integrity Research Journals

Advanced Journal of Plant Biology
Volume 5(1), pages 16-20, June 2024
Article Number: 16F698AE2
ISSN: 2992-4928
https://doi.org/10.31248/AJPB2024.033
https://integrityresjournals.org/journal/AJPB

Full Length Research

Antimalarial evaluation of the stem bark extract of *Anacardium occidentale* stem bark *(Anacadeceae):* Cashew stem bark

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Received 30th April 2024; Accepted 26th June 2024

ABSTRACT: Malaria is a threat to life and one of the most notorious of all parasitic diseases. It is a protozoa disease caused by the genus *Plasmodium* and transmitted through the bite of an infected female Anopheles mosquito. Five species of this plasmodium exist and have been identified to infect humans: Plasmodium *falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale and Plasmodium knowlesi. P. falciparum* causes the most severe form of the disease in man. Antimalarial drugs exist for the treatment of malaria. Still, the development of resistance to antimalarial drugs by the parasites poses one of the greatest threats to malaria control, and this happens to be the main cause of recent increases in malaria morbidity and mortality. Some antimalarial drugs that have been used in the treatment of malaria diseases include quinine, and its derivatives, artemisinin in combination states, such as amodiaquine, mefloquine, lumefantrine or sulfadoxine-pyrimethamine, including some plant extracts for treatment of drug-resistant malaria and some antibiotics such as doxycycline, and tetracycline. Despite the use of synthetic drugs in the treatment of malaria, some medicinal plant like *Alstonia boonei (Apocynaceae)*, *Vernonia amygdalina* (bitter leaf), *Acanthospermum hispidum, Keetia leucantha, Carpolobia lutea aerial, Cymbopogon citratus* (lemongrass) and *Azadirachta indica* have been noted in the treatment of malaria with no exception to *A. occidentale.* Therefore, the present study investigated the anti-malarial activity of the stem bark extract and fractions of *A. occidentale.* The n-hexane: ethylacetate fraction of stem bark extract of *A. occidentale* demonstrated potential anti-malarial activity with percentage chemo-suppression of 56.18% and 65.85% at 1000mg/kg body weight after the fifth and seventh day of treatment, respectively.

Keywords: *Anacardium occidentale,* antimalarial, cashew stem bark, medicinal plants, *Plasmodium falciparum.*

INTRODUCTION

Historically, it is known that traditional herbal medicines have been on the forefront in the treatment of malaria for thousands of years in several parts of the world. The innovative antimalarial drug used in the Western hemisphere was extracted from the bark of *Cinchona* (Rubiaceae) species, the alkaloid quinine, still principally used. In the early centuries, infusions of the plant bark were used in the treatment of human malaria (Baird *et al.*, 1996). As research became obligatory, quinine was isolated and characterized (Saxena *et al*, 2003), thus becoming the ancient and most essential antimalarial drug still in existence.

Another discovery in the ancient medicinal plant used in the West is *Artemisia annua*, rediscovered in China in the 70th as a fundamental source of the antimalarial artemisinin (Bruce-Chwatt, 1982, Klayman, 1985).

Artemisinin-combined therapies (ACT) were formally adopted as the first-line treatment of uncomplicated and resistant malaria in Nigeria from 2005 onwards (Mokuolu *Et al*, 2007). Regardless of the efficacy of ACT, it is not widely and commonly used by all because of its high costs, limited production of artemisinin derivatives to Good Manufacturing Practices (GMP) standards and toxicity (Haynes, 2001; Malomo e*t al*., 2001; Adebayo *et al*., 2002; Afonso *et al*., 2006; Boareto e*t al*., 2008).

Ever since the isolation and characterization of Quinine and Artemisinin, the search for more traditional herbal medicine became significant, hence the need to investigate the efficacy of *Anacardium occidentale* stem bark for the treatment of malaria. *A. occidentale* is a plant belonging to the family Anacardiaceae. It is commonly called cashew and has been noted over the years as a multipurpose plant with numerous parts used in traditional medicine to treat several ailments, including malaria. Although, its claim for malaria treatment lacks sufficient scientific validation. There are reports that it possesses anti-diabetic, anti-inflammatory, anti-ulcerogenic, antifungal, antibacterial and analgesic properties, (Echindu, 1991, Akinpelu and Ojewole, 2001).

