Alternative & Integrative Medicine

Josué et al., Altern Integ Med 2014, 3:2
http://dx.doi.org/10.4172/2327-5162.1000159

Research Article Open Access

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

Wabo Poné Josué*, Noumedem Anangmo Christelle Nadia, Komtangi Marie Claire, Yondo Jeannette and Mpoame Mbida

Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon

***Corresponding author:** Wabo Poné josué, Department of Animal Biology, Faculty of Science, University of Dschang, PO Box 067 Dschang, Cameroon, Tel: + 237 75390318/99521863; E-mail: waboponejosue@yahoo.fr

Rec date: Mar 12, 2014; **Acc date:** Mar 31, 2014; **Pub date:** Apr 02, 2014

Copyright: © 2014 Josué WP, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

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 IC_{50} 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 jloccosum* [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.

Citation: Josué WP, Nadia NAC, Claire KM, Jeannette Y, Mbida M (2014) *In Vitro* Sensitivity of *Plasmodium falciparum* Field Isolates to Methanolic and Aqueous Extracts of *Cassia alata* (Fabaceae). Altern Integ Med 3: 159. doi:10.4172/2327-5162.1000159

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 on icebox. This was quickly transported to the laboratory of Biomedical Sciences for analysis. In the laboratory the specimens were distributed in eppendorf tubes and centrifuge 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 antiplasmodial 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:

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

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.

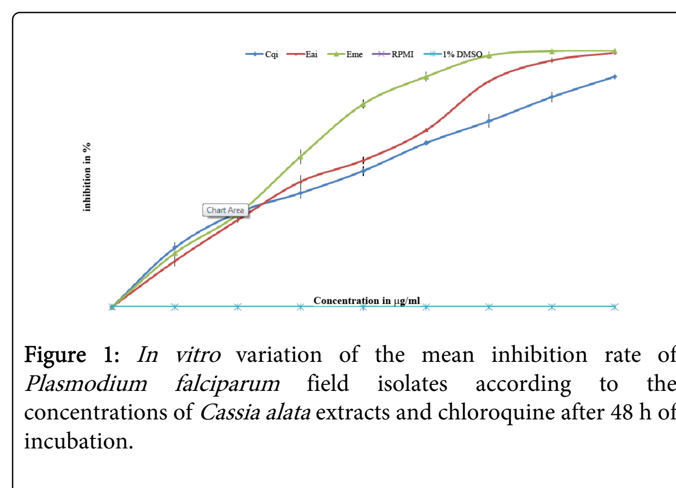

Figure 1: *In vitro* variation of the mean inhibition rate of *Plasmodium falciparum* field isolates according to the concentrations of *Cassia alata* extracts and chloroquine after 48 h of incubation.

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 antiplasmodial 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_{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_{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_{50} and

IC_{90}) 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.

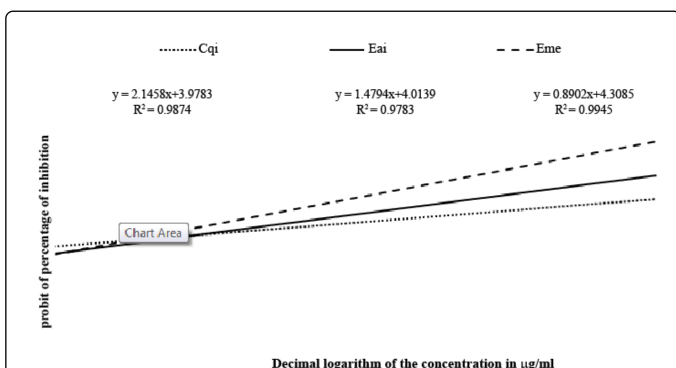

Figure 2: Inhibition (in probit) of the *Plasmodium falciparum* field isolates according to the decimal logarithm of the concentrations of *Cassia alata* extracts and chloroquine after 48 h of incubation.

