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IN VITRO TEST OF FIVE ETHIOPIAN MEDICINAL PLANTS FOR ANTIMALARIAL ACTIVITY AGAINST *PLASMODIUM FALCIPARUM*

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ABSTRACT: The *in vitro* antimalarial activity of crude extracts from aerial parts of *Ajuga remota* Benth., *Artemisia afra* Jacq. and *Artemisia rehan* Chiov., seeds of *Lepidium sativum* (L.) and roots of *Securidaca longipedunculata* Fresen. were studied by utilizing the inhibition of uptake of [^{3}H]hypoxanthine into *P. falciparum* line FCA-2/Ethiopia. Of the five extracts tested, extracts from seeds of *L. sativum* and *S. longipedunculata* showed no antimalarial activity at the highest concentration [50 $\mu g(ml)^{-1}$] used in the present study. However, ethanol extracts from aerial parts of *A. afra*, *A. rehan* and *A. remota* possessed antimalarial activity with IC_{50} values of the order of 7-23 $\mu g(ml)^{-1}$. *A. afra* showed the highest activity [IC_{50}=7 $\mu g(ml)^{-1}$] as compared to that of *A. rehan* [IC_{50}=14 $\mu g(ml)^{-1}$] and *A. remota* [IC_{50}=23 $\mu g(ml)^{-1}$]. Results of this study showed that the extracts from three of the plants did have antimalarial activity, which suggests further investigations of cytotoxicity, chemical isolation and *in vivo* studies of those plants with potential antimalarials.

Key words/phrases: Crude extracts, *in vitro*, *Plasmodium falciparum*

INTRODUCTION

Effective control of malaria has proved difficult in tropical rural Africa, where the disease is responsible for a high proportion of deaths. In view of the increasing prevalence of strains of *Plasmodium falciparum* that display resistance to most of the currently available antimalarial drugs, there is an

urgent need for new and more effective agents either synthetic or from plants (Peters, 1985).

Many plants used in traditional medicine have in fact been an important source of drugs for the treatment of malaria. For example, quinine from the bark of cinchona, is a plant product which has been in use for centuries in the treatment of malaria (Peters, 1980). At present artemisinin and its derivatives represent the most potent and safe new antimalarial agent. These were isolated in China from the annual herb *Artemisia annua* and are particularly effective against multi-drug resistant strains of *P. falciparum* (Hien and White, 1993). The discovery of artemisinin as a very potent and safe drug has stimulated interest for the search of other antimalarially active plants.

There have been several anti-malaria screening studies of medicinal plant extracts, and significant levels of antimalarial activity were reported for some. In 1947, crude extracts of about 600 different plants were screened for their *in vivo* activities against *Plasmodium gallinaceum* in chicks and against *P. cathemerium* and *P. lophurae* in ducklings (Spencer *et al.*, 1947). In these studies, species from some 300 plant genera gave positive results and the most significant levels of activity were from species of Simaroubaceae and the Amaryllidaceae. Crude extracts of *Azadirachta indica*, and nimbolids, the major constituent of *A. indica* were found to inhibit chloroquine resistant strains of *P. falciparum in vitro* (Rochanakij *et al.*, 1985). Certain quassinoids from plants of the family Simaroubaceae have been found to show high activity against drug resistant strains of *P. falciparum in vitro* (Trager and Polonsky, 1981). Out of 49 Tanzanian medicinal plants, extracts from *Cyperus rotundus, Hosiudia opposita* and *Lantana camara* were found to have significant antimalarial activity (Weenen *et al.*, 1990). Further more, in a comprehensive review on the progress made in the search for antimalarial agents from plants, many medicinal plants from several countries have been reported to have significant antimalarial activity against *P. falciparum* and other *Plasmodium* species (Nkunya, 1992). In *in vitro* antimalarial studies of Ethiopian medicinal plant extracts, *Hagenia abyssinica* (Moges Kassa and Ward, 1993), *Croton macrostachys*, *Calpurnia aurea*, *Dodonia angustifolia* (Solomon Sorssa, 1992), *Berssama abyssinica* (Moges Kassa *et al.*, 1996), *Withania somnifera* and *Vernonia amygdalina* (Mesfin Bogale and Beyene Petros, 1996) were found to

have significant antimalarial activity against *P. falciparum*. We report here, the *in vitro* antimalarial activity of extracts of other reputed Ethiopian medicinal plants against *P. falciparum*.

