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 clearance

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 with EDTA and put in an icebox. This was quickly transported to the laboratory of Biomedical Sciences for analysis. In the laboratory the specimens were distributed in eppendorf tubes and centrifuged at 800g for 10 min [13]. These preparations were then washed 3 times by centrifugation using 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 +.

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 to 2% 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 candle jar was then put in an incubator for 48 h at 37°C [14]. At 24 h of incubation, the culture medium in each well was replaced by fresh ones containing the same concentration of products. After 48h, the supernatants were removed using Pasteur pipettes. A small drop (10-15 µl) of erythrocytes from the bottom of each well was put on a clean glass microscope slide for the preparation of thin blood films. The percentage of inhibition (PI in %) was determined using the following:

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

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\textsubscript{50} and IC\textsubscript{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.

\[ \text{Figure 1: In vitro variation of the mean inhibition rate of Plasmodium falciparum field isolates according to the concentrations of Cassia alata extracts and chloroquine after 48 h of incubation.} \]

This figure shows that RPMI 1640 and 1% DMSO did not affect (0% inhibition rate) the development of P. falciparum. In the treated wells, the inhibition rate increases with increased concentration of the tested products. For concentrations greater than 4 µg/ml, the effect of chloroquine was less than the effect of extracts with a significant difference (p<0.05), but below these concentrations the antimalarial activity was similar. At concentrations of 4 to 32 µg/ml the effect of the methanolic extract was higher than that of aqueous extract (58.7 to 98.13% and 48.9 to 58.1% respectively; p<0.05). After the transformation of the inhibition rate to probits (Figure 2), a linear relationship was obtained with the logarithm of the concentrations.

From the equations of the regression lines the following results were obtained: 50 % inhibition concentration (IC\textsubscript{50}) of 0.48 ± 0.02, 0.67 ± 0.11 and 0.77 ± 0.08 µg/ml for methanolic extract, aqueous extract and chloroquine respectively. Also, the IC\textsubscript{90} determined from the same regression lines was 0.7± 0.02, 1.53 ± 0.11; and 2.22 ± 0.08 µg/ml respectively for methanolic extract, aqueous extract and chloroquine.

The inhibition gradient of the development of P. falciparum was: Eme > Eai > Cqi. Figure 3 shows the inhibitory concentrations (IC\textsubscript{50} and IC\textsubscript{90} for methanolic and aqueous extracts of Cassia alata (Fabaceae). Altern Integ Med 3: 159. doi:10.4172/2327-5162.1000159
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.

**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 antimalarial activity of the extracts against field isolates parasites was dose-dependent. The reported antimalarial activity of *C. alata* may be attributed to terpenes and tannins compounds present in the extracts [19-24].

**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.

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