Journal of Advances in Microbiology

11(1): 1-11, 2018; Article no.JAMB.41897
ISSN: 2456-7116

Assessment of Antiplasmodial and Anti-anaemic Activities of *Hoslundia opposita*, an Ivorian Medicinal Plant

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Authors' contributions

This work was carried out in collaboration between all authors. Author KCM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TK, ADSD, GAA, BS and YSS managed the analyses of the study. Authors BAP and TAO managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/41897
Editor(s):
(1) Adekunle Sanyaolu, Epidemiology Division, Nigeria Center for Disease Control, Federal Ministry of Health, Abuja, Nigeria.
Reviewers:
(1) Morebise, Olugbenga, Al Saints University School of Medicine, Dominica.
(2) Voravuth Somsak, Walailak University, Thailand.
Complete Peer review History: http://www.sciencedomain.org/review-history/25096

Original Research Article

Received 20th March 2018
Accepted 30th May 2018
Published 12th June 2018

ABSTRACT

Aims: The aim of this study was to investigate the antiplasmodial and anti-anaemic activities of different extracts of *Hoslundia opposita*
Place and Duration of Study: This study was carried out from March to November at Institut Pasteur of Côte d'Ivoire.
Methodology: Extracts of *Hoslundia opposita* were prepared using various solvents. The *in vitro* antiplasmodial activity was investigated on both clinical isolates and Chloroquine-resistant K1 strain of *Plasmodium falciparum*, using SYBR green I based assay. Moreover, the anti-anaemic activity was assessed on phenyl hydrazine induced anaemia in albino rats.
Results: Two (2) among the five (5) extracts were not active. However, the hydroethanolic, aqueous and ethyl acetate extracts exhibited a moderate (IC_{50}= [22.88 – 49.65] µg/mL), promising (IC_{50}=

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[5.99 – 12.1] µg/mL), and very active (IC_{50}= 1.57 µg/mL) on clinical isolates. The hydroethanolic and aqueous extracts showed a promising antiplasmodial activity (IC_{50}= 11.68 and 12.23 µg/mL respectively) on Chloroquine-resistant K1 strain of *P. falciparum*. Whereas, the ethyl acetate extract exhibited a potent activity (IC_{50}= 0.78 µg/mL) on it. Extract showing good antiplasmodial activity were used for *in vivo* anti-anaemic test. Among selected extracts, the hydroethanolic extract was the most active with hematologic recovery percentages better than those of vitamin B_{12}.
Conclusion and Prospect: *Hoslundia opposita* could be a potential source for new antimalarial drugs. Furthermore, this plant extract might be used as an improved traditional medicine.

Keywords: Plasmodium falciparum; Hoslundia opposite; chemosensitivity.

1. INTRODUCTION

Malaria is a deadly parasitic infection, widespread in the tropical area. This disease is caused by a protozoan transmitted to humans through a bite of a female *Anopheles* mosquito and characterized by fever, caused by hemolysis due to the presence and development of parasites in red blood cells [1], sometimes leading to severe anaemia. According to WHO, anaemia prevalence is higher in areas where malaria is endemic, basically in Africa, with a rate ranging from 31% to 90% for children. Anaemia is common in malaria and severe anaemia may cause death through hypoxia and heart failure [2]. Despite considerable measures taken to kick off malaria, it remains a major cause of morbidity and mortality for most tropical countries. Africa is disproportionately bearing the global burden of malaria with 90% of cases, and 92% of deaths in 2015. Children under 5 years of age account for 70% of death worldwide [3].

Unfortunately, chemo-resistance outbreak is a serious public health issue, as several studies outlined low susceptibility of *Plasmodium falciparum* clinical isolates to antimalarial drugs in endemic areas [4]. Therefore, new active compounds are needed to diversify antimalarial drugs and the use of medicinal plants for therapeutic purposes has long been demonstrated [5]. Thus, due to the success of quinine and artemisinin derivatives, the treatment against *Plasmodium* resistant strains prompted the interest of researchers in medicinal plants to look for new antimalarial drugs [6,7]. The low cost, availability, accessibility and effectiveness are some reasons due to widespread use of medicinal plants [8]. It was then that [9,10], as well as many authors, evaluated the antianemic activity of plants in order to find new antianemic drugs.

For this purpose, the present study was designed to investigate on the antiplasmodial and antianemic potentials of different extracts of *Hoslundia opposita*, an Ivorian medicinal plant to seek for new antimalarial and anti-anaemic drugs.

2. MATERIALS AND METHODS

2.1 Animal Material

Albino rats of Wistar strain of both sexes weighting between 120 and 210 g obtained from the animal husbandry of Institut Pasteur of Côte d'Ivoire were acclimated for one week prior to experiment. They were marked for individual identification and then kept in plastic cages. Rats were regularly fed with food pellets manufactured by FACI Company and given water *ad libitum.* It should be noticed that experiments in this study were performed in compliance with the international standards of animal welfare as recommended by the European Union legislation for the protection of animals used for scientific purposes (Directive 2010/63/EU).