MATERIALS AND METHODS

Preparation of plant materials

Aerial part of *Artemisia afra* (Compositae), *Artemisia rehan* and seeds of *Lepidium sativum* (Cruciferae) were purchased from the local market. Roots of *Securidaca longipedunculata* (Polygalaceae) and aerial part of *Ajuga remota* (Labiatae) were collected from Robe and Bedelle, respectively. These have been identified by a taxonomist at the Department of Drug Research, Ethiopian Health and Nutrition Research Institute (EHNRI). A sample of the dried and finely ground plant materials, weighing 300 g was left to macerate in 96% ethanol for 72 hrs and filtered. The ethanol was then removed by evaporation. In only one case, that of *L. sativum*, methanol was used in place of ethanol. In this case, stock solutions of the extracts were prepared by dissolving in ethanol and dimethyl sulfoxide (DMSO). These were then sterilized by gamma irradiation for one hour.

Preparation of parasites:

P. falciparum, line FCA-2/Ethiopia (Moges Kassa and Mshana, 1997) was used. This culture line was initiated at the Armauer Hansen Research Institute (AHRI) on 7 April, 1994 directly from parasitized human blood collected from Zewai, Ethiopia. For the purpose of the present study we used the material that had been frozen in liquid nitrogen after two months in culture. This was grown continuously for two months before being used according to published techniques (Trager and Jensen, 1976). Cultures were maintained *in vitro* in human type O+ve blood cells diluted to 5% hematocrit with complete RPMI-1640 which consisted of 12% human O+ve serum. By the time of drug testing, growth of the culture was synchronized using 5% sorbitol.

Test procedure

The test procedure was following Desjardins *et al.* (1979) and O'Neill *et al.* (1985). Stock solutions of crude extracts and chloroquine diphosphate were

serially diluted aseptically with complete RPMI-1640 on the day the experiment was set up. The ethanol and DMSO concentrations never exceeded 0.1% and controls demonstrated that there was no effect on [^{3}H]hypoxanthine incorporation in this test system. Aliquot of 100 μl diluted drugs were dispensed into 96-microtiter plates so as to yield final concentrations of 50, 5, 0.5, 0.05 and 0.005 μg(ml)$^{-1}$. More accurate determination of 50% inhibition concentration (IC_{50}) values were achieved on the basis of 2 fold dilution at concentrations within the range of values obtained by 10 fold dilutions. All tests were performed in duplicate. To each well was added 10 μl of human RBC (O+ diluted to 5% hematocrit) with 0.5-1% parasitemia. Two series of controls were performed, one with parasitized cells without drug and another with uninfected blood without drug. The plates were incubated in a CO_2-incubator at 37° C for 24 h. After the 24 h incubation period the plates were removed and 5 μl of [^{3}H]ypoxanthine (40 μCi, Amersham) was added to each well and incubation was continued for a further 18–24 hrs.

Harvesting and scintillation count

After the last 18–24 h incubation period, the cells were harvested with multicell harvester (Skatron AS, Norway) through a filtermat predamped with distilled water. After being washed with distilled water for 20 sec., the filtermat was dried and individual areas representing the wells were removed and placed into scintillation vials. Toluene scintillator (4 ml) was added into each vial and counting was carried out in 1216 Rackbeta II scintillation counter (LKB LTD, Croydon, UK).

