W3 strains of *Plasmodium falciparum* of 10 plants. We then evaluate the inhibitory activity of the synthesis of hemozoin and the antioxidant activity of plants which among the ten showed an antiplasmodial activity.

2. Material and Methods

2.1. Plant material

Leaves, stem and root bark, aerial parts, or whole plant, according to the organ used in traditional medicine in Bagira, of the 10 plants mentioned above were harvested with the practitioner of traditional medicine between January and February 2014 in Bukavu. Seagrass beds formed during the harvest were deposited at the Meise herbarium where the identity of the plant was assigned. The plant drug was dried in the open air at a temperature of 32 °C and the dry matter was ground using an electric stainless-steel mill (Plymix PX-MFC 90 D, Belgium).

2.2. Plasmodial strains

Two standard strains of *Plasmodium falciparum* were used, the chloroquinosensitive strain 3D7 (MRA-102, ATCC® Manassas Virginia) and the chloroquine resistant strain W2 (MRA-156, MR4, ATCC® Manassas Virginia) to evaluate antiplasmodial activity. The different strains of plasmodium were frozen and stored at -195.79 °C in liquid nitrogen without handling. Strains were cultured continuously [25].

2.3. Positive controls and substrate

Analytical grade quinine hydrochloride (Sigma Aldrich: 48457, USA) and chloroquine diphosphate (Sigma Aldrich: C6628, UK) served as positive controls in the evaluation of antiplasmodial activity [26]. As in previously study, chloroquine also served as a positive control when evaluating the inhibitory activity of hemozoin synthesis [27] and L-ascorbic acid (Sigma Aldrich: A7506, China) a positive control in antioxidant activity assay as in the past [28]. It made it possible to prepare a standard curve with 5 successive dilutions of order 2 made from a solution of ascorbic acid at 40 µg / ml ($y = 0.0298X + 0.0071$; $r^2 = 0.9997$). DPPH (Sigma Aldrich: 1898-66-4, UK) was used as a substrate for the evaluation of antioxidant activity. It was prepared at 0.002% (w / v) in methanol. The hemin used in the present study was provided by Sigma Aldrich (51280-5G; Netherlands). It served as a substrate for the synthesis of β hematin in test to assess the inhibitory activity of hemozoin synthesis. It made it possible to prepare a solution of hematin at 7.5 mM in 0.1M NaOH [29].

2.4. Obtaining extract

n-hexane extracts were prepared by maceration of 100 g of the drug sprayed in 1 L of n-hexane for 72 h. After filtration, the marc was taken up 3 times with 100 ml of n-hexane and all the filtrates were combined, thus constituting the n-hexane extract (EH). The marc was taken up by methanol following the same procedure and led to the production of the methanolic extract (EM). The aqueous extracts (EA) were obtained according to the protocol used in traditional medicine in the treatment of malaria in Bagira (Table 1). The filtrates were concentrated on a rotary evaporator and the concentrates kept cool before use.

Table 1: Protocol for the preparation of aqueous extracts

N	Plant species	HM	Protocol used in traditional medicine in Bagira	s□□□□□□□□SD□□
1	Aframomum laurentii	BR0000018879292	Infusion for 20 minutes of 65 g of the aerial parts in 1.5 L of water.	15,2 ± 2,5
2	Chenopodium opulifolium	BR0000018879247	Decoction for 1 hour of 35 g of the aerial parts in 1L of water.	21,3 ± 3,5
3	Dalbergia katangensis	BR0000019598468	Infuse for 25 minutes 100 g of the bark of the fresh ground roots in 3 L of water or "e'cibabe".	12,4 ± 0,8
4	Dialium angolense	BR0000018879285	Decoction for 1 hour of 32 g of fresh leaves in 1L of water.	12,1 ± 0,5
5	Ekebergia benguellensis	BR0000018879278	Decoction for 15 minutes of 35 g of the 2 fresh root barks in 1.5 L of water.	23,6 ± 0,7
6	Julbernardia paniculata	BR0000024710657	Infusion of 100 g of fresh leaves in 1.5 L of water for 20 minutes.	15,4 ± 0,5
7	Ochna schweinfurthiana	BR0000024710633	Décoction pendant 50 minutes de 65 g des écorces tige fraîches dans 1,5 L d'eau.	17,8 ± 0,6
8	Psorospermum corymbiferum	BR0000018879261	Maceration of 65 g of the root bark in 1.5 L of water.	38,5 ± 0,5
9	Rothmannia engleriana	BR0000024710626	Maceration for 48 hours of 65 g of the crushed leaves in 1L of water.	17,4 ± 0,4
10	Senecio cineraria	BR0000024711256	Decoction of a handful of the whole fresh plant in (1.5) L of water for 60 minutes.	12,3 ± 0,5