2.2 Plant Material

Plant material encompassed leaves of *Hoslundia opposita* known as “Anomalié” in “Baoulé”, “Dialésogroso” in “Gouro” or “deli” in “Madingo” (Ivorian’s local languages). *Hoslundia Opposita* leaves were collected in September 2016 in Moronou, in the Department of Toumodi (Côte d'Ivoire). Plant harvesting time was set in the morning (8 O’clock) and after collection they were brought to Abidjan. Samples were identified at the National floristic Center of the University of Félix Houphouët-Boigny, Abidjan (Ivory Coast). Leaves of *Hoslundia opposita* were dried in a shaded place for two weeks at room temperature (25°C) and then ground into powder using a mechanical grinder (IKAMAG RCT® "Staufen, Allemagne"). Plant extracts were therefore prepared from powder.

2.3 Preparation of Crude Extracts

Five successive extractions using increasing polarity solvents, according to the Protocols of [11,12] were performed. In this order, five solvents, such as hexane, ethyl acetate, ethanol, methanol, and distilled water were used.

2.3.1 Hexane extraction

One hundred grams (100 g) of drug powder was dissolved in 1 L of hexane. The hexane mixture was stirred for 24 hours at room temperature (25°C) using a magnetic agitator "IKAMAG RCT" (Staufen, Germany). The homogenate was filtrated on white linen tissue and then twice on hydrophilic cotton and finally on WHATMANN paper (3 mm). Filtrate was first reduced using a rotary evaporator at 40°C, before being dried in an oven (Venticel) at 45°C for 24 hours. The hexanic crude extract was obtained.

2.3.2 Ethyl acetate extraction (ACoET)

The residual marc obtained after extraction with hexane was dried. The powder was dissolved in 1 L of ethyl acetate and extraction was carried out following the same process as that of the hexane extraction.

2.3.3 Hydro-ethanolic extraction

The residual marc obtained after the ethyl acetate extraction was dried. The extraction was performed according to previous method but in this case the ethyl acetate was replaced by a hydro-alcoholic solvent constituted of distilled water (30%) and pure ethanol (70%).

2.3.4 Methanolic extraction

The residual marc obtained after the hydroethanolic extraction was dried. Extraction was carried out according to previous method using methanol.

2.3.5Aqueous extraction

The previous process was repeated on the residual Marc of the methanolic extraction with 1L of distilled water. After being dried in an oven for 72 hours, the aqueous crude extract was obtained.

2.4 Yield of Crude Extract

This yield is calculated according to the following formula:

$$Y\ (\%) = (We / Wv) \times 100$$

Y (%): Yield of extraction in percentage (%).
We: weight of extract after solvent evaporation.
Wv: weight of powder used for extraction [13].

2.5 Phytochemical Study

The phytochemical study consists in carrying out a qualitative chemical analysis based on coloring or precipitation reactions more or less specific to each class of secondary metabolites. Phytochemical groups (sterols, polyterpenes, alkaloids, tannins, polyphenols, flavonoids, quinones and saponins) in extracts were sought according to the methods described by [13].

2.5.1 Identification of sterols and polyterpenes (Liebermann reaction)

A quantity of 0.1 g of extract was dissolved in 1 mL of acetic anhydride and then taken up in a test tube, 0.5 mL of concentrated H_2SO_4 were poured. The appearance, at the interphase, of a purple and violet ring, turning to blue then green, indicating a positive reaction (revealing the presence of sterols and triterpenes).

2.5.2 Identification of saponins (foam index)

To identify saponins, 10 mL of each aqueous extracts were put in a test tube. The tube was stirred for 15 seconds and then left resting for 15 minutes. Persistent foam height greater than 1 cm indicates the presence of saponins.

2.5.3 Identification of alkaloids (Burchard reaction)

To identify alkaloids, the reagents of DRAGENDORFF (reagent of iodo-bismuthate) and BOUCHARDAT (iodoiodurized reagent) were used. Indeed, six (6) mL of each solution was evaporated to dryness. The residue was taken up in 6 mL of alcohol at 60°C. Addition of 2 drops of Dragendorff reagent to the alcoholic solution causes a precipitate or an orange colour. The addition of 2 drops of Bouchardat reagent to the alcoholic solution causes a reddish-brown precipitate and indicates a positive reaction.

2.5.4 Identification of polyphenols: ferric chloride reaction ($FeCL_3$)

To identify polyphenols, the ferric chloride reaction ($FeCL_3$) was used. Thus, a drop of

