In Vitro Sensitivity of Plasmodium falciparum Field Isolates to Methanolic and Aqueous Extracts of Cassia alata (Fabaceae)

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Abstract

Objective: The aim of this study was to evaluate the in vitro activity of aqueous and methanolic extracts of Cassia alata leaves on the development of Plasmodium falciparum field isolates.

Method: The Trager and Jensen method with slight modifications was used. For the culture, RPMI 1640 and Albumax were used to replace human serum. The extracts as well as the reference drug (chloroquine) were diluted using RPMI medium. The P. falciparum field isolates were incubated with 8 concentrations ranging from 128 to 1 µg/ml in a 96-well microplate and incubated for 48 h in a candle jar. RPMI and 1% DMSO were used as negative controls.

Result: The extraction yields of C. alata were 7.96 and 13.23% for aqueous and methanolic extracts respectively. RPMI and DMSO didn’t have any harmful effect on the growth of P. falciparum. On the other hand, in the wells treated with extracts of C. alata leaves, inhibition of P. falciparum growth was registered with increasing concentrations of extracts. The inhibitory effect of the methanolic extract was stronger and we obtained the maximum mean inhibition rate of 100 ± 0.00% and 99.87 ± 0.62% at the concentrations 128 and 64 µg/ml respectively. As for the aqueous extract, it yielded a mean inhibitory rate of 99.2 ± 0.76% at the concentration of 128 µg/ml. Given the IC50 obtained that is 0.48 ± 0.02; 0.67 ± 0.11 and 0.77 ± 0.08 µg/ml for methanolic extract, aqueous extract and chloroquine respectively. The extracts of C. alata may be classified as active. This activity may be due to the presence of terpenes and tannins in the extracts.

Keywords: Antiplasmodial; Activity; Cassia alata; Plasmodium falciparum; Cameroon

Introduction

Malaria is a parasitic disease caused by a protozoan of the genus Plasmodium and transmitted by Anopheles mosquito vectors. In endemic regions, more than 300 million cases of malaria occur annually [1]. This disease is responsible for about 200 million of cases worldwide and each year it kills about 600 000 of people [2], of which 1 million are children of less than 5 years old. In Cameroon, malaria transmission is permanent and intense [3]. It remains a major public health problem in Cameroon as elsewhere in sub-saharan Africa [4]. These past 30 years, malaria parasites especially P. falciparum have rapidly developed resistance to commonly used antimalarial drugs [5]. New, more effective and affordable anti-malarial drugs are needed [6]. Medicinal plants play a key role in the control of malaria, especially where access to modern health services is limited. Tropical rainforest plants represent a fertile source of potential candidates for the development of new alternative anti-malarial drugs. In Cameroon, many plants are used by traditional healer to cure fever. In certain rural areas, anti-malarial traditional medicine is even preferred to pharmaceutical drugs, suggesting that herbal preparations are useful and active products [7]. More than 200 different species of plants from Cameroon possess antiplasmodial properties; but only 26 species have been investigated [4]. Cassia alata extract was shown to possess antifungal activity on some dermatophytes especially on Trichophyton verrucosum and Epidermophyton floccosum [8]. This plant has also demonstrated antibacterial activity on Vibrio cholera, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli [9]. It is in this light that the present study designed to assess, using the Trager and Jensen culture technique, the antiplasmodial efficacy of Cassia alata was tested in vitro, on P. falciparum field isolates. C. alata belongs to the Fabaceae plant family, the most exploited by tradipractitioners for the treatment of malaria [10].

Materials and Methods

Ethical clairance

To carry out this research, an ethical clearance was obtained from the National Ethics Committee of Cameroon, in order to ensure the consent and the confidentiality of the participants.

Plant material

Fresh leaves of Cassia alata (Fabaceae) were collected from Dschang-Cameroon in November 2011. The plant was identified in the National Herbal of Cameroon where a specimen was kept under number 18572/SRF-CAM. The leaves of the plant were air dried and reduced to powder before extractions were undertaken.