These unit quantities have been optimized according to the need for crude extract. HM: represents the number of the herbarium granted to the Meise herbarium, s□□□□□□□□SD□□extraction efficiency in percentage ± standard deviation, N = 3

2.5. pLDH antiplasmodial activity test

Antiplasmodial activity is evaluated by the colorimetric method developed above [30]. This method is based on the ability of plasmodial lactate dehydrogenase or pLDH to use APAD or 3-Acetylpyridine Adenine Dinucleotide as a coenzyme to convert lactate to pyruvate. This reaction is approximately 300 times faster than that carried out by human LDH. In the presence of APAD, *Plasmodium falciparum* produces APADH, which in the presence of nitrotetrazolium blue chloride (NTB) is transformed into formazan blue. The latter is measured by colorimetry at 630 nm. All tests were performed using 2% parasitaemia and 1% hematocrit. For each crude extract, a series of 8 dilutions of order 2 (from 100 to 0.78125 µg / mL) was prepared, placed in two rows of a 96-well microplate, and tested in triplicate. Chloroquine and quinine were used as standards. Infected and uninfected erythrocytes were added as positive and negative controls, respectively. After 48 h of incubation at 37 ° C., the level of parasitaemia was estimated by measuring the activity of lactate dehydrogenase with a spectrophotometer at 630 nm. The percentage of antiplasmodial activity was calculated by the formula: AAP (%) = (Ab-Ae) * 100% / Ab. Where *Ab* is the absorbance measured for the growth control, *Ae*, the absorbance measured in the presence of the extract and AAP

(%) is the proportion of metabolically active cells. Results are expressed as average IC_{50}, the concentration of an extract that reduced the level of parasitaemia to 50%, using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

2.6. b-hematin formation inhibition test

Inhibitory activity of β-hematin synthesis was evaluated by the inhibition test of the formation of β-hematin [31]. Briefly, a stock solution of 200 µg / ml is prepared from which eight dilutions of order 2 are prepared. The latter are then interacted with the substrate in the mixture successively containing 50 µl of hemin (0.5 mg / ml dissolved in DMSO), 100 µl of sodium acetate buffer (0.5 M, pH = 4.450) and 50 µl of different concentrations of the plant extract. The mixture is then incubated at 37 ° C for 18-24 hours. After incubation, centrifugation takes place at 4000 rpm for 10 minutes, followed by removal of the supernatant. The unreacted free hematin is then eliminated, by washing with 200 µl of DMSO followed by centrifugation at 4000 rpm for 10 minutes then rejection of the supernatant. It follows the addition of 200 µl of NaOH and then the reading in a spectrophotometer UV-VIS at 405 nm. The negative control consists of double distilled water and chloroquine diphosphate is used as the positive control. The percentage of inhibition is obtained by the formula: IHz (%) = (Ab-Ae) / Ab × 100% where IHz (%) means the percentage of inhibition, Ab means absorbance of the negative control and Ae corresponds to absorbance of the test. GraphPad can then generate IC_{50} from the dose-response equations.

2.7. Antioxydant activity assay

Antioxidant activity was evaluated using DPPH method [29]. It consists in trapping the free radical DPPH (2, 2-Diphenyl-1-Picrylhydrasyl) with the extract with antioxidant potential and then reading the remaining amount of DPPH with a spectrophotometer and thereby deducing the anti-radical power of the extract. To do this, 50 µL of extract or positive control prepared at different dilutions of order 2 in methanol from a 100 µg / ml solution were interacted with 1950 µL of 0.002% DPPH in a plate. 96 wells (Nunc WVR, Germany). After mixing and incubating in the dark for 30 minutes, the solution was read at 492 nm (Thermo Fisher Scientific Inc., Waltham, USA). The tests were carried out in triplicate and the 0.002% DPPH solution was used as a negative control. The percentage of antioxidant activity was calculated by the formula: AAO (%) = (Ab-Ae) * 100 / Ab. Where: Ab = absorbance measured in the presence of the negative control; Ae = absorbance measured in the presence of the extract, AAO (%) = Percentage of inhibition. This percentage of activity made it possible to generate the concentration versus response equations thanks to which the IC_{50} or concentration at which the extract has 50% activity were calculated using GraphPad Prism Version 6 software to categorize the extracts.

