



IN VITRO ANTI-PLASMODIAL ACTIVITY OF *ANNONA SENEGALENSIS* PERS AGAINST THE 3D7 STRAIN OF *PLASMODIUM FALCIPARUM*

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ABSTRACT

The antiplasmodial activity of 70% aqueous methanol crude extract of *Annona senegalensis* leaf, stem bark and root were evaluated against the 3D7 strain of *Plasmodium falciparum* with the aim of searching for new plant derived drug candidates against malaria infection. The study was carried out using the SYBRGreen 1 assay in an *ex vivo* culture of parasitized red blood cells complimented with a Giemsa stained microscopy study of same to ascertain the validity of the results. The stem bark extracts gave antiplasmodial activity while the leaf and root extracts showed no antiplasmodial activity. This study shows that the stem bark of *Annona senegalensis* has some antimalarial pharmacophores, which if isolated, would be a lead to developing new antimalarials.

Keywords: *Annona senegalensis*, *Plasmodium falciparum*, 3D7 strain, SYBRGreen 1 assay, antimalarials.

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INTRODUCTION

Malaria is the most important parasitic disease with vast distribution across the tropics and subtropics [1]. It is closely tied to very high morbidity and mortality especially amongst children below the ages of 5 and pregnant women. In the last decade, the prevalence of malaria has been escalating at an alarming rate, especially in Africa, with an estimated 1.5 to 2.7 million deaths and 300 to 500 million cases recorded annually [2].

In addition to its burden in terms of morbidity and mortality, the economic effects of malaria infection can be tremendous. These include direct costs for treatment and prevention, as well as indirect costs such as loss of productivity from morbidity and mortality, time spent seeking treatment and diversion of household resources. The annual economic burden of malaria infection in 1995 was estimated at US\$ 8 billion for Africa alone [3]. This heavy toll can hinder economic and community development activities throughout the region [4].

Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. Resistance to antimalarial drugs has been described for two of the four species of malaria parasite that naturally infect humans namely, *P. falciparum* and *P. vivax*. *Plasmodium falciparum* has developed resistance to nearly all antimalarials in current use and this has further complicated the struggle towards the eradication of the disease burden.

Plants and other natural products have been resourceful in the treatment of various ailments of human and other organisms and with the current incidence of parasite resistance to mainstay drugs, the need to investigate new pharmacophores cannot be underscored. *Annona senegalensis* is a shrub or small tree that grows largely in the savannah. It is known locally by the names 'Gwandar daji' (Hausa), 'Uburu-ocha' (Igbo), 'Abo' (Yoruba) and Wild custard apple (English common name) [5]. This study was designed to investigate its antiplasmodial potential in a bid to add to the pool of new leads for possible better antimalarials.

MATERIALS AND METHODS

Plant materials

The leaves (W25L), stem bark (W26S) and root bark (W6R) of *Annona senegalensis* (Plate 1a) were collected from the wild in Zaria (11°05'N, 7°43'E), northern Nigeria. The samples were taken to the Herbarium Section of Department of Biological Sciences, Ahmadu Bello University, Zaria, where they were properly identified and documented, and assigned a voucher number 190.

Preparation of plant extracts

Plant materials were air dried at room temperature for 1-2 weeks, after which each was ground to powder. 150 g of the leaves, 144 g of the root bark and 250 g of the stem bark were weighed and macerated in 1L of 70% aqueous methanol for 72 hr. The filtrates obtained were evaporated to dryness using an evaporating dish on a water bath at 45-50 °C and were kept under aseptic condition till usage [6].

In vitro cultivation of *Plasmodium falciparum* 3D7 strain and antiplasmodial activity assay

The plant extracts were subjected to an *in vitro* antimalarial screening using the chloroquine sensitive 3D7 *Plasmodium falciparum* strain, obtained from the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India. These were maintained in media composed of RPMI 1640 with L-glutamine and 25 mM Hepes buffer (16.2 g/L), supplemented with NaHCO₃ (2 g/L), 5% Albumax Hypoxanthin (100 ml/L) and Gentamycin of 10 mg/ml (20 mg/L) in a secador box filled with gas mixture of 5% O₂, 5% CO₂ and 90% nitrogen at 37°C in a CO₂ incubator [7]. SYBR green I-based fluorescence assay was used to determine cell viability after inoculation of parasites with the test plant materials as described by Smilkstein [8]. Sorbitol synchronized parasites were incubated under normal culture conditions at 2% hematocrit and 1% parasitemia in varying concentrations of plant extracts. Artemisinin and chloroquine were used as