Anacardium occidentale L. (the “cashew”) is well known as an essential source of alkyl-phenols, isolated from its fruit (the “cashew nuts”). These compounds have numerous biological actions depicted amongst them are: antioxidant, cytotoxicity to cancer cell lines, larvicidal, antibacterial, molluscicidal, and schistosomicidal (Kubo *et al*., 1994).

Therefore, the objective of this study is to determine the anti-malarial activity of the plant extracts and its fractions.

MATERIALS AND METHODS

Sample collection and preparation of *Anacardium occidentale*

The fresh stem bark of *A. occidentale* were collected from the Faculty of Agricultural Science farm, University of Benin, Ugbowo Campus, Benin City, Nigeria. The plant stem bark sample was authenticated by Prof. MacDonald Idu of the Department of Plant Biology and Biotechnology University of Benin and Voucher number UBH-A389 issued. The fresh samples were rinsed with running water, dried at ambient temperature (30ºC) and ground to powder using a mechanical grinder. The powdered stem bark was stored in an air-tight sample bottle and kept for further analysis.

In vivo antimalarial assay

Parasite inoculation

The Chloroquine sensitive *Plasmodium berghei* (NK-65 strain) infected mice were obtained from the Nigerian Institute of Medical Research (NIMR), Lagos, Nigeria and kept at the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City prior to commencement of the experiments. Each mouse used in the experiment was infected intraperitoneally with 0.2 mL of infected blood containing about 1 x 10^7 *P. berghei* – parasitized erythrocytes. Dilution was made with 3 mL of 7.2 pH Phosphate Buffer Saline (PBS), giving rise to 1x10^8 of parasitized red blood cells PRB/mL working solution. The parasitized mice were grouped into twenty-one groups of five mice each labelled 1-25.

Preparation of Giemsa solution

Giemsa powder (3.4 g) was dissolved in 250 mL of methanol and after the dissolution, 250 mL of glycerol was added and the mixture was thoroughly shaken. The procedure was done in a dark room. The solution was poured into a dark reagent bottle and kept in a dark cupboard for a week. The resulting solution was properly filtered before use.

Preparation of phosphate buffered saline (PBS)

Sodium dihydrogen phosphate (NaH_2PO_4) (10.9 g) and 3.2 g of disodium hydrogen phosphate (Na_2HPO_4) were dissolved in distilled water and the solution was made up to 1000 mL using distilled water. The pH of the solution was adjusted to 7.2, with concentrated HCl.

Evaluation of suppressive activity (4 Day Test)

This test was used to investigate the antiplasmodial activity of the samples and chloroquine against *P. berghei* in mice. The tests were performed in a 4-day suppressive standard test using the methods of Knight and Peters (1980). On the first day (D_1), one hundred and five Swiss albino mice were inoculated (intraperitoneally) with 0.1 mL of parasitized erythrocyte (containing about 1 x 10^7 *P. berghei*), and they were randomly divided into twenty one groups (Group 1-21) of five mice each and treated for the next four consecutive days (D_1-D_4). Groups 1-18 received daily doses of the extract (300, 500 and 1000 mg of extract/kg body weight of animal) by the oral route, group 19 received no treatment, (negative control), while group 20 received 5 mg/kg of chloroquine and group 21 received 1.75 mg/kg of artesunate daily by the oral route at the same time. The required dose was given according to the weight of the animals two hours after inoculation of parasites on D_1, then once daily for three more days (D_2–D_4)

Evaluation of parasitaemia

On day five (D_5) of the study, thin films were prepared with blood collected from the tail of each mouse. The thin film was fixed with methanol and stained with Giemsa and the thin blood films of infected and treated mice were

Table 1. Day 5 result of percentage parasitaemia of different fractions of the stem bark of *A. occidentale* with standard drugs (Chloroquine and Artesunate).