Two types of extracts (aqueous and methanolic) were prepared and tested on P. falciparum field isolates.
Preparation of extracts

The methanolic extract was obtained using the procedure described by Wabo Poné et al. [11]. Briefly, 100 g of stored powder were macerated in 1.5 l methanol 90% which removes the active ingredients of plants. The mixture was daily stirred and 72 hours later, the solution was sieved and filtered using filter paper of pore size 2.5 µm. The extract was evaporated using a rotavapor Buchi-R-124 model heated at 65°C for 8 h.

A similar procedure was carried out for the aqueous extract, except that hot (distilled) water was used as solvent. The infusion took 3 h and evaporated for 7 days in a ventilated oven at a temperature of 50°C.

Dilution of extracts

200 µg of methanolic extract was diluted in 100 µl of Dimethylsulfoxide (DMSO). A quantity of RPMI was added to obtain a total volume of 1000 µl and thus a stock solution of 200 µg/ml. A series of dilutions were made with RPMI medium to obtain concentrations of 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml [10].

Reference drug and chemicals

The reference drug, pharmaceutical chloroquine, used in this study was bought from a local pharmacy. RPMI 1640 and Albumax were obtained from SIGMA and GIBCO respectively. Chloroquine was chosen due to its availability, and also because some authors have used it for in vitro trials. This drug was diluted with RPMI in order to obtain the same concentrations with the organic and aqueous extracts. Negative controls used for the bioassay were 1% DMSO and culture medium (RPMI 1640 +Albumax).

Antiplasmodial assay

About 4ml of blood were collected by vein puncture from patients suffering from malaria at the District Hospital of Dschang using a manual syringe of 10ml. This blood was transferred in sterile tubes containing 5% glucose, 5% Albumax and filter through a STERIVEX GS Millipore of 0.22 µm. The extract was sieved and filtered using filter paper of pore size 2.5 µm. The mixture was daily stirred and 72 hours later, the solution was sieved and filtered using filter paper of pore size 2.5 µm. The extract was sieved and filtered using filter paper of pore size 2.5 µm. The extract was sieved and filtered using filter paper of pore size 2.5 µm.

Evaluation of the antimalarial activity

To evaluate the effects of the various extracts on P. falciparum field isolates, 21 µl of infected red blood cells with a parasitemia of about 1% were distributed in 81 wells of the 96-well microplate and mixed with a volume of 189 µl of a specified tested products at various concentrations diluted with a culture medium (RPMI 1640, supplemented with 25 Mm HEPES, 0.2% Sodium bicarbonate and glucose, 5% Albumax and filter through a STERIVEX GS Millipore of 0.22 µm) [12]. The microplate was covered and placed in a candle jar. This container was totally closed when the candle was about to go off. The culture medium in each well was replaced by RPMI 1640 supplemented with sodium bicarbonate until all white blood cells were removed. The prepared blood was diluted with a washed blood, of group O+

clean glass microscope slide for the preparation of thin blood films. The percentage of inhibition (PI in %) was determined using the following:

\[
PI(\%) = \frac{\text{Parasitemia in control wells} - \text{Parasitemia in treated wells}}{\text{Parasitemia in control wells}} \times 100
\]

All tests were repeated three times for each treatment and control in the same conditions.

Statistical analysis

Comparisons of different inhibition rates on P. falciparum growth were made using the Chi-square test. Results were regarded as significant at P<0.05. The 50% and 90% inhibitory concentrations (IC\(_{50}\) and IC\(_{90}\)) were determined from linear regression curve obtained between the inhibition rate expressed in probit and the decimal logarithm of the concentrations (µg/ml).

Results

The yields obtained after extraction with methanol and hot water solvents from 100 g of C. alata leaves powder were 13.23 % and 7.96 % respectively. The variation of the mean inhibition rate of the growth of P. falciparum field isolates according to the different concentrations of C. alata and chloroquine is shown in Figure 1.
IC_{50} for the growth of *P. falciparum*. From this figure, we observed that, no matter the tested products the IC_{50}s are less than the IC_{90}s.

![Figure 2](image-url) 
**Figure 2:** Inhibition (in probit) of the *Plasmodium falciparum* field isolates according to the decimal logarithm of the concentrations of *Cassia alata* extracts and chloroquine after 48 h of incubation.