positive controls, while DMSO at 0.4% was used as negative control. After 48 hr. of incubation, 100 µl of SYBR Green I solution (0.2 µl of 10,000 X SYBR Green I (Invitrogen)/mL) in Lysis Buffer (Tris (20 mM; pH 7.5), EDTA (5 mM), Saponin (0.008%; w/v), and Triton X-100 (0.08%; v/v)) was added to each well and mixed twice gently with multi-channel pipette and incubated in the dark at 37 °C for 1 hr. Fluorescence was measured with a Victor fluorescence multi-well plate reader (Perkin Elmer) with excitation and emission wavelength bands centered at 485 and 530 nm, respectively. The fluorescence counts were plotted against the drug concentrations and the 50% inhibitory concentration (IC₅₀) was determined by analysis of dose-response curves [7].

Dilution of test plant extracts and control drugs

All the test plant extracts and artemisinin stock solutions were prepared in dimethyl sulfoxide (DMSO) while chloroquine stock solution was prepared in Milli-Q grade autoclaved water. All the required concentrations were attained by dilutions with culture medium and ensuring that a 0.4% DMSO was attained in all the final solutions with the exception of the chloroquine and artemisinin (0.4% DMSO was found to be non-toxic to the parasites). Flat-bottomed tissue culture plates (96 wells) were used in the dilutions and eventual screening tests.

RESULTS AND DISCUSSION

The results obtained are shown in Table 1, Figure 1 and 2, and Plate 1b, 1c and 1d in which the crude plant root extract (W6R) and the crude plant leaves extract (W25L) showed no activity (Plate 1b and 1d) with an IC₅₀ > 100 µg/ml while the crude plant stem bark extract (W26S) gave a promising activity with an IC₅₀ at 45 µg/ml, which was further validated by the complete parasite clearance observed (Plate 1c). These results give validity to the traditional use of the plant in the treatment of malaria-like conditions and also agrees with the findings of Ajaiyeoba *et al.* [9], Ibrahimia *et al.* [10] and Good [2] of its *in vivo* antimalarial activity against *Plasmodium berghei*. Also, this plant leaves and stem bark have been reported as remedy for the treatment of cancer tumors and as vermifuge for horses. Also, the root bark has been implicated as an additive for intestinal troubles, purgative, insecticide, diarrhea, curing tooth aches and venereal disease [5]. This study has further revealed that the phytochemicals contained in the stem bark of this plant has antiplasmodial properties and their further isolation holds good promise for possible new lead antimalarial pharmacophores against the most deadly human strain of the malarial parasite, *Plasmodium falciparum*.

Table 1: Antimalarial activity of crude 70% aqueous methanol extracts of *Annona senegalensis* root, leaf and stem bark

Plant name	Plant Part used	Starting weight/ Recovery weight (g)	Yield (%)	<i>P. falciparum</i> 3D7 IC ₅₀ (µg/ml)
<i>Annona senegalensis</i>	Root (W6R)	150/21	14.0	>100
	Leaf (W25L)	120/25	20.4	>100
	Stem bark (W26S)	150/19	12.8	45

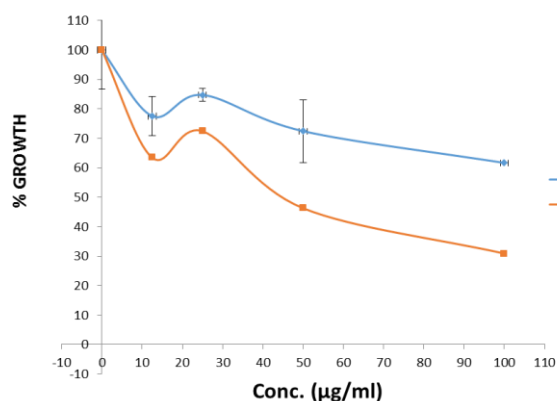


Fig.1: Dose-dependent growth inhibition activity of 70% aqueous methanolic leaf (W25L) and stem (W26S) crude extract against *Plasmodium falciparum* 3D7 strain