Conc. of extract (mg/kg)	Chloroquine	Artesunate	Crude extract	n-Hexane	n-Hex/ Ethyl acetate	Ethyl acetate	Ethyl acetate /Methanol	Methanol
1.75	-	2.75±0.07	-	-	-	-	-	-
300	-	-	12.00±0.02	11.40±0.22	11.47±0.01	11.97±0.02	9.95±0.02	11.18±0.21
500	-	-	9.27±0.08	8.46±0.01	10.82±0.02	9.27±0.02	8.43±0.10	9.38±0.14
1000	-	-	7.53±0.01	5.85±0.01	7.56±0.02	7.69±0.01	6.97±0.12	7.35±0.32
5	2.63±0.05		-	-	-	-	-	-

Table 2. The day 7 result of percentage parasitaemia of different fractions of the stem bark of *A. occidentale* and those of standard drug (chloroquine/Artesunate).

Conc. of extract (mg/kg)	Cloroquine	Artesunate	Crude extract	n-Hexane	n-Hex/ Ethyl acetate	Ethyl acetate	Ethyl acetate /Methanol	Methanol
1.75	-	1.35 ± 0.04	-	-	-	-	-	-
3	-	-	10.82 ± 0.03	9.49 ± 0.42	11.45 ± 0.09	10.77 ± 0.05	9.35 ± 0.06	10.48 ± 0.08
500	-	-	8.82 ± 0.02	7.49 ± 0.44	9.73 ± 0.04	8.64 ± 0.18	7.70 ± 0.09	8.60 ± 0.07
1000	-	-	6.19 ± 0.77	4.67 ± 0.19	6.74 ± 0.25	6.60 ± 0.23	6.28 ± 0.19	5.85 ± 0.05
5	1.24 ± 0.04	-	-	-	-	-	-	-

examined with 100x magnification for parasitemia level under the microscope (in oil immersion) from day five through day seven (D_5 – D_7). Red blood cells were counted in 10 fields of vision and the parasitized cells were noted. The percentage parasitemia in a group was calculated as:

$$\% \text{ parasitemia} = \frac{No.\ of\ parasitised\ RBC}{total\ No.\ of\ RBC} \text{x } 100 \text{ ----- (1)}$$

Where: RBC = red blood cells.

The average percentage suppression for each dose of each sample was calculated in comparison with controls as follows:

$$\text{Average } \% \text{ suppression} = \frac{X-Y}{X} x\ 100 \text{ ----- (2)}$$

Where: X= Average % parasitaemia negative control; Y= Average % parasitemia treated groups.

Statistical analysis

The results obtained were enunciated as mean ± standard error of the mean (SEM) of six duplicates. The obtained data were subjected to one-way analysis of variance (ANOVA) and the difference between means was determined by Duncan's multiple range tests by the use of the Statistical Analysis System (SPSS Statistics 17.0) where applicable. P-values <0.05 were considered significant in this research.

RESULTS AND DISCUSSION

Tables 1 and 2 show the effects of feeding the stem bark of *A. occidentale* extract and fractions on mice infected with chloroquine sensitive *Plasmodium berghei* (NK-65 strain) to determine the anti-plasmodial. The anti-plasmodial activity on the stem bark of *Anarcadium occidentale* stem bark on mice was monitored on days 5 and 7.

The emergence of widespread resistance of Plasmodium species to most antimalarial drugs has led to a more vigorous and concerted research into medicinal plants for the treatment of malaria. *A. occidentale* is commonly known traditionally for its efficacious use in the treatment of different ailments including malaria in Nigeria.

According to the previous findings by some researchers and authors, the antiplasmodial activity of extracts and isolated compounds were classified as follows; highly active for oral doses of 100-250 mg/kg/day providing ≥ 60% suppression of parasitaemia, moderate activity at oral doses of 100-250 mg/kg/day providing ≥ 30-59% suppression of parasitaemia, promising/evidence of anti-malaria activity at ora l doses of 300-650 mg/kg/day

Table 3. The Day 5 result of percentage chemo-suppression of *P. berghei* by the stem bark of *A. occidentale* and standard drugs (chloroquine and Artesunate).