2.8. Statistical data analysis

Statistical analysis of the data was made possible by GraphPad Prism version 6 software (GraphPad Software, La Jolla, USA). The comparison between two means was made by Student's t-test with the Welch correction; Multivariate analysis used the one-way variance analysis test and compared several variables. Bonferroni's correction was applied to him and the materiality threshold was set at 95%.

3. Result and Discussion

3.1. Antiplasmodial activity of different extracts from 10 plants on chloroquinosensitive 3D7 plasmodial strain.

The antiplasmodial activity on the 3D7 strain was evaluated for the methanolic (EM), aqueous (EA) and n-hexanic (EH) extracts from 10 plants thus making a total of 30 extracts. The overall results of this antiplasmodial screening on 3D7 shows that 21 extracts (70%) have an activity with an IC_{50} value <50 µg / mL These extracts represent 6 plants out of 10 (Table 2).

Table 2: Antiplasmodial activity on the 3D7 chloroquino-sensitive strains of 10 plant species known to be antimalarial in Bagira (DR Congo)

N°	Plant species	PU	EM	EA	EH
				CI_{50} ± SD (µg/mL)	
	Chloroquine			0,02 ± 0,01	
	Quinine			1,65 ± 0,6	
1	*Aframomum laurentii*	PE	63,6 ± 9,2	69,5 ± 2,3[a]	121 ± 2,3
2	*Chenopodium opulifolium*	PA	4,9 ± 0,1[a]	7,1 ± 0,2[a]	123,1 ± 0,2
3	*Dalbergia katangensis*	F	2,9 ± 0,2	5,6 ± 0,3[b]	4,7 ± 0,3[a]
4	*Dialium angolense*	F	4,5 ± 0,1[a]	3,1 ± 0,1	17,2 ± 0,3
5	*Ekebergia benguellensis*	ER	15,9 ± 0,5	68,4 ± 0,1[c]	132 ± 2,3
6	*Julbernardia paniculata*	F	51,5 ± 0,1	59,7 ± 0,5[a]	87 ± 2,3
7	*Ochna schweinfurthiana*	ET	11,7 ± 0,7	16,4 ± 0,3[a]	136 ± 2,1
8	*Psorospermum corymbiferum*	ER	5,5 ± 0,1	19,4 ± 0,7[b]	6,7 ± 0,3
9	*Rothmannia engleriana*	F	6,1 ± 1,7	16,3 ± 0,5[b]	45,1 ± 2,3
10	*Senecio cineraria*	PE	54,6 ± 0,7	61,2 ± 0,1[b]	71,4± 0,7

Results expressed as a mean ± standard deviation, N = 3, HM: Meise herbarium code, EM: methanolic extract, EA: aqueous extract, EH: n-hexane extract, a if p <0.01, b if p <0.001, c if p <0.0001.

Depending on their IC_{50} values, these 30 extracts have been grouped into 4 classes: *(i)* class 1 extracts with an $IC_{50} \leq 5$ µg / mL considered as very active extracts; *(ii)* class 2 extracts including 5 µg / mL $\leq IC_{50} \leq$ 15 µg / mL, these extracts are considered to be moderately active; *(iii)* class 3 of extracts including 15 µg / mL $< IC_{50} <$ 50 µg / mL, these extracts are considered as weakly active and at the end *(iv)* class 4 whose extracts have an $IC_{50} \geq 50$ µg / mL, the extracts of this class

are considered inactive.