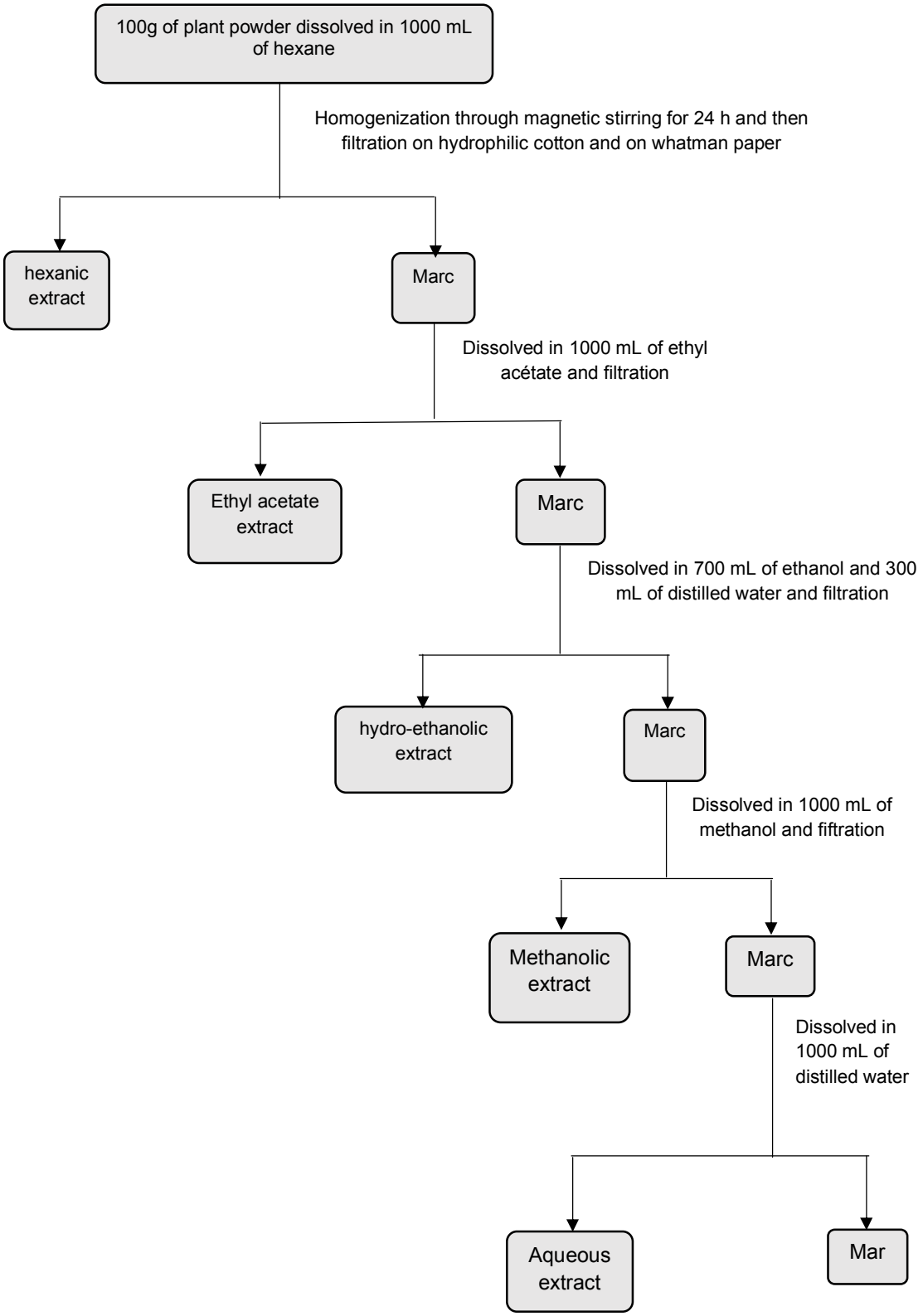

Fig. 1. Synoptic pattern of *Hoslundia opposita* extracts [12]

alcoholic solution of ferric chloride at 2% was added to 2 mL of each solution. The appearance of blue-blackish or green colour indicates a positive reaction.

2.5.5 Identification of flavonoids (cyanidin reaction)

To identify flavonoids, the cyanidin reaction was used. Two (2) ml of each extract were evaporated and the residue was taken up in 5 mL of a twofold diluted hydrochloric alcohol. By adding 2 to 3 magnesium chips, there was a heat release and then a pink-orange or purplish colour. The addition of 3 drops of isoamyl alcohol intensified this colour confirming the presence of flavonoids. An alcoholic solution of quercetin was used as standard.

2.5.6 Identification of tannins (1% ferric chloride reaction)

Two (2) mL of distilled water were poured in a test tube containing 1 mL of extract and then one to two drops of ferric chloride 1% were added. The appearance of a blue, blue-black or black colour indicates the presence of gallic tannins; the green or dark green colour indicates the presence of catechin tannins.

2.5.7 Identification of quinones (Borntraëger reaction)

An aliquot of extract was dissolved in 5 mL of 1/5 fold diluted HCl and heated in a boiling water bath for 30 minutes, then extracted with 20 mL of CHCl 3 after cooling. To the organic phase 0.5 mL of NH_4OH diluted at 50% were added . A red or purple colour indicated the presence of quinones.

2.6 *In vitro* Antiplasmodial Assay

The chloroquine-resistant K1 strain of *Plasmodium falciparum* (ATCC MRA-159, MR4, ATCC Manassas, Virginia), as well as four clinical isolates of *Plasmodium falciparum* (ANKTC037, ANKTC038, ANKTC046, and ANKTC047) collected from patients with uncomplicated malaria at Anoukoua-Kouté Community Health Centre (Abidjan, Cote d'Ivoire) were used for this study.