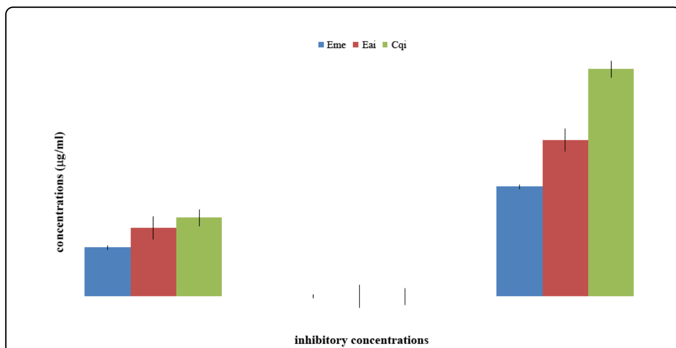

Figure 3: Inhibitory concentration (IC_{50} and $_{IC90)}$ of extracts of *Cassia alata* and chloroquine after 48 h of incubation

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 antiplasmodial activity than the aqueous extract at all concentrations. Similar observations were reported by Sotheara 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 antiplasmodial activities of the extracts against field isolates parasites were dose-dependent. The reported antiplasmodial 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 Sotheara et al. [17] with the methanolic extracts of *Brucea javanica*, *Eurycoma longifolia*, *Phyllanthus urinaria*, *Stephania rotunda*, *Azadirachta indica*, *Flueggea virosa*, *Vernonia 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* chloroquinoresistant 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* chloroquinosensitive strain. We can therefore consider that *P. falciparum* field isolates used in this study was a chloroquinoresistant strain. Taking into consideration the threshold of *in-vitro* antiplasmodial 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/m 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

The authors wish to express sincere gratitude to the Laboratory of Applied Biology and Ecology (LABEA) and the Laboratory of Biomedical Sciences of the University of Dschang. We also thank the traditional healers of Dschang-Cameroon and M. FOPA François for the information on the plant specimen and for helping in the collection of *P. falciparum* field isolates used in this study respectively.

References

1. Köhler I, Jenett-Siems K, Siems K, Hernández MA, Ibarra RA, et al. (2002) In vitro antiplasmodial investigation of medicinal plants from El Salvador. Z Naturforsch C 57: 277-281.
2. WHO (2012) The world malaria report 2012. Geneva World Health Organization.
3. Saotoing P, Vroumsia T, Tchobsala, Tchuenguem FFN (2005) Culicidae diversity in the Northern part Cameroon and the effect of essential oils

from local plants on adults of Anopheles gambiae, PhD, 3rd Round, University of Yaounde I, Cameroon, 118.