![Figure 3](image-url) 
**Figure 3:** Inhibitory concentration (IC_{50} and IC_{90}) of extracts of *Cassia alata* and chloroquine after 48 h of incubation.

**Discussion**

The extracts obtained from the *C. alata* leaves presented different yields. The higher yield (13.23%) was obtained with methanolic extract. This finding is similar to that reported by Muganga et al. [15] with Fuerstia Africana. In fact, these authors obtained a yield of 13.3% for methanolic extract and 5.3% for aqueous extract of this plant. These differences observed in the various studies may be due in one hand, to the nature of the solvent and in the other hand to the method used [16].

From the normal growth observed in the negative control wells, the variation of parasitemia shown in the treated wells was due to the effect of tested products. The methanolic and aqueous extracts of *C. alata* were active against *P. falciparum* with IC_{50} values of 0.48 µg/ml and 0.67 µg/ml respectively. Even though water is used as the solvent in the traditional preparation, the methanolic extract demonstrated a slightly higher antimalarial activity than the aqueous extract at all concentrations. Similar observations were reported by Sotheera et al. [17] and Douki et al. [18] comparing the methanolic extracts of *Brucea javanica* and *Staudtia gabonensis* to aqueous extracts. This suggests that, more active compounds were extracted with this solvent. The antimalarial activities of the extracts against field isolates parasites were dose-dependent. The reported antimalarial activity of *C. alata* may be attributed to terpènes and tannins compounds present in the extracts [19-24]. In general, the IC_{50}s obtained in this work are closer to those reported by other researchers. The IC_{50}=0.48 µg/ml obtained with methanolic extracts of *C. alata* was similar to the ones reported (0.48 µg/ml) by Kayembe et al. [24] when testing the antiplasmodial effects of the same plant. However, IC_{50}=0.25 µg/ml obtained by Kayembe et al. [21] with *Cassia occidentalis* extracts is significantly less than the one obtained in the present study. On the contrary, higher IC_{50}s were reported by Sotheera et al. [17] with the methanolic extracts of *Brucea javanica, Euycorma longifolia, Phyllanthus urinarina, Stephania rotundula, Azadirachta indica, Flueggea virosa, Vernonnia cinerea, Fagraea fragrans, Bixa orellana, Spondias pinnata, Anneslea fragrans, Cananga latifolia* and *Andrographis paniculata* with IC_{50} of (1.7 ; 1.8 ; 2.5 ; 9 ; 16;31,5 ; 32,1 ; 33,4 ; 33,5 ; 41,2 ; 41,5 ; 48,7 ; 50 µg/ml respectively). By comparing either the IC_{50} of the methanolic extract or aqueous extract to that of chloroquine, we noticed that the IC_{50} of plant extracts were less than that of the reference drug (0.77 µg/ml). This IC_{50} was similar to the one obtained (IC_{50}=0.8 µg/ml) by El Tahir et al. [25] when testing the effect of chloroquine on a *P. falciparum* chloroquine-resistant strain. However, they determined an IC_{50} of 0.23 µg/ml during evaluation of the activity of the same product on a *P. falciparum* chloroquine-sensitive strain. We can therefore consider that *P. falciparum* field isolates used in this study was a chloroquine-resistant strain. Taking into consideration the threshold of_in-vitro* antimalarial activity proposed by Rosoanaivo et al. [26], the effect of the plant extract tested in this study can be considered active. The IC_{50} obtained with both extracts falls in the range 0.1 to 1 µg/ml.

**Conclusion**

RPMI and 1% DMSO allowed the normal growth of *P. falciparum* field isolates. The two tested extracts inhibited the growth of this *P. falciparum* strain with the means IC_{50} of 0.48 ± 0.02 and 0.67 ± 0.11 µg/ml for methanolic and aqueous extracts respectively. Methanolic extract was the most potent on the development of *P. falciparum* field isolates with a maximum mean inhibition rate of 100% at 128 µg/ml concentration. Even though the IC_{50} of chloroquine was 0.77 ± 0.08 µg/ml, its effect remains less than the ones obtained with the tested extracts. This could be due to the resistance developed by the parasite.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgement**

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