Analysis of results

Counts per minute were converted to disintegrations per minute (DPM) by using an external standard and mean DPM was calculated for each concentration and control wells. The percentage inhibition was calculated from the following equation (O'Neill *et al.*, 1985):

$$\%\ \text{Inhibition} = 100 - \frac{A-C}{B-C}\ X\ 100$$

where, A = DPM infected blood with plant extract
B = DPM infected blood with no plant extract
C = DPM uninfected blood

Concentration-versus-percent inhibition curves and IC_{50} values, with their correlation coefficient were determined by a linear regression analysis using SPSS PC+ effect analysis computer program.

RESULTS

Crude extracts of five Ethiopian medicinal plants were tested *in vitro* for their antimalarial activities by utilizing the inhibition of $[^3H]$hypoxanthine into *P. falciparum* line FCA-2/Ethiopia. Antimalarial activities of the extracts and IC_{50} values calculated from these data on the basis of two fold dilutions are shown in Fig. 1 and Table 1, respectively. Of the five plant extracts, seeds of *L. sativum* and roots of *S. longipedunculata* showed no growth inhibition across the concentration range employed in this study, indicating lack of activity against *P. falciparum* FCA-2/Ethiopia *in vitro*. However, extracts from aerial part of *A. afra, A. rehan* and *A. remota* were found to possess antimalarial activity with IC_{50} values of 7, 14 and 23 $\mu g(ml)^{-1}$, respectively. Chloroquine which was used as a standard antimalarial drug exhibited an IC_{50} value of 0.23 $\mu g(ml)^{-1}$ against *P. falciparum* FCA-2/Ethiopia tested under identical condition with the plant extracts.

Table 1. ***In vitro* antimalarial activity of crude extracts of some Ethiopian medicinal plants against *P. falciparum* line FCA-2/Ethiopia.**

Plant/drug	Part used	Approximate IC_{50} ($\mu g/ml$)[a]	Linear Correlation Coefficient
Artemisia afra	Aerial	7	0.99
Artemisia rehan	Aerial	14	0.98
Ajuga remota	Aerial	23	0.99
Chloroquine diphosphate		0.23	0.92

[a] Inhibition of uptake of $[^3H]$hypoxanthine; IC_{50} values based on two fold dilutions.