The reference strain K1 was maintained in culture in a human type O positive erythrocytes, according to the method described by [14] in RPMI 1640 medium (Gibco®, Life Technology, UK) containing 12.60 mL of HEPES buffer (25 mM), 100 mL of hypoxanthine, 312.5 µL of gentamycin (40 mg / mL) and glucose (20 g / L, Wagtech).

The chemosensitivity test was performed in duplicate in 96-well microplate with concentrations ranging from 100 to 1.56 µg / mL for crude extracts and from 1600 to 3.125 nM for chloroquine. Compounds were incubated in a total volume of 200 µL (RPMI, 2% hematocrit and 1.5% parasitemia) for 72 h in a modular incubator chamber saturated by CO_2 at 37°C in 96 well microplate.

After 72 h of incubation, 100 µL of each well was transferred to a new 96-well microplate and 100 µL lysis buffer containing SYBR green I (5 µL of SYBR Green were added to 25 mL of lysis Buffer) were added to each well and incubated in darkness at 37°C for 1 h. Fluorescence was measured using a spectrofluorimeter (BIOTEK, FLX 800) with excitation and emission wavelengths of 485 and 530 nm, respectively. The IC_{50} (concentration of a substance inhibiting 50% of parasite growth) was determined by the analysis of dose-response curves using IVART (*In Vitro* Analysis and Reporting Tool) software from WWARN [15].

2.7 Evaluation of Antianemic Activity

Anemia was induced by intraperitoneal injection of phenylhydrazine (Phz) at a dose of 40 mg / kg for 2 days, as described by [13]. After injections, rats were divided into nine groups of six rats.

-rats of group N (Control) received distilled water;

-rats of group A (negative control) received a dose of 40 mg/kg Phz daily for 2 days;

-those of lot V (positive control) received 1 ml / kg of body weight of vitamin B12 syrup from Day 2(D2) to Day 22 (D22);

those from groups 3, 4 and 5 respectively received 100 mg of ethyl acetate, aqueous and hydro-ethanolic extract of *Hoslundia opposita* per kg of body weight from D2 to D22; rats from groups 8, 9 and 10 respectively received 200 mg of ethyl acetate, aqueous and hydroethanolic extracts of *Hoslundia opposita* per kg of body weight from D2 to D22;

At the end of the experiment, blood was collected from the tails of anesthetized rats into collection tubes containing EDTA to determine biochemical parameters using an automatic blood cell counter (Sysmex XN 1000).

2.8 Ethical Consideration

Prior to conducting this study, a written informed consent was obtained from each patients and an approval letter was issued by the Ivorian National ethical committee.

2.9 Statistical Analyses

The data were analyzed using the Graph Pad Prism 5.01 software (San Diego California, USA). Values were expressed as mean ± standard of deviation. Data analysis was carried out using one way analysis of variance (ANOVA ONE WAY), followed by Dunnett test when P is significant at 0.05.

3. RESULTS

3.1 Yield of Different Extractions

The yield of extraction depends on plant species, the content of each species in secondary metabolites, the nature and the polarity of the solvent used for extraction. From the five crude extractions, the hydroethanolic extract gave the highest yield (9.2%) while that of methanol was the lowest (1.05%), Fig. 1.

3.2 Phytochemical Analysis

The phytochemical analysis of the different extracts of *Hoslundia Opposita* revealed the presence of major chemical compounds such as: alkaloids, polyphenols, sterols, terpenes, Gallic tannins, flavonoids and Quinones (Table 1). However, catechin tannins and saponosides were absent in two extracts.

3.3 *In vitro* Antiplasmodial Assay

Hoslundia opposita crude extracts were tested on both clinical isolates and Chloroquine-resistant K1 strain of *Plasmodium falciparum*.

Table 2 shows the IC_{50} values of crude extracts after data analysis. From the five extracts, two did not show any activity. The other three extracts, namely the ethyl acetate, the hydroethanolic and aqueous extracts, showed an antiplasmodial activity on the erythrocyte stage of *Plasmodium falciparum.* Thus, the hydroethanolic and aqueous extracts exhibited a promising antimalarial activity (IC_{50} = 11.68 and 12.23 µg/mL, respectively). While the ethyl acetate extract has a potent antimalarial activity (IC_{50} = 0.78 µg/mL) on chloroquine-resistant K1 strain of *Plasmodium falciparum* (Table 2).

3.4 Anti-anaemic Activity

After injection of phenylhydrazine to the nine groups of rats, except for the normal control, there was a significant decrease in red blood cells (49.59% ± 1.135), hemoglobin level (34.61% ± 1.717) and hematocrit rate (49.31% ± 0.528) on day 2 (Table 3, 4 and 5). After 10 days of treatment, these hematological parameters almost increased significantly ($P < .001$). However, the hydro-ethanolic extract was the most active with an increase in hematological parameters. This extract seems to be even better than the reference molecule (vitamin B12).

