Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 885-888
Received: 12-09-2017
Accepted: 14-10-2017

Abbas AY
Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

Muhammad I
Department of Biochemistry, Faculty of Natural and applied Sciences, Umaru Musa Yaradua University, P.M.B. 2218, Katsina, Nigeria

Bilbis LS
Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

Saidu Y
Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

Onu A
Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

Correspondence
Abbas AY
Department of Biochemistry, Faculty of Science, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

In vitro antimalarial activity of some Nigerian medicinal plants

Abbas AY, Muhammad I, Bilbis LS, Saidu Y and Onu A

Abstract
Malaria remains one of the major public health problems in sub-Saharan Africa and Nigeria in particular. In recent time, the problem was exacerbated by increased development of resistance by the major etiological agent for human malaria *Plasmodium falciparum* to malaria treatment drugs. Therefore, novel antimalarial drugs entities are imperative. The *in vitro* antimalarial activity of eight medicinal plants was evaluated. The evaluation was done using World Health Organisation (WHO) Mark III test method and the plant materials were extracted sequentially using water-ethanol, hexane, petroleum ether and chloroform extracts. The eight plant evaluated include *A. africanus, C. farisona, T. macroptera, B. buonopozense,* C. *papaya, C. citrates, K. senegalensis* and *H. floribunda.* The results obtained have shown that each of the tested plants possesses at least one very active (< 5 µg/ml) or active (5 µg/ml < IC_{50}< 50 µg/ml) extract. This preliminary work has indicated that these plants are promising for further investigation of novel antimalarial agents.

Keywords: Malaria, medicinal plant, *Plasmodium falciparum*, resistance

Introduction
Malaria is an infectious disease caused by the protozoan parasites, *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium vivax.* The disease is confined mainly to tropical and subtropical regions of the world and is transmitted by the female anopheles mosquito. According to the WHO report, there were 212 million new cases of malaria worldwide in 2015 (range 148–304 million) [1]. The African Region accounted for most global cases of malaria (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) with estimated 235,000-639,000 deaths in 2015, mostly among children under five years and pregnant women [1]. Malaria is directly responsible for one in five childhood death in Africa [2]. In Nigeria, malaria is the most significant public health problem. It accounts for 25 % of under-five mortality, 30 % of childhood and 11 % of maternal mortality. About 50 % of the Nigerian population will have at least one episode of malaria annually while children below the age of five (about 24 million) will have two to four attacks annually [1]. The economic cost of malaria in Nigeria can be as high as 1.3 % of economic growth per annum. This is largely due to rising cost of treatment, loss of productivity and earning due to days lost from illness [1].

Since time immemorial, medicinal plants have been used as a source of medicine in virtually all cultures [3]. In Africa, medicinal plants extract are still widely used in the treatment of many ailments including malaria and about 80 % of African population use traditional medicine for primary health care [4]. Several medicinal plants have been used in different part of the world for the treatment of malaria. For example, quinine extracted from the back of cinchona tree was used as an antimalarial agent as far back as 1632 [5]. And this was later developed as antimalarial agents in form of primaquine, quinacrine, chloroquine and other quinine family of drugs [6]. There is increased and widespread resistance of the malarial parasites especially *P. falciparum* (the major etiological agent for human malaria) to the current standard malaria treatment drugs [1]. Hence, there is urgent need for the development of new novel drugs for the treatment of malaria. This is couple with the fact that most medicinal plants currently used for the treatment of malaria have little scientific data to validate their claimed antimalarial activity [3]. So, investigation of their antimalarial property is paramount in order to establish their efficacy as well as their potential as sources of novel antimalarial drugs.

In the present work, we have reported an *in vitro* antimalarial evaluation of some traditional medicinal plants used either as antimalarial or for other ailments in Nigeria.

Materials and Methods

Chemicals and Reagents

All the chemicals and reagents used for this study were of analytical grade and were purchased from Sigma-Aldrich, Germany, May and Baker Ltd, England, Ranbaxy, India or BDH Chemical Ltd, England.

Plant Materials

All plant materials used in this study were obtained from Sokoto, North-Western Nigeria. The plants were authenticated at the Herbarium, Botany unit, Usmanu Danfodiyo University, Sokoto, Nigeria and voucher specimens were deposited at the Herbarium. Different plant parts (Leaves and bark) of the selected were open-air-dried under the shade, pulverized (with pestle and mortar) into a coarse powder. The powder obtained was sieved (1 mm^2 sieve) into a fine powder and stored (at room temperature) in a sealed plastic container until required.