The first class contains 5 extracts belonging to 3 plants. These include *Chenopodium opulifolium*, *Dalbergia katangensis* and *Dialium angolense* (table 2). Extracts with these IC_{50} values ≤ 5 µg / mL are considered in several antiplasmodial screens [32–34] as very promising and likely to lead to isolation of antimalarial compounds of interest. this interesting antiplasmodial activity of these three plants has just been reported for the first time in this study. Statistical analysis with ANOVA shows a statistically significant difference between these 5 extracts in comparison to the chloroquine used as a positive control ($p < 0.001$); however, no statistically significant difference was observed with quinine. These extracts therefore have an activity as interesting as quinine. The second class contains 6 extracts belonging to 5 plants, notably *Chenopodium opulifolium*, *Dalbergia katangensis*, *Ochna schweinfurthiana*, *Psorospermum corymbiferum* and *Rothmannia engleriana* (table 2). Antiplasmodial activity of extracts from this class is also of interest since it can also lead to the isolation of antimalarial compounds as in previous studies [35,36]. The third class contains 5 extracts belonging to 5 plants that are *Dialium angolense Ekebergia benguellensis*, *Ochna schweinfurthiana*, *Psorospermum corymbiferum*, *Rothmannia engleriana* (table 2). The interest of extracts of this class can be revealed in the research for the development of an improved traditional medicine either in monoherbal or in polyherbal therapies. The fourth class contains 14 extracts with low antiplasmodial activity. It can nevertheless be used in recipes for combinations based on several plants. In this class there are three plants including Aframomum laurentii, Julbernardia paniculata, Senecio cineraria. none of which showed activity with an $IC_{50} \leq 50$ µg / mL

Antiplasmodial screening on the 3D7 strain shows that only 7 plants have antiplasmodial activity on the chloroquine-sensitive strain. However, in the WHO African region, which supports more than 90% of the global incidence of the disease, these results are not satisfactory, more so since in the region, chloroquino-resistant strains are almost permanently observed. remaining. It is that during this study we also evaluated the antiplasmodial activity of plants which have passed the previous test on chloroquine-resistant strains W2.

3.2. Antiplasmodial activity of 7 plants reputed to be antimalarial in Bagira on chloroquino-resistant W2 plasmodial strain

The antiplasmodial activity on the W2 strain was carried out on the 15 extracts which showed an interesting activity on the 3D7 strain. The results obtained show that 3 extracts are very active, 6 extracts are moderately active, and two extracts are inactive (table 3).

Table 3: Antiplasmodial activity on the W2 chloroquine resistant strains of seven plant species known to be antimalarial in Bagira (DR Congo)

			CI_{50} ± SD (µg/mL)		
N	**Plant species**	**PU**	**EM**	**EA**	**EH**
	Chloroquine			66,2 ± 0,1	
	Quinine			1,52 ± 0,5	
1	*Chenopodium opulifolium*	PA	6,9 ± 0,3	14, 1 ± 0,2	ND
2	*Dalbergia katangensis*	F	3,6 ± 0,1	7, 2 ± 0,1	6,5 ± 0,3
3	*Dialium angolense*	F	3,9 ± 0,1	4,5 ± 0,1	18,5± 0,1
4	*Ekebergia benguellensis*	ER	20,1± 1,7	ND	ND
5	*Ochna schweinfurthiana*	ET	14,7 ± 0,7	61,2 ± 0,1	ND
6	*Psorospermum corymbiferum*	ER	58,5 ± 0,1	78,1 ± 0,1	91,5 ± 0,1
7	*Rothmannia engleriana*	F	14,1 ± 1,1	ND	ND

Results expressed as a mean ± standard deviation, N = 3, EM: methanolic extract, EA: aqueous extract, EH: n-hexane extract, a if $p < 0.01$, b if $p < 0.001$, c if $p < 0.0001$.

The very active extracts belong to 2 plants, *Dalbergia katangensis* (EM, IC_{50} = 3.6 ± 0.1 µg / mL) and *Dialium angolense* (EM, CI_{50} = 3.9 ± 0.1 µg / mL and EA, CI_{50} = 4.5 ± 0.1 µg / mL). One of the 7 plants, *Psorospermum corymbiferum* with $IC_{50} \geq 50$ µg / mL of its 3 extracts, does not show antiplasmodial activity on the resistant Chloroquino strain W2 (Table 3). Statistical analysis of the variances in the IC_{50} values of extracts of *Dialium angolense* and *Dalbergia katangensis*, the two best plants in the series, on strains 3D7 and W2, show no statistically significant difference between them. These two plants would therefore have equivalent activity both on chloroquine-sensitive strains and on chloroquine-resistant strains.

3.3. Hemozoin synthesis inhibitory activity

The inhibitory activity on the synthesis of hemozoin only affected the 6 plants which were found to be active on the chloroquine-resistant strain. The results obtained show that no plant has exhibited strong inhibitory activity on the synthesis of hemozoin. 5 extracts belonging to 3 plants nevertheless showed moderate activity according to our classification scale. These are *Dalbergia katangensis* (EM, IC_{50} = 7.3 ± 0.1 µg / mL and EA, IC_{50} = 10.1 ± 0.6 µg / mL), *Dialium angolense* (EM, IC_{50} = 7.1 ± 0.1 µg / mL and EA, IC_{50} = 9.5 ± 0.5 µg / mL), *Ochna schweinfurthiana* (EM, IC_{50} = 13.8 ± 0.8 µg / mL) and the 3 remaining plants did not show inhibiting activity of hemozoin synthesis (Table 4).