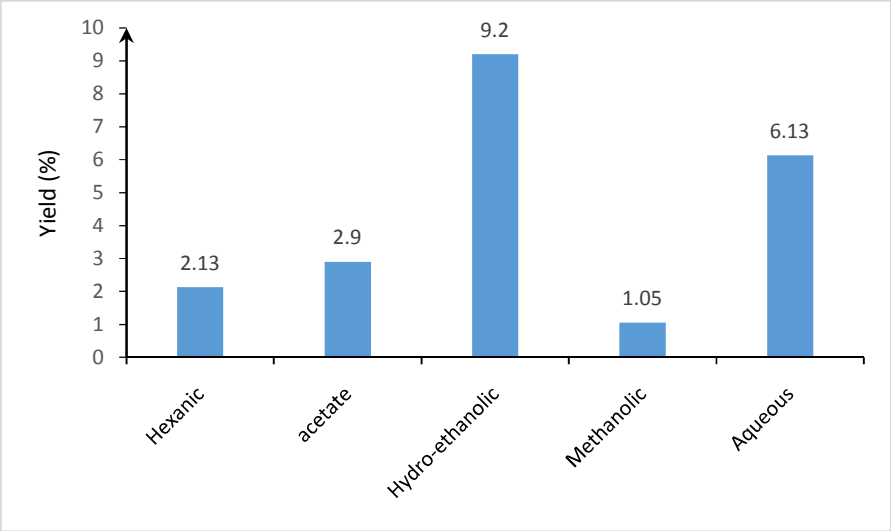

Fig. 2. Yields of different extracts of *Hoslundia opposita*

Table 1. Phytochemical analysis of different extracts of *Hoslundia opposite*

		Sterol et Polyter-penes	Poly-phenols	Flavo-noïds	Tannins		Quinonic compound	Alkaloids		Saponins
					Gal	Cat		D	B	
Crude extracts of *Hoslundia opposita*	Hexane	+	-	-	-	-	-	+	+	-
	Ethyl Acetate	+	-	-	-	-	-	+	+	-
	Hydro-ethanol	+	+	+	-	-	+	+	+	-
	methanol	+	-	-	-	-	-	+	+	-
	Aqueous	+	+	-	+	-	-	+	+	+

+ : Presence of compound
- : Absence of compound

Table 2. IC_{50} of different extracts of *Hoslundia opposita* on clinical isolates and *Plasmodium falciparum K1* strain

	IC_{50}				
Extracts (µg/ml)	**Isolate 2**	**Isolate 3**	**Isolate 4**	**Isolate 5**	***Plasmodium falciparum* K1 strain**
Hhex	> 50	> 50	> 50	> 50	Non valid
Hac	22.98	42.69	1.57	49.45	0.78
Hhe	12.04	12.1	5.99	1.52	11.68
Hme	> 50	> 50	2.87	> 50	Non valid
Haq	6.33	11.93	23.27	22.88	12.23
CQ (nM)	22.42	25.27	24.38	22.42	819.55

Hhex: H.opposita hexanic extract; Hac: H.opposita ethyl acetate extract; Hhe: H.opposita hydroethanolic extract ; Hme: H.opposita methanolic extract; Haq : H.opposita aqueous extract; CQ : Chloroquine

Table 3. Effect of *Hoslundia opposita extracts* on red blood cell counts in Phenylhydrazine induced anaemia in rats

Products	**Red blood cells (10^6cells/µL)**						
	before Phz injection	**D2 after Phz injection**		**D12 after Phz injection**		**D22 after Phz injection**	
Normal control (DW 10 ml/kg) from D2 to D22	9.01 ± 0.26	8.42 ± 0,30		8.91 ± 0.41		8.71 ± 0.30	
Negative control (DW 10 ml/kg) from D2 to D22	9.29 ± 0.19	5.22 ± 0.23	-43.81 ^{a***}	5.85 ± 0.20	+12.07 ^{b}	6.23± 0.23	+19.34 ^{b*}
Vit B12 (1ml/day) from D2 to D22	8.18 ± 0.16	4.03 ± 0.37	-50.70 ^{a***}	6.24 ± 0.20	+54.72 ^{b***}	7.12 ± 0.15	+76.47 ^{b***}
Hac (100 mg/kg) from D2 to D22	8.32± 0.05	4.46 ± 0.19	-46.40 ^{a***}	6.09 ±0.38	+36.61 ^{b***}	6.36 ± 0.20	+42.62 ^{b***}