Preparation of the Extract

The fine powder of each medicinal plant (50 g) was extracted with ethanol-water (1:1, 500 mL) and separated at room temperature over night [7]. The filtrates obtained were sequentially extracted using organic solvents of different polarity. The filtrate (ethanol-water, 1:1) for each plant was first mixed with 100 mL of hexane in a separating funnel and mixed thoroughly. The mixture was then allowed to stand for an hour so that two clear immiscible layers are formed. The ethanol-water layer (which high density) will separate to the bottom of the funnel, while the hexane layer will separate to the top of the funnel. The hexane layer was decanted into an evaporating flask and concentrated by rotary vacuum evaporation. This procedure was repeated for petroleum ether and chloroform. The concentrated dried extracts were then stored at 4 ^{0}C until their use in antimalarial assays.

In vitro culture of *Plasmodium falciparum* and antimalarial activity

The World Health Organisation (WHO) Mark III test [8] was used in evaluating the *in vitro* antimalarial activity of the organic solvent extracts of the selected medicinal plants against *P. falciparum*. The extracts were reconstituted with distilled water into concentrations of 1 µg/ml, 5 µg/ml, 10 µg/ml 50 µg/ml and 100 µg/ml for the assays. In brief, the blood medium mixture (BMM) was prepared by mixing 0.9 mL of RPMI 1640 medium containing 25 mM HEPES (Sigma-Aldrich, Germany), 50 mM hypoxanthine (Sigma-Aldrich, Germany) with 100 µl of washed malaria parasite infected blood sample (clinical isolates of *P. falciparum*) obtained from Microbiology laboratory of Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto-Nigeria. Gentamycin (0.01 mg/ml) from May and Baker Ltd, England was added to BMM to prevent bacterial contamination. The extracts sensitivity antimalarial assays were done on 96-well plate. In each well of the plate, 50 µl of BMM, 50 µl RPMI 1640, 20 µl of type O^+ heat inactivated blood serum and 30 µl of different extracts concentrations were added. Six of the wells were labeled negative control and no extract was added (30 µl of RPMI 1640 was added to make up the volume to 150 µl) and another six well were labeled positive control and 30 µl chloroquine (Ranbaxy, India) was added. The mixtures were gently mixed to ensure homogeneity of the mixtures. The plates with the cultures were kept at 37 ^{0}C under an atmosphere of 5 % O_2 and 5 % CO_2 for 24 to 48 [9]. After the culture period, the mixtures were then harvested (after the negative control wells showed schizont formation) by gentle siphoning up the culture medium and transferring the erythrocyte sediment on a clean glass slide to make a thin film and fixed with 1-2 drop water-free methanol. The film was then air-dried and stained with Giemsa stain (BDH Chemical Ltd, England). The microscopic (Microscope Nikon corporation, Europe) examination was done using X 100 objective lens after a drop of immersion oil was applied to the film. Area of the film, where the total number of erythrocytes is approximately 250 per field was observed. The number of parasitized erythrocytes (presence of schizonts) in 20-30 fields for each slide was viewed and counted. The counts from the test wells and positive control were compared with that of the negative control. The results were expressed as percentage of growth inhibition. The concentration, which inhibited the growth by 50 % (IC_{50}) in comparison to negative control, was determined graphically by dose-response curves.

Results and Discussion

A number of Nigerian medicinal plants have been found to demonstrate an interesting antimalarial potential and have been evaluated for possible development of antimalarial drug as resistant to current standard drugs is reaching an alarming position [10, 11]. The current study evaluated the antimalarial activity of eight Nigerian medicinal plants for the purpose of scientific validation of the therapeutic efficacy and potential for their development as antimalarial drugs.