Table 4: Inhibitory activity on hemozoin synthesis of six plant species known to be antimalarial in Bagira (DR Congo)

			CI_{50} ± SD (µg/mL)		
	Plant species	**PU**	**EM**	**EA**	**EH**
	Chloroquine			0,018 ± 0,2	
	Quinine			1,65 ± 0,6	
1	*Chenopodium opulifolium*	PA	98,4±1,2	29,5±0,2	ND
2	*Dalbergia katangensis*	ER	7,3 ± 0,1	10,1 ± 0,6	21,1 ± 0,1
3	*Dialium angolense*	F	7,1 ± 0,1	9,5 ± 0,5	30,6 ± 0,2
4	*Ekebergia benguellensis*	ER	98,6±0,5	ND	ND
5	*Ochna schweinfurthiana*	ET	13,8±0,8	ND	ND
6	*Rothmannia engleriana*	F	98,3±1,6	ND	ND

Results expressed as a mean ± standard deviation, N = 3, EM: methanolic extract, EA: aqueous extract, EH: n-hexane extract, a if p <0.01, b if p <0.001, c if p <0.0001.

The comparison made between the variances of the IC_{50} values of *Dalbergia katangensis*, *Dialium angolense* and *Ochna schweinfurthiana* obtained during the evaluation of the antiplasmodial activity on the W2 strain and the values obtained during the evaluation of the inhibitory activity of the hemozoin synthesis shows a statistically significant difference (p <0.01). This proves that the antiplasmodial activity of the extracts of the above plants is superior to their inhibitory activity on the synthesis of hemozoin. These extracts would therefore contain compounds which act not only by inhibiting the synthesis of hemozoin but also by other mechanisms. These results also show that among the 6 plants active on the chlo

Roquine-resistant strains, *Chenopodium opulifolium*, *Ekebergia benguelensis* and *Rothmania engleriana* act by other mechanisms than the inhibition of the synthesis of hemozoin.

3.4. Antioxidant activity of 6 plants that showed antiplasmodial activity on the W2 strain

The antioxidant activity focused on 18 extracts from 6 plants actives on the strain W2. The extracts were categorized according to their IC_{50} values as for the antiplasmodial activity. The results obtained show that 7 extracts are very active, 5 extracts are moderately active, 3 extracts are weakly active, and 3 extracts are inactive (table 5).

Table 5: Antioxidant activity on DPPH of six plants reputed to be antimalarial in Bagira (DR Congo)

			CI_{50} ± SD (µg/mL)		
	Plant species	**PU**	**EM**	**EA**	**EH**
	Ascorbic acid			1 ± 0,4	
1	*Chenopodium opulifolium*	PA	5,9 ± 0,7	72,3 ± 0,2	4,9 ± 0,1
2	*Dalbergia katangensis*	F	0,8 ± 0,1	0,9 ± 0,1	4,8 ± 0,1
3	*Dialium angolense*	F	0,9 ± 0,1	1,6 ± 0,1	6,1 ± 0,3
4	*Ekebergia benguelensis*	ER	66,2 ± 3,1	62,8 ± 0,6	7,3 ± 0,4
5	*Ochna schweinfurthiana*	ET	4,8 ± 0,4	5,5 ± 0,1	12,5 ± 0,4
6	*Rothmannia engleriana*	F	15,7 ± 1,4	5,5 ± 0,1	31,7 ± 0,5

