In Vitro Screening of Antiplasmodium Activity of Momordica Charantia

Shehab Ali Yousif. Department of Environmental Health, Faculty of public and Environmental Health, University of Bahri,
Khartoum Sudan, Shehab.ali@bahri.edu.sd
Date Received 7/8/2014 Date Accepted 28/9/2014

Abstract- Extracts/fractions of the fruit coat of Momordica charantia, (common name; bitter melon, Balsam, pear, sopropo, arsorossie, Ku gu foo, peria, Karela, balsamina and mara) were tested in-vitro for its antiplasmodial activity. Principally P. falciparum malaria parasite was collected and grown in vitro. The medium used to initiate the culture of Plasmodium falciparum was RPMI-1640, which was originally developed for the in vitro cultivation of leucocytes. The IC50 value of the crude extracts being 52.0 ± 0.265 μg/ml. Compared to the IC50 value of Chloroquine where the IC50 value is 0.025 μg/ml. Only chloroform fraction from the crude extract had shown good antiplasmodial activity with IC50 of 1.83 ± 0.029 μg/ml.

Index Terms- Antiplasmodium Activity, Antimalarial activity, Momordica charantia, Plasmodium falciparum

I. INTRODUCTION
Malaria is one of the most prevalent diseases in the world. Each year this disease infects about 500 million people with 2.3 million deaths.1 research shows that the malaria parasite has evolved and spread alongside humans and is at least as old as the event of the human expansion out of Africa 60-80,000 years ago.2 Malaria control requires an integrated approach comprising mainly prevention, including vector control and the use of effective prophylactic antimalarial, as well as treatment of infected patients with effective antimalarial.3 Malaria is one of the most important health problems in sub-tropical and tropical countries. The World Health Organization estimates that 2.300 million people, or 41% of the total world population, live in areas with malaria risk. More than 300 to 500 million clinical cases are reported annually resulting in at least 1.5 to 2.7 million deaths. Approximately 1 million deaths among children under 5 years old are attributed to malaria alone or in combination with other diseases4-5. Over 80% of malaria deaths occur in Africa and 15% in Asia. In the Americas, 14% of the population is at risk although the mortality is relatively low in this region. The emergence of Chloroquine-resistant strain of P. falciparum, the most deadly species of Malaria parasites and the resistance of vectors (Anopheles spp) to insecticides, in combination with poverty and lack of good quality health care, are the main causes for the increase of malaria morbidity and mortality.6 There is a consensus that new drugs to treat malaria are urgently needed. Many approaches to antimalarial drug discovery are available.7-9 In previous study analyzed and screened crude extracts of A. occidentale for antimalarial activity, the ethanol extract gave the highest antimalarial activity of 75.64 %, with IC50 value of 11.7 μg/ml compared to aqueous extract which had antimalarial activity of 72.3 8 % with IC50 of 16.0 μg/ml.10 It has been reported that “extracts prepared from the combination of four plants (C. papaya, C. aurantifolia, P. guajava and M. indica) demonstrated the best antiplasmodial activities with IC50 values of 15.07±1.74μg/ml and 0.36±0.15μg/ml respectively.11 Scientist reported that; the in vitro antimalarial activity of ethanol, hexane, chloroform and ethylacetate extracts of Dendrathema indicum a plant used in traditional medicine in Nigeria is reported. The ethyl acetate fraction after fractionation showed the highest activity of 68% parasite elimination at the end of the 4-day incubation as compared to chloroform extract which had antimalarial activity of 61%.12 The results of the anti-P. falciparum activity of Justicia betonica and Aloe dawei leaf extracts using the chloroquine diphosphate as control, showed that these extracts had Anti-Plasmodia activity with the 50% schizonts suppression per 200 white blood cell, (EC50) values of 13.36 (95% CI: 9.03 to 22.24) μg/ml, 7.97 (95% CI: 3.56 to 17.85) μg/ml and 24.86 (95% CI: 9.24 to 66.9) μg/ml, respectively.13 In study on Anti-plasmodium activity, the extraction with methanol and hot water solvents from 100 g of Cassia alata leaves powder were 13.23 % and 7.96 % respectively, the growth of P. falciparum, at different concentrations of C. alata and chloroquine is shows that; RPMI 1640 did not affect (0% inhibition rate) the development of P. falciparum, in the treated wells, while 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, 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.14 Recent study in Burkina Faso study, the Terminalia avicenoides produced the most effective antimalarial extracts, with four coming from its leaves and three from its stem bark, also three extracts from Combirotum...
There is a crack in Malaria’s resistant drugs recently had been reported, that the parasite *P.falciparum* seem to be able to hide from vaccines, and to outwit each new generation of drugs. That, the parasite routinely produces a protein, known only as Dihydrofolate reductase (DHFR), to keep itself alive the drug pyrimethamine blocks the function of DHFR and this controls the infection. But pyrimethamine has been widely used over the last 40 years, and inevitably, the parasite had developed resistance. It did so, according to the two researchers, by altering its DHFR. They genetically engineered laboratory bacteria to produce large quantities of DHFR, and then studied the way the protein can change to protect itself, however severe falciparum Malaria had many clinical features favour the diagnosis of severe falciparum malaria.17

It has been reported that *P. falciparum* is becoming resistant to different anti-malarial drugs viz: quinine, sulfodoxine / pyrimethamine, mefloquine etc. in different parts of India”. 18 Jenson stated that “Thirteen percent of its total genetic material is in variable genes that it can switch on and off to fool the immune response any time it wants. It is in the business of trying to avoid a vaccine and destroy everything we have done-and it’s got the genes to do it. Malaria parasites are very, very famous for producing a smokescreen of immune responses.19