For each plant, four organic solvent extracts prepared by sequential extraction of the plant material with water-ethanol (W-E), hexane (HE), Petroleum ether (PE) and chloroform (CE) were tested. The results are summarized in Table 1. The activity of the extracts was defined according to the IC_{50} values obtained from dose-response curves after treatment with the extracts. Plant extract with an IC_{50} value < 5 µg/ml was classified very active. An extract showing an IC_{50} value 5 µg/ml < IC_{50}< 50 µg/ml, 50 µg/ml < IC_{50}< 100 µg/ml and IC_{50}> 100 µg/ml were considered active, moderately active and inactive respectively (Table 2). From the results, five of the plants were found to be very active (Table 2) against the malarial parasites. In *A. africanus*, water-ethanol and hexane extracts showed an IC_{50} of < 1 µg/ml (Table 1). For *C.citratus*, the water-ethanol extract showed an IC_{50} of 4 µg/ml while the hexane and chloroform extracts of *T. macroptera* showed an IC50 of <1 µg/ml (Table 1). The hexane and petroleum ether extracts of *H. floribunda* and *B. buonopozense* showed an IC50 of 2 µg/ml and 4 µg/ml (Table 1) respectively. The hexane (Table 1) extract of *C.farisona* (42 µg/ml), *C.citratus* (6 µg/ml) and *C.papaya* (20 µg/ml) were found to be active (Table 2) against the malarial parasites. Also, the water-ethanol extract (Table 1) of *T. macroptera* (9 µg/ml), *H. floribunda* (12 µg/ml) and *K. senegalensis* (8 µg/ml) were active (Table 2) against the parasites. The chloroform extract of *C. farisona* (8 µg/ml) and *C. papaya* (10 µg/ml) as well as the *C. citratus* (20 µg/ml) were also found to be active against the malarial parasites. Moderate antimalarial activity (Table 2) was found in the water-ethanol extract (Table 1) of *C. farisona* (59 µg/ml) and *B. buonopozense* (76 µg/ml). It was also observed in chloroform extract (Table 1) of *C. citrates* (52 µg/ml) and *H. floribunda* (62 µg/ml). However, the petroleum ether, chloroform, water-ethanol and hexane extracts (Table 1) of *A. africanus, C. farisona, H. floribunda, B. buonopozense, K. senegalensis and C. papaya* were found to be inactive (Table 2) with an IC_{50} of > 100 µg/ml.

Table 1: *In vitro* antimalarial activity (IC_{50} values) of the plants extract against malaria parasite growth.

Plant Name	Part Used	Extracts	Yield (%)	IC_{50} (µg/ml)
Asparagus africanus	leaves	Water-ethanol	4.1	< 1
		Petroleum ether	6.0	>100
		Hexane	2.0	< 1
		Chloroform	1.4	>100
Cadaba farisona	Leaves	Water-ethanol	3.5	59
		Petroleum ether	4.5	>100
		Hexane	4.6	42
		Chloroform	2.0	8
Cymbopogon citratus	Leaves	Water-ethanol	3.2	4
		Petroleum ether	1.0	26
		Hexane	6.4	6
		Chloroform	5.0	52
Terminalia macroptera	Bark	Water-ethanol	6.1	9
		Petroleum ether	1.6	50
		Hexane	1.4	< 1
		Chloroform	2.8	< 1
Holarrhena floribunda	Leaves	Water-ethanol	2.2	12
		Petroleum ether	0.4	>100
		Hexane	1.6	2
		Chloroform	2.4	62
Bombax buonopozense	Bark	Water-ethanol	7.8	76
		Petroleum ether	0.6	4
		Hexane	0.5	>100
		Chloroform	1.2	>100
Khaya senegalensis		Water-ethanol	4.5	8
		Petroleum ether	1.8	66
		Hexane	2.8	>100
		Chloroform	1.3	>100
Carica papaya		Water-ethanol	5.3	>100
		Petroleum ether	4.0	>100
		Hexane	1.5	20
		Chloroform	0.8	10
Chloroquine	-	-	-	< 1

Table 2: Antimalarial activity status of the plants extracts.

Measure of Extracts Activity	Plant Extract
Very active (IC_{50} < 5 µg/ml)	*A africanus* (W-E and HE)
	C. citratus (W-E)
	T. macroptera (HE and CE)
	H. floribunda (HE)
	B. buonopozense (PE)
Active (5 µg/ml < IC_{50}< 50 µg/ml)	*C. farisona* (HE and CE)
	C. citratus (HE and PE)
	T. macroptera (W-E)
	H. floribunda (W-E)
	B. buonopozense (PE)
	K. senegalensis (W-E)
	C. papaya (HE and CE)
Moderately active (50 µg/ml < IC_{50}< 100 µg/ml)	*C. farisona* (W-E)
	C. citratus (CE)
	T. macroptera (PE)
	H. floribunda (CE)
	B. buonopozense (W-E)
	K.senegalensis (PE)
Inactive (IC_{50}> 100 µg/ml)	*A africanus*(PE and CE)
	C. farisona (PE)
	H. floribunda (W-E)
	B. buonopozense (HE and CE)
	K. senegalensis (HE and CE)
	C. papaya (W-E and PE)