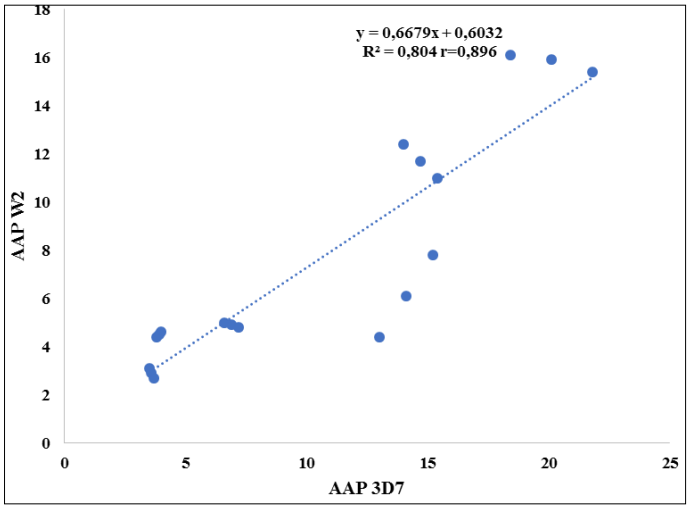
Results expressed as a mean ± standard deviation, N = 3, EM: methanolic extract, EA: aqueous extract, EH: n-hexane extract, a if p <0.01, b if p <0.001, c if p <0.0001.

The active extracts belong to 5 plants, in particular *Chenopodium opulifolium* (EH, CI_{50} = 4.9 ± 0.1 µg / mL), *Dalbergia katangensis* (EM, CI_{50} = 0.8 ± 0.1 µg / mL, EA, CI_{50} = 0, 9 ± 0.1 µg / mL and EH, IC_{50} = 4.8 ± 0.1 µg / mL), *Dialium angolense* (EM, CI_{50} = 0.9 ± 0.1 µg / mL and EM, IC_{50} = 1.6 ± 0.1 µg / mL) and *Ochna schweinfurthiana* (EM, IC50 = 4.8 ± 0.4 µg / mL) (Table 5). Statistical treatment with ANOVA does not show any difference between the IC_{50}s of methanolic and aqueous extracts of *Dalbergia katangensis* and *Dialium angolense* compared to the ascorbic acid used as a positive control. On the other hand, their IC_{50} of n-hexane extracts exhibit weak activities in comparison with the same positive control. The methanolic and aqueous extracts of *Dal*

bergia katangensis and *Dialium angolense* therefore exhibit an antioxidant activity equivalent to that of ascorbic acid, unlike their n-hexane extracts, the activity of which is lower