Products	Red blood cells (10^6cells/µL)							
	before Phz injection	D2 after Phz injection		D12 after Phz injection		D22 after Phz injection		
Haq (100 mg/kg) from D2 to D22	9.5 ± 0.15	5.15 ± 0.17	-45.77 ^{a***}	7.17 ± 0.12	+36.15 ^{b***}	7.47 ± 0.09	+44.97 ^{b***}	
Hhe (100 mg/kg) from D0 to D22	8.55 ± 0.15	4.01 ± 0.29	-53.10 ^{a***}	6.29 ± 0.20	+56.86 ^{b***}	6.71 ± 0.07	+67.41 ^{b***}	
Hac (200 mg/kg) from D2 to D22	9.17 ± 0.19	4.7 ± 0.26	-48.72 ^{a***}	6.79 ± 0.23	+44.55 ^{b***}	7.27 ± 0.17	+54.77 ^{b***}	
Haq (200 mg/kg) from D2 to D22	9.59 ± 0.13	5.08 ± 0.19	-47.04 ^{a***}	7.26 ± 0.15	+42.97 ^{b***}	7.71 ± 0.18	+51.87 ^{b***}	
Hhe (200 mg/kg) from D0 to D22	8.28 ± 0.07	4.12 ± 0.09	-50.18 ^{a***}	6.65 ± 0.16	+61.24 ^{b**}	7.52 ± 0.34	+82.46 ^{b***}	

Mean ± SEM (n=6). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

a: Pourcentage of variation as compared to D0; b: Pourcentage of variation as compared to D2

DW: Distilled Water; Vit B12: Vitamin B12

Hac: H.opposita ethyl acetate extract; Haq: H.opposita aqueous extract

Hhe: H.opposita hydroethanolic extract D: Day Phz: Phenylhydrazine

Table 4. Effect of *Hoslundia opposita extracts* on the rate of hemoglobin in Phenylhydrazine induced anaemia in rats

Products	Hemoglobin (g/dl)							
	Before Phz injection	D2 after Phz injection		D12 after Phz injection		D22 after Phz injection		
Normal control (DW 10 ml/kg) from D2 to D22	14.80 ± 0.61	14.07 ± 0.41		14.50 ± 0.72		14.27 ± 0.55		
Negative control (DW 10 ml/kg) from D2 to D22	15.06 ± 0.55	10.46 ± 0.53	-23.90 ^{a***}	12.02 ± 0.59	+22.34 ^{b}	12.9 ± 0.31	+28.45 ^{b**}	
Vit B12 (1ml/day) from D2 to D22	14.23 ± 0.33	8.3 ± 0.59	-41.67 ^{a***}	13.70 ± 0.52	+65.06 ^{b***}	14.27 ± 0.52	+71.92 ^{b***}	
Hac (100 mg/kg) from D2 to D22	14.20 ± 0.12	9,9 ± 0,51	-30.28 ^{a***}	12.75 ± 0.38	+28.79 ^{b*}	13.25 ± 0.88	+33.83 ^{b**}	
Haq (100 mg/kg) from D2 to D22	15.14 ± 0.30	10.52 ± 0.28	-30.51 ^{a***}	14.80 ± 0.33	+40.68 ^{b***}	14.30 ± 0.13	+35.93 ^{b***}	
Hhe (100 mg/kg) from D0 to D22	14.76 ± 0.24	8.4 ± 0.39	-43.09 ^{a***}	13.97 ± 0.35	+66.31 ^{b***}	13.33 ± 0.13	+58.69 ^{b***}	
Hac (200 mg/kg) from D2 to D22	14.66 ± 0.24	9.98 ± 0.47	-31.92 ^{a***}	13.28 ± 0.50	+33.06 ^{b***}	13.30 ± 0.40	+33.27 ^{b***}	
Haq (200 mg/kg) from D2 to D22	15.98 ± 0.26	10.56 ± 0.33	-33,92 ^{a***}	14.36 ± 0.51	+35,98 ^{b***}	14.52 ± 0.06	+37.5 ^{b***}	
Hhe (200 mg/kg) from D0 to D22	14.22 ± 0.12	8.03 ± 0.21	-43.53 ^{a***}	14.33 ± 0.35	+78.45 ^{b***}	14.53 ± 0.48	+80.95 ^{b***}	

Mean ± SEM (n=6). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

a: Pourcentage of variation as compared to D0; b: Pourcentage of variation as compared to D2

DW: Distilled Water; Vit B12: Vitamin B12

Hac: H.opposita ethyl acetate extract; Haq: H.opposita aqueous extract

Hhe: H.opposita hydroethanolic extract D: Day

Phz: Phenylhydrazine

Table 5. Effect of *Hoslundia opposita extracts* on the rate of hematocrit in Phenylhydrazine induced anaemia in rats