W-E, water-ethanol extract; HE, hexane extract; PE, petroleum ether extract; CE, chloroform extract

The results have shown that all the eight plants evaluated have at least one of its extract having either very active or active activity against malarial parasites (Table 2). The antimalarial activity of C. *papaya* [12], *C. citrates* [13], *K. senegalensis* [14] and *H.* floribunda [15] have been previously investigated, but to the best of our knowledge, this is the first time that *A. africanus*, *C. farisona*, *T. macroptera and B. buonopozense* antimalarial activity are evaluated. The antimalarial activity of

medicinal plants is associated with phytochemicals constituents in them especially the alkaloids content [16]. For example, the antimalarial activity of *C. fenestratum* was reported to be as a result of their protoberberine alkaloids [17].

Conclusion

This preliminary study warrant further phytochemical and *in vivo* studies including isolation and characterization of possible active ingredient(s) responsible for antimalarial activity of the promising extracts of the plants evaluated. This have already started in earnest and is ongoing.

References

1. World Malaria Report, Geneva, World Health Organization, 2016.
2. World Malaria Report, Geneva, World Health Organization, 2012.
3. Hoareau L, Dasilva EJ. Medicinal plants: a re-emerging health aid. Elec. J. of Biotech. 1999; 2(2):56-69.
4. WHO Traditional Medicine Strategy, Geneva, World Health Organization, 2002-2005.
5. Amusan OOG, Adergan EK, Makinde JM. Antimalarial active Principles of *Spathodeacampenulata* stem bark. Phytother. Res. 1996; 10(8):692-693.
6. Duker-Eshun G, Joroszewski JW, Asomaning WA, Oppong-Boachie F, Christensen SB. Antiplasmodial constituents of *Cajanus cajan*. Phytother. Res.2004; 18(2):128-130.
7. Morris KS, Aziz K. Tumour inhibitors 114 Aloe emodin: Antileukemic-principle isolated from *Rhamnus frangular* L. Lloydia. 1976; 39:223-224.
8. WHO/CTD/MAL/97 20 Rev 2, Geneva, WHO, 2001.
9. Trager W, Jensen JB. Human Malaria Parasite in continous Culture Science. 1976; 193(4254):673-5.
10. Ibrahim HA, Imam IA, Bello AM, Umar U, Muhammad S, Abdullahi SA. The potential of Nigerian Medicinal Plant As Antimalarial Agent: A review. Int. J. Sci. Tech. 2012; 2(8):600-5.
11. Government in action. Report from presidential research and communication unit, office of public communication, State House, Abuja, 2005.
12. Ayoola GA, Coker HAB, Adesogun SA, Adepoju-Bello AA. Phytochemical Screening and Antioxidant activities of some selected Medicinal Plants Used for Malaria therapy in south-western Nigeria. Trop. J Pharm. Res. 2008; 7(3):1019-1024.
13. Mann A, Muhammad G, Abdulkadir NA. Medicinal and Economic plants of Nupe land, Jube-Eram and publications, Lagos, 2003, 190.
14. Ngemenya MN, Tane P, Berzins K, Titanji VPK. Antiplasmodial Activity of some Medicinal Plants used in Cameroon:- preliminary toxicity studies of highly active extracts . XIth Annual Conference of The Cameroon Bioscience Society, 2004.
15. Fotie J, Bothle SD, Leimanis ML, Georges E, Rukunga G, Nkenfack AE . Lupeol long chain fatty acid ester with antimalarial activity from *Holarrhena floribunda* J. Nat. Prod. 2005; 69(1):62-67.
16. Maria CM, Ana PM, Milene G, Jorge P, Anto´nio PC, Virgı´lio R. Antimalarial activity of medicinal plants used in traditional medicine in S. Tome´ and Prı´ncipe islands. J. Eth. Pharm. 2002; 81:23-29.
17. Quan Le T, Yasuhiro T, Jun-ya U, Nhan TN, Yukiko M, Khurshida B *et al*. *In vitro* antiplasmodial activity of antimalarial medicinal plants used in Vietnamese traditional medicine. J. Eth. Pharm. 2003; 86:249-252