4. Titanji VPK, Zofou D, Ngemenya MN (2008) The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. Afr J Tradit Complement Altern Med 5: 302-321.
5. Idowu OA, Soniran OT, Ajana O, Aworinde DO (2010) Ethnobotanical survey of antimalarial plants used in Ogun State, Southwest Nigeria. African Journal of Pharmacology 4: 55-60.
6. Koudouvo K, Karou SD, Ilboudo DP, Kokou K, Essien K, et al. (2011) In vitro antiplasmodial activity of crude extracts from Togolese medicinal plants. Asian Pac J Trop Med 4: 129-132.
7. Zofou D, Tene M, Ngemenya MN, Tane P, Titanji VP (2011) In vitro antiplasmodial activity and cytotoxicity of extracts of selected medicinal plants used by traditional healers of Western cameroon. Malar Res Treat 2011: 561342.
8. Sule WF, Okonko IO, Joseph TA, Ojezele MO, Nwanze JC, et al. (2010) In vitro Antifungal Activity of Senna alata Linn. Crude Leaf Extract. Research Journal of Biological Sciences 5: 275-284.
9. Saheli C, Sabyasachi C, Sikha D (2012) An Overview on the Ethnophytopathological Studies of Cassia alata-an Important Medicinal Plant and the Effect of VAM on its Growth and Productivity. International Journal of Research in Botany 2: 13-19.
10. Saotoing P, Vroumsia, Tchobsala, Tchuenguem F, Nioga N (2011) Medicinal plant use in traditional treatment of malaria in Cameroon. Journal of Ecology and the Natural Environment 3: 104-117.
11. Wabo Poné J, Bilong Bilong CF, Mpoame M (2010) In vitro nematicidal activity of extracts of Canthium mannii (Rubiaceae), on different life-cycle stages of Heligmosomoides polygyrus (Nematoda, Heligmosomatidae). J Helminthol 84: 156-165.
12. Kemgne EA, Mbacham WF, Boyom FF, Zollo PH, Tsamo E, et al. (2012) In vitro sensitivity of Plasmodium falciparum field isolates to extracts from Cameroonian Annonaceae plants. Parasitol Res 110: 109-117.
13. Trager W, Jensen JB (1976) Human malaria parasite in continuous culture. Science 193: 673-675.
14. Jensen JB, Trager W (1977) Plasmodium falciparum in culture: use of outdated erthrocytes and description of the candle jar method. J Parasitol 63: 883-886.
15. Muganga R, Angenot L, Tits M, Frédérich M (2010) Antiplasmodial and cytotoxic activities of Rwandan medicinal plants used in the treatment of malaria. J Ethnopharmacol 128: 52-57.
16. Wabo poné J (2007) Effets des extraits de Canthium mannii, Hiern, 1877 (Rubiacées) sur deux nématodes parasites strongylides : Ancylostoma caninum Ercolani, 1859 et Heligmosomoides polygyrus Dujardin, 1845. Thèse de Doctorat / PhD, Département de Biologie et Physiologie Animales, Faculté des Sciences, Université de Yaoundé I, Cameroun.
17. Hout S, Chea A, Bun SS, Elias R, Gasquet M, et al. (2006) Screening of selected indigenous plants of Cambodia for antiplasmodial activity. J Ethnopharmacol 107: 12-18.
18. Lekana-Douki JB, Bongui JB, Oyegue Liabagui SL, Zang Edou SE, Zatra R, et al. (2011) In vitro antiplasmodial activity and cytotoxicity of nine plants traditionally used in Gabon. J Ethnopharmacol 133: 1103-1108.
19. Edeoga HO, Okwu DE, Mbaebie BO (2005) Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 4: 685-688.
20. Okwu DE (2005) Phytochemicals, vitamins and mineral contents of two Nigeria medicinal plants. International Journal of Molecular Medicine and Advance Sciences 1: 375-381.
21. Bahorun T, Neergheen VS, Aruoma OI (2005) Phytochemical constituents of Cassia fistula. African Journal of Biotechnology 4: 1530-1540.
22. Georges K, Jayaprakasam B, Dalavoy SS, Nair MG (2008) Pest-managing activities of plant extracts and anthraquinones from Cassia nigricans from Burkina Faso. Bioresour Technol 99: 2037-2045.
23. Kayembe JS, Taba KM, Ntumba K, Tshiongo MTC, Kazadi TK (2010) In vitro anti-malarial activity of 20 quinones isolated from four plants used by traditional healers in the Democratic Republic of Congo. Journal of Medicinal Plants Research 4: 991-994.
24. Kayembe JS, Taba KM, Ntumba K, Kazadi TK (2012) In vitro Antimalarial Activity of 11 Terpenes Isolated from Ocimum gratissimum and Cassia alata Leaves, Screening of Their Binding affinity with haemin. Journal of Plant Studies.
25. El Tahir A, Satti GM, Khalid SA (1999) Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on Maytenus senegalensis (Lam.) Exell. J Ethnopharmacol 64: 227-233.
26. Rosoanaivo P, Dehaho E, Ratsimamanga-Uverg S, Frapier F (2004) Guidelines for the nonclinical evaluation of the efficacy of traditional antimalarias. Traditional Medicine Plants a Malaria.CRCPress, LLCBocaRaton London 255-270.