Products	Hematocrit (%)						
	Before Phz injection	D2 after Phz injection		D12 after Phz injection		D22 after Phz injection	
Normal control (DW 10 ml/kg) from D2 to D22	51.70 ± 2.08	47.80 ± 1.65		51.90 ± 2.10		50.83 ± 2.07	
Negative control (DW 10 ml/kg) from D2 to D22	52.00 ± 1.56	27.16 ± 1.07	-43.92 ^{a***}	33.36 ± 2.408	+14.4 ^{b}	35.06 ± 1.627	+20.23 ^{b*}
Vit B12 (1ml/day) from D2 to D22	48.85 ± 0.99	23.13 ± 1.62	-48.56 ^{a***}	49.47 ± 1.75	+76.68 ^{b***}	49.48 ± 1.07	+85.97 ^{b***}
Hac (100 mg/kg) from D2 to D22	48.56 ± 0.49	24.28 ± 0.16	-50 ^{a***}	41.28 ± 2.66	+70.02 ^{b***}	44.78 ± 1.2	+84.43 ^{b***}
Haq (100 mg/kg) from D2 to D22	53.34 ± 0.89	26.74 ± 0.84	-49.67 ^{a***}	48.84 ± 1.04	+82.65 ^{b***}	50.42 ± 0.49	+88.56 ^{b***}
Hhe (100 mg/kg) from D0 to D22	50.32 ± 0.64	22.08 ± 1.77	-50.16 ^{a***}	46.97 ± 0.99	+87.28 ^{b***}	48.73 ± 0.47	+94.30 ^{b***}
Hac (200 mg/kg) from D2 to D22	49.40 ± 0.87	24.30 ± 0.84	-50.81 ^{a***}	42.3 ± 1.69	+74.07 ^{b***}	47.9 ± 1.19	+97.11 ^{b***}
Haq (200 mg/kg) from D2 to D22	54.60 ± 0.90	27.26 ± 0.77	-50.07 ^{a***}	50.14 ± 1.14	+83.93 ^{b***}	52.54 ± 0.66	+92.74 ^{b***}
Hhe (200 mg/kg) from D0 to D22	48.90 ± 0.62	24.28 ± 0.73	-50.35 ^{a***}	48.23 ± 0.77	+98.64 ^{b***}	51.35 ± 2.04	+111.49 ^{b***}

Mean ± SEM (n=6). $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.
a: Pourcentage of variation as compared to D0
b: Pourcentage of variation as compared to D2
DW: Distilled Water
Vit B12: Vitamin B12
Hac: H.opposita ethyl acetate extract;
Haq: H.opposita aqueous extract
Hhe: H.opposita hydroethanolic extract
D: Day
Phz: Phenylhydrazine

3. DISCUSSION

The *in vitro* antiplasmodial activity of five extracts of *Hoslundia opposita* was assessed on 4 clinical isolates and on chloroquine resistant K1 strain of *Plasmodium falciparum*. According to the Natural Substances Classification scale established by [16], the hexane and methanolic extracts of *Hoslundia opposita* were not active (IC_{50} > 50 µg/mL). However, the acetate extract was found to be active on clinical isolates. The hydroethanolic extract has a good and promising activity. While the aqueous extract has promising and moderate activity on these clinical isolates. According to the same classification, the hydroethanolic and aqueous extracts showed a promising antimalarial activity on *Plasmodium falciparum* K1 strain, respectively). As for the ethyl acetate extract, it exhibited a potent effect on *Plasmodium falciparum* K1 strain.

Therefore, extracts exhibiting good antiplasmodial activity were selected for the *in vivo* antianaemic activity. Though all extracts showed an antianaemic activity, the hydroethanolic extract was the most active with red blood cells recovery percentages better than those of vitamin B12. These results are in accordance with those of [17]. A study conducted in Côte d'Ivoire showed that *Hoslundia opposita* contained iron and its decoction could be used to treat anaemia. It is, therefore, possible that these extracts contain one or more compounds that could interact and stimulate the formation and secretion of the erythropoietic growth factor. Indeed, erythropoietin systems have been reported to improve rapid synthesis of blood cells [18].

The phytochemical analysis of the three extracts revealed the presence of polyphenols, flavonoids, polyterpenes and alkaloids. Indeed, the cumulative presence of compounds such as polyphenols, triterpenes and alkaloids in the extracts demonstrates that this medicinal plant might have a synergistic effect on *Plasmodium falciparum* [4,19].

These results are in agreement with some previous works in which different parts of *Hoslundia opposita* collected in Kenya showed a potent antiplasmodial activity against *Plasmodium falciparum* chloroquine sensitive (D6) and resistant (W2) strains with IC_{50} values ranging from 2 to 4 µg/mL [20]. It has also been shown that organic root bark extracts of *Hoslundia opposita* harvested in Tanzania had a high *in vitro* antimalarial activity against Chloroquine-resistant K1 strain *of Plasmodium falciparum* [21].

In addition, the phytochemicals such as polyphenols, flavonoids and alkaloids, found in the hydro-ethanolic extract, could be responsible for its good hematologic recovery. Indeed, according to [22], these compounds play a key role in the absorption of iron in the body; which is very important in hematopoiesis.

4. CONCLUSION

On completion of this study, we noticed that the ethyl acetate, the hydroethanolic and aqueous extracts of *Hoslundia opposita* showed a good antiplasmodial activity. Furthermore, It was also demonstrated that all extracts exhibited a good anti-anaemic activity and the most active was the hydro-ethanolic extract. These results showed that some extracts of *Hoslundia opposita* (Hydroethanolic and aqueous) have both antiplasmodial and antianemic activities. *Hoslundia opposita* could be a potential source for new antimalarial drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Levine ND. Progress in taxonomy of the Apicomplexan protozoa. J. Protozool. 1988;35:518-520.
2. Menendez C, Fleming AF, Alonso PL. Malaria-related anemia. Parasitol Today. 2000;16(11):469-76.
3. WHO. World malaria report. Geneva. 2016; Consulté le 26/04/2018. Available:http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/
4. Nassirou RS, Ibrahim ML, Ilagouma AT, Mahamadou A, Mamoudou M, Abdoulaye A, et al. Évaluation *in vitro* de l'activité antiplasmodiale d'extraits de plantes issues de la pharmacopée traditionnelle du Niger. Journal of Applied Biosciences. 2015;89:8291–8300.
5. Ogbonna DN, Sokari TG, Agomuoh AA. Antimalarial activities of some selected traditional herbs from Southeastern Nigeria against Plasmodium Species. Res J Parasitol. 2008;3(1):25-31.