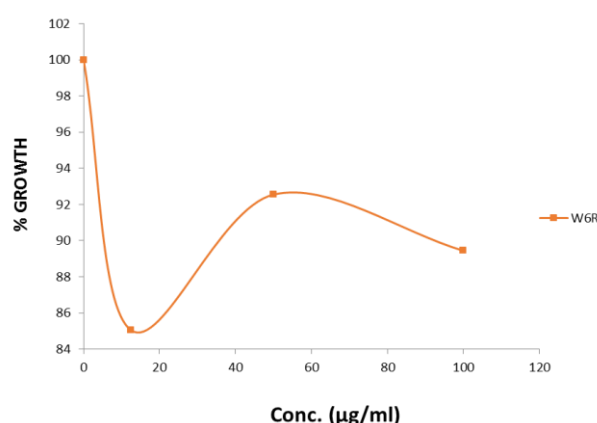


Fig.2: Dose-dependent growth inhibition activity of 70% aqueous methanolic root (W6R) crude extract against *Plasmodium falciparum* 3D7 strain

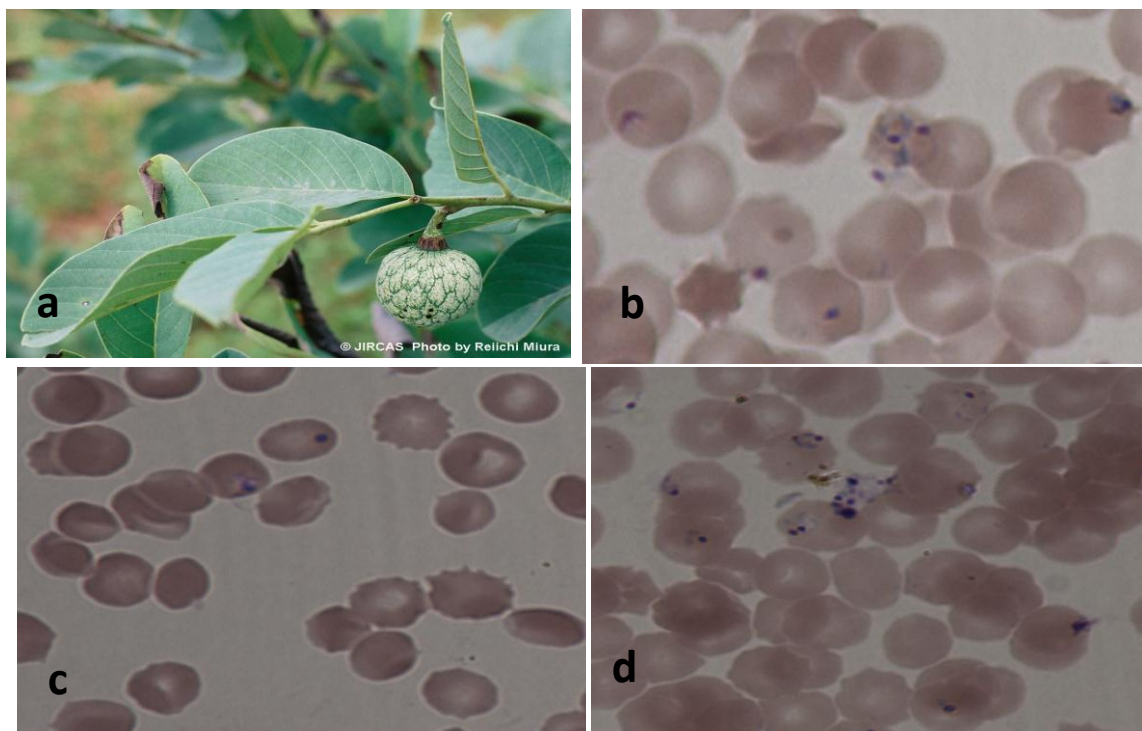


Plate 1: a) *Annona senegalensis* plant b) No growth inhibition by the crude root extract, c) Complete growth inhibition of parasite growth d) No growth inhibition by leaf crude extract.

CONCLUSION

The antimalarial potential of *Annona senegalensis* stem bark extract has been proven using both SYBRGreen 1 dye assay and microscopy to validate the claim. This finding has ascertained the folkloric claim of this plant's traditional usage in treating malaria and fever-like disease conditions in Nigeria and other parts of Africa. Further work is recommended on this plant part to isolate the active compound (s) in a bid to serve as possible new antimalarial drug candidates.

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