3.5. Correlation between different activities

The correlation between the different activities related to the 6 most active plants in the study particulary on methanolic and aqueous extracts was determined for the antioxidant, antiplasmodial and inhibitory activities of the synthesis of hemozoin (Fig 1). We observe a positive relationship between the different activities taken two by two with a correlation coefficient r ranging from 0.459 (aqueous extracts, AAO vs ISHZ) to 0.982 (aqueous extract, ISHZ vs AAP W2).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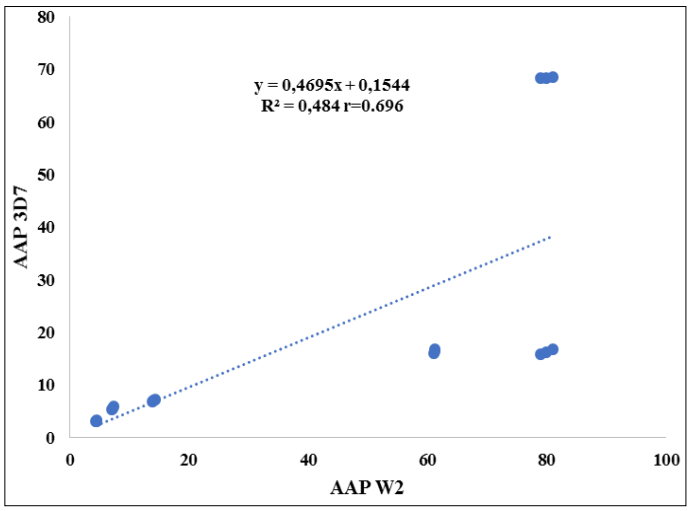
R² = 0,804 r=0,896
AAP W2
AAP 3D7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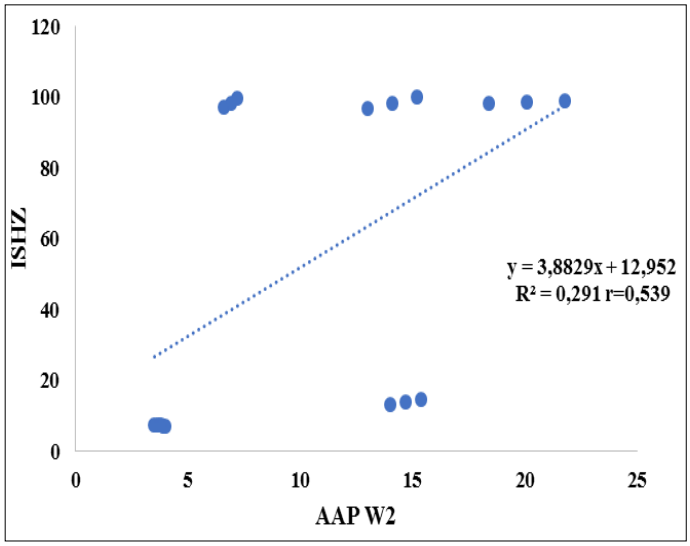
R² = 0,484 r=0.696
AAP 3D7
AAP W2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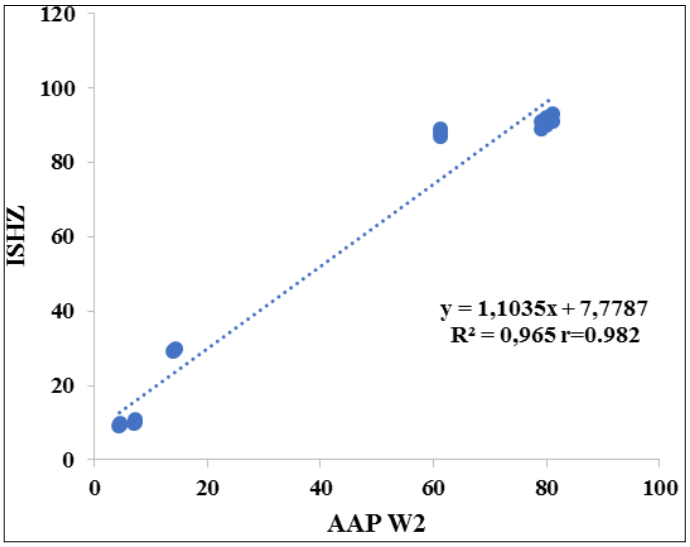
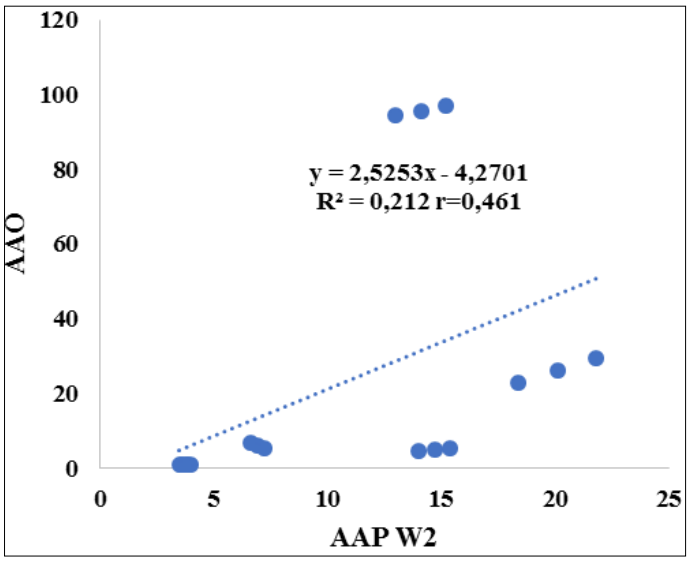
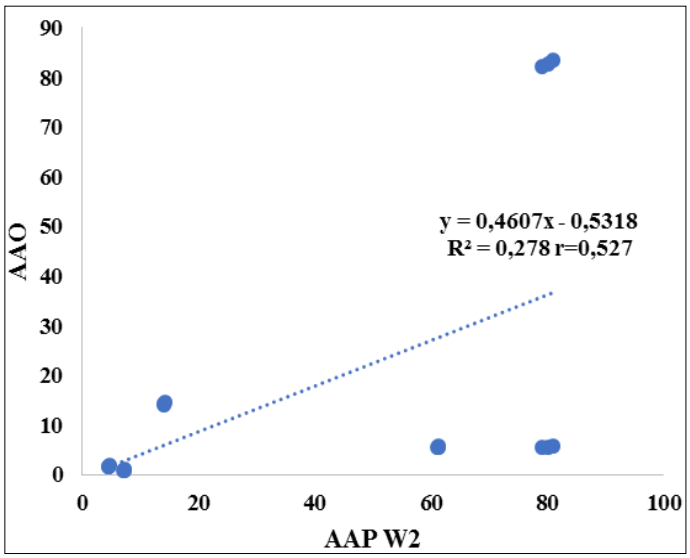
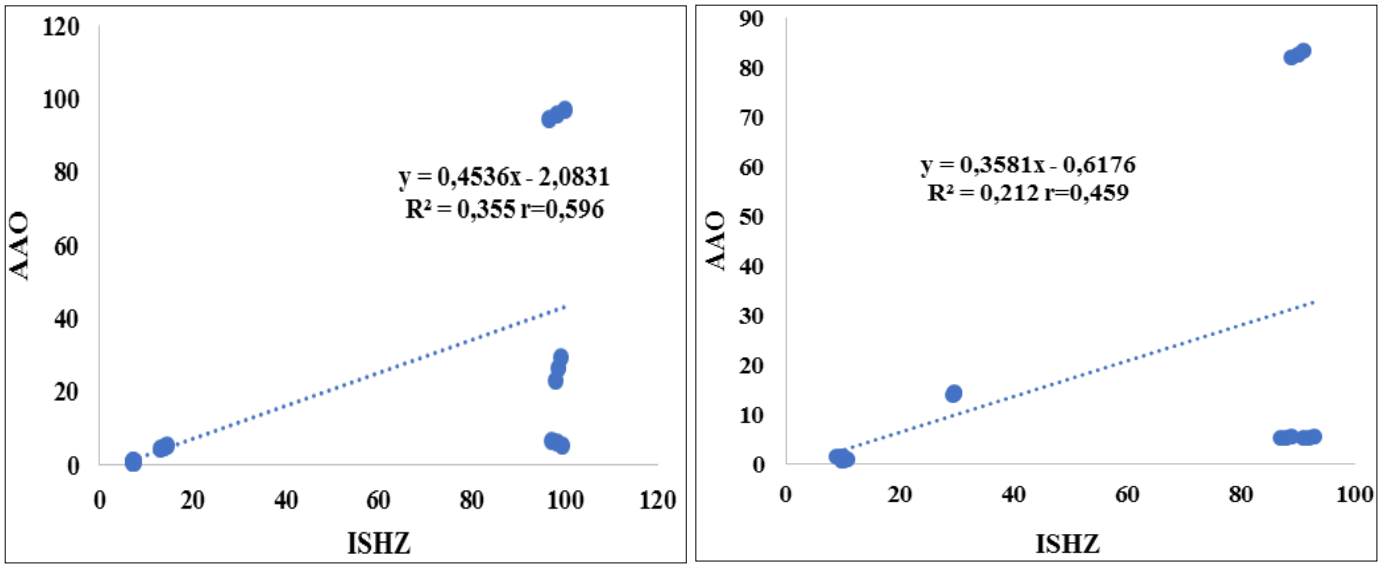
R² = 0,291 r=0,539
ISHZ
AAP W2