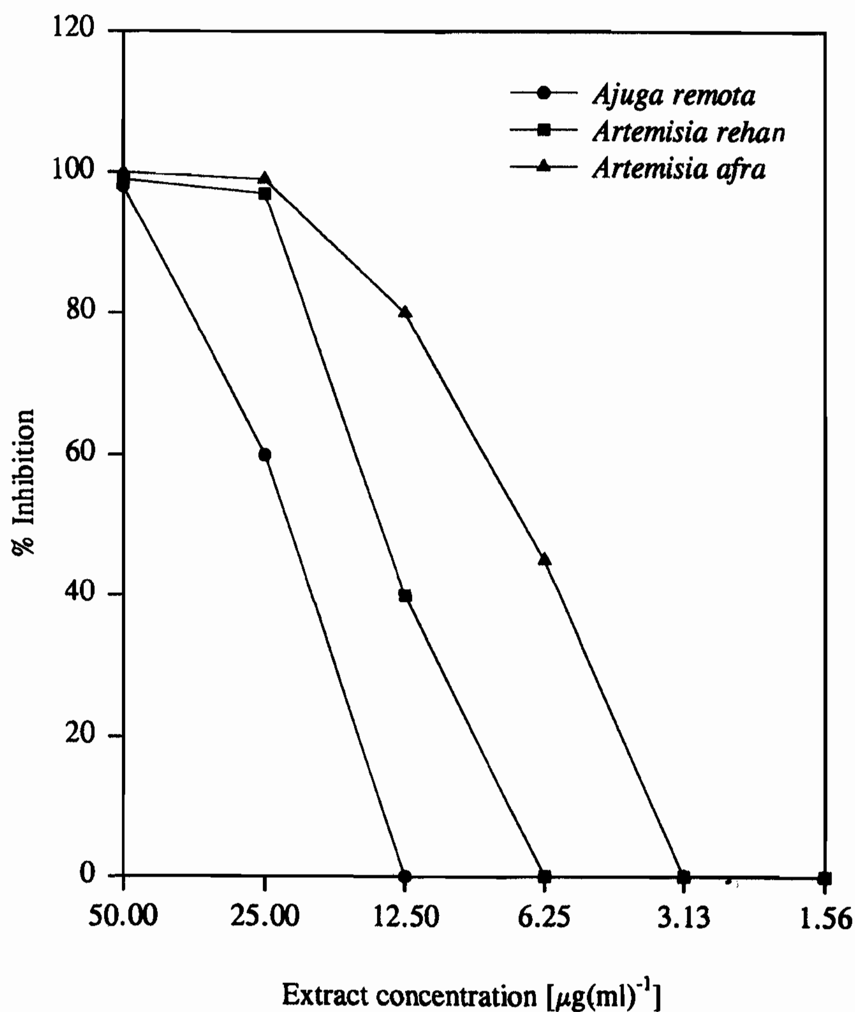

Fig. 1. Effect of crude extracts of some Ethiopian medicinal plants *in vitro* on *P. falciparum* FAC-2/Ethiopia.

DISCUSSION

In vitro antimalarial plant extract studies in different laboratories have made use of different long term culture lines of *P. falciparum* originating mainly from Southeast Asia (Desjardins *et al.*, 1979; O'Neill *et al.*, 1985; Rochanakij *et al.*, 1985; Weenen *et al.*, 1990). However, in the present study assessment of the *in vitro* antimalarial activity of extracts of Ethiopian medicinal plants was carried out using an Ethiopian isolate of *P. falciparum* established in our laboratory. Conclusions drawn from the analysis of such isolates may strongly support or disprove the traditional claim about the medicinal use of the plants as anti-malaria in this area.

Results of the present study on the *in vitro* antimalarial activity indicate that, of the five plant extracts tested, methanol extract of seeds of *L. sativum* and ethanol extract of roots of *S. longipedunculata* proved to be inactive against *P. falciparum* line FCA-2/Ethiopia at 50 $\mu g(ml)^{-1}$, the highest concentration tested. These findings agree with earlier workers (Mesfin Bogale and Beyene Petros, 1996) who observed that petroleum ether, chloroform and methanol extracts of *S. longipedunculata* had no *in vitro* antimalarial activity against chloroquine resistant Cameroonian isolate of *P. falciparum* (FCM-29) up to a concentration of 50 $\mu g(ml)^{-1}$

As shown in Table 1 and Fig. 1, ethanol extracts of *A. afra* and *A. rehan*, which are in the same genus with that of the Chinese *A. annua*, and extracts of *A. remota* were found to have *in vitro* antimalarial activity with IC_{50} values of the order of 7–23 $\mu g(ml)^{-1}$. Plant extracts exhibiting IC_{50} values less than 10 $\mu g(ml)^{-1}$ were considered as having significant antimalarial activity (Weenen *et al.*, 1990). Interestingly, *A. afra* showed the highest activity as compared to *A. rehan* and *A. remota*. Its IC_{50} value was 7 $\mu g(ml)^{-1}$, which is close to that of *A. annua* [4 $\mu g(ml)^{-1}$] (O'Neill *et al.*, 1985) and *Azadirachta indica* [5 $\mu g(ml)^{-1}$] (Rochanakij *et al.*, 1985), which are plants of potential antimalarials reported in the literature. It is interesting to note that these previous reports were based on activities against Thai multi-drug resistant strain of *P. falciparum* whereas we obtained similar results with an Ethiopian isolate.