Conc. of extract (mg/kg)	Chlororquine	Artesunate	Crude extract	n-Hexane	n-Hex/Ethyl acetate	Ethyl acetate	Ethyl acetate methanol	Methanol
1.75	-	79.19	-	-	-	-	-	-
300	-	-	10.40	14.55	13.95	12.45	26.99	16.12
500	-	-	25.65	36.53	18.90	29.00	36.83	29.66
1000	-	-	43.49	56.18	43.28	42.34	47.71	44.86
5	80.70	-	-	-	-	-	-	-

Table 4. The Day 7 result of the percentage chemo-suppression of *P. berghei* by the stem bark of *A. occidentale* and standard drug (chloroquine/Artesunate).

Conc. of extract (mg/kg)	Chloroquine	Artesunate	Crude extract	n-Hexane	n-Hex/Ethyl acetate	Ethyl acetate	Ethyl acetate methanol	Methanol
1.75	-	89.99	-	-	-	-	-	-
300	-	-	18.61	27.23	15.18	18.94	30.43	21.33
500	-	-	33.75	46.68	26.70	35.73	42.87	35.64
1000	-	-	50.71	65.83	50.88	51.83	53.87	56.35
5	90.95	-	-	-	-	-	-	-

providing 47.0-84.5% suppression of parasitaemia (Mota *et al*., 1985; Gathirwa *et al.*, 2011; Mesfin *et al*., 2012; Falade *et al,* 2014; Upadhyay *et al.,* 2014).

The results obtained as presented in Tables 1 and 2 show that the percentage mean parasitized red blood cells at various concentrations were dose-dependent. The mean parasitized red blood cell gives information on the number of parasites present in the red blood cells. From the observations, it was seen that the n-hexane/ethyl acetate fraction showed a more promising antimalarial activity owing to its ability to exert more effect on the parasites, thereby reducing the parasite count more prominently as compared to the other fractions. The five fractions however exhibited significant antimalarial activity and this result revealed that the fractions of *A. occidentale* stem bark may contain some biologically active substances that are useful in the management of malaria.

Chemo-suppression is the ability of the extract to kill the parasite at the blood stage and it is monitored in days. The results obtained for Day 5 and Day 7 are represented in Tables 3 and 4 respectively. The chemo-suppression investigation revealed that the n-hexane/ethyl acetate fraction exhibited the highest chemo-suppression at 1000 mg/kg body weight in both days 5 and 7. More so, the n-hexane fraction exhibited the lowest percentage of chemo-suppression at 300 mg/kg body weight in both days. This result agrees with those reported by Odugbemi *et al*. (2007) who acknowledged the potency of *A. occidentale* in the treatment of fever and malaria.

Alkaloids are one of the major classes of compounds possessing antimalarial activity and one of the oldest and most important antimalarial drugs, quinine belongs to this class of compounds (Odugbemi *et al*., 2007). Possibly, the presence of alkaloids in *A.occidentale* extract might have contributed to the antimalarial activity exhibited by the plant extracts. Also, flavonoids and terpenoids are compounds with a widespread occurrence in the plant kingdom which have also been detected in Artemisia species. They are reported to have exhibited significant *in vitro* antimalarial activity against *P. falciparum* (Dharani *et al*., 2008). The presence of these in *A. occidentale* extract, justifies the antimalarial activity exhibited by the plant extracts. Hence, the results of the present study revealed that *A. occidentale* exhibited potent antimalarial activity against *P. falciparum* isolates *in vivo*.

Recommendation for further work

It is recommended that an *in vitro* antimalarial activity be carried out on the plant extract for better comparison of the plant's potency.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ACKNOWLEDGMENTS

The authors wish to appreciate the staff of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin city, Edo State Nigeria.

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