y = 1,1035x + 7,7787
R² = 0,965 r=0.982
ISHZ
AAP W2


y = 2,5253x - 4,2701
R² = 0,212 r=0,461
AAO
AAP W2


y = 0,4607x - 0,5318
R² = 0,278 r=0,527
AAO
AAP W2



AAO: *antioxydant activity*, AAP W2: *antiplasmodial activiy on W2 strain*, AAP 3D7: *Antiplasmodial activity on 3D7 strain*, ISHZ: *inhibitor of hemozoin synthesis activity*.

Fig 1: Correlation between severals activities evaluated in this study.

A very strong correlation is established between the inhibitory activity of the synthesis of hemozoin and the antiplasmodial activity on the resistant chloroquino strain for the aqueous extracts (r = 0.982) and between the antiplasmodial activity on the resistant chloroquino strain in comparison withantiplasmodial activity on the sensitive chloroquino strain (r = 0.896). This strong correlation suggests that the compounds responsible for the antiplasmodial activity on the two strains in the methanolic extracts are the same. It is the same for the inhibitory activity of the synthesis of hemozoin and the antiplasmodial activity of aqueous extracts.

4. Conclusion

This study proves that among the ten plants used in traditional medicine in Bagira RD Congo in the treatment of malaria, several of them, as *Dalbergia katangensis* and *Dialium angolense* have an antiplasmodial activity and act by several mechanisms including the inhibition of hemozoin synthesis. They also have a very interesting antioxidant potential which gives them added value compared to antimalarials used in clinical practice. This study suggests bioguided fractionation for the isolation of new compounds that are both antimalarial and antioxidant.

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