O' Neill *et al.* (1985) suggested that cytotoxicity of natural products could give false positive results in screening procedures utilizing this *in vitro* system. Therefore, the initial information on the *in vitro* antimalarial activity of the plant extracts against *P. falciparum* needs further investigations which might include cytotoxicity tests and isolation of the active constituents, along with *in vivo* studies in rodent or simian malaria parasites.

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REFERENCES

1. Desjardins, R.W., Canfield, C.J., Hynes, J.D. and Chulay, J.D. (1979). Quantitative assessment of antimalarial activity *in vitro* by a semi-automated microdilution technique. *Antimicrob. Agents Chemother.* **16**:710–718.
2. Hien, T.T. and White, N.J. (1993). Qinghaosu. *Lancet* **341**:603–608.
3. Mesfin Bogale and Beyene Petros (1996). Evaluation of the antimalarial activity of some Ethiopian traditional medicinal plants against *Plasmodium falciparum in vitro*. *SINET: Eth. J. Sci.* 19:233–243.
4. Moges Kassa and Ward, S. (1993). Antimalarial activity of local medicinal plants against *Plasmodium falciparum in vitro. Eth. Pharm. J.* **11**:39–45.
5. Moges Kassa and Mshana, R. (1997). Establishment of two lines of Ethiopian isolates of *Plasmodium falciparum in vitro. Ethiop. J. Health Dev.* **11(3)**:235--239.
6. Moges Kassa, Mshana, R. and Aschalew Hunde (1996). Antimalarial Activity of *Bersama abyssinica* against *Plasmodium falciparum in vitro. Eth. Pharm. J.* **14**:16–21.

7. Nkunya, M.H.H. (1992). Progress in the search for antimalarials. *NAPRECA Monograph*, Series No. 4. Natural Products Research Network for Eastern and Central Africa, Addis Ababa University, Addis Ababa, Ethiopia.

8. O'Neill, M.J., Bray, D.H., Boardman, P., Phillipson, J.D. and Warhurst, D.C. (1985). Plants as source of antimalarial drugs, Part 1. *In vitro* test method for the evaluation of crude extracts from plants. *Planta. Med.* **61**:394-398.

9. Peters, W. (1980). Chemotherapy of malaria. **In**: *Malaria*, pp. 143-283, (Kreier, J.P., ed.), Academic Press, New York.

10. Peters, W. (1985). The problem of drug resistance in malaria. *Parasitol.* **90**:705--515.

11. Rochanakij, S. Thebaranonth, Y., Yenjai, C. and Yuthavong, Y. (1985). Nimbolide a constituent of *Azadirachta indica*, inhibits *Plasmodium falciparum* in culture. *Southeast Asia J. Trop. Med. Pub. Hlth.* **16**:66-72.

12. Solomon Sorssa (1992). *In vitro* evaluation of the activity of some Ethiopian traditional medicinal plant crude extracts against *Plasmodium falciparum.* MSc Thesis, School of Graduate Studies, Addis Ababa University.

13. Spencer, C.F., Konivszy, F.R., Rogers, E.F., Kuehl, F.A., Philips, R.F., Walti, J., Folkers, K., Malanga, C. and Seeler, A.O. (1947). Survey of plants for antimalarial activity. *Lloydia* **10**:145-175.

14. Trager, W. and Polonsky, J. (1981). Antimalarial activity of quassinoids against Chloroquine resistant *Plasmodium falciparum in vitro*. *Am. J. Trop. Med. Hyg.* **30**:531-537.

15. Trager, W. and Jensen, J.B. (1976). Human malaria parasites in continuous cultures. *Science* **193**:673-675.

16. Weenen, N., Nkunya, M.H.H., Bray, D.H., Mwasumbi, L.B., Kinabo, L.S. and Kilimali, V.A.E.B. (1990). Antimalarial activity of Tanzanian medicinal plants. *Planta Med.* **56**:368-370.