6. Olasehinde GI, Ojurongbe O, Adeyeba AO, Fagade OE, Valecha N, Ayanda IO, et al. *In vitro* studies on the sensitivity pattern on Plasmodium falciparum to anti-malaria drugs and local herbal extracts. Malaria J. 2014;13(1):63-69.
7. Akuodor GC, Amos GM, Essien AD, Essien DO, Akpan JL, Ezeokpo BC. Antimalarial potency of the leaf extract of *Aspilia africana* (Pers.) C.D Adams. Asian Pac J Trop Med. 2012;5(2):126-129.
8. Ijioma SNI, Okafor AI, Ndukuba P, Nwankwo AA, Akomas SC. Hypoglycemic, hematologic and lipid profile effects of *Chromolaena odorata* ethanol leaf extract in alloxan induced diabetic rats. Annals of Biological Sciences. 2014;2(3):27-32.
9. Droucoula GC, Kouakou SL, Miezan BAP, Tchimon DP, Yapi HF. Antianemic properties of *Trema guineensis* aqueous and ethanolic extracts. European Journal of Biotechnology and Bioscience. 2017;5 (2):20-24
10. Ponmozhi E, Ramya B. Anti-anemic activity *Murraya koenigii* leaves on phenylhydrazine induced anemia in rats. World Journal of Science and Research. 2015;1(1):1-8.
11. Bidié AP, Koffi E, N'guessan JD, Djaman AJ, Guédé-Guina F. Influence of *Mitragyna ciliata* (MYTA) on the microsomal activity of ATPase Na+/K+ dependent extract on a rabbit (heart). African Journal of Traditional, Complementary and Alternative Medicines. 2008;5(3):294-301.
12. Tuo K. Criblage phytochimique, activité antioxydante et antiplasmodiale in vitro de cinq plantes utilisées traditionnellement en Côte d'Ivoire contre le paludisme. Doctorat d'Université Thèse Unique, Université Félix Houphouët Boigny. 2015;200.
13. Koffi AJ, Bla KB, Yapi HF, Bidie AP, Djaman AJ. Phytochemical screening of some medicinal plants in Côte D'ivoire and evaluation of their extraction efficiency. International Journal of Pharmacognosy and Phytochemical Research. 2015;7(3); 563-569.
14. Trager W, Jensen JB. Human malaria parasite in continuous culture. Science. 1976;193:673-675.
15. Le Nagard H, Vincent C, Mentre F, Le Bras J. Online analysis of in vitro resistance to antimalarial drugs through nonlinear regression. Computer Methods and Programs in Biomedicine. 2011;104:10-18.
16. Jansen O, Tits M, Angenot L, Nicolas JP, De Mol P, Nikiema JB, Frédérich M. Anti-plasmodial activity of *Dicoma tomentosa* (Asteraceae) and identification of urospermal A-15- O-acetate as the main active compound, Malaria Journal. 2012; 11:1-9.
17. Koné WM, Koffi AG, Bomisso EL, Tra Bi FH. Ethnomedical study and iron content of some medicinal herbs used in traditional medicine in Cote d'ivoire for the treatment of anaemia. Afr J Tradit Complement Altern Med. 2012;9(1):81-87.
18. Fasola TR, Oloyede GK, Bello SI. Phytochemical investigation of guava (*Psidium guajava* linn.) Stem bark for haematological indices in albino swiss rats. EJEAFChe. 2012;11(4):315-327.
19. Camacho CM Del R, Croft SL, Phillipson JD. Natural products as sources of antiprotozoal drugs. Curr. Opin. Anti-Infect. Invest. Drugs. 2000;2:47–62.
20. Gathirwa JW, Rukunga GM, Mwitari PG, Mwikwabe NM, Kimani CW, Muthaura CN, et al. Traditional herbal antimalarial therapy in Kilifi district, Kenya. Journal of Ethnopharmacology. 2011;134(2):434-442.
21. Weenen H, Nkunya MH, Bray DH, Mwasumbi LB, Kinabo LS, Kilimali VA. Antimalarial activity of Tanzanian medicinal plants. Planta Med.1990;56(4):368-370.
22. N'guessan K, Kouassi KH, Ouattara D. Plantes utilisées pour traiter l'anémie, en médecine traditionnelle, par Abbey et Krobou, dans le sud de la Côte-d'Ivoire. J Appl. Sci Res. 2010;6:1291-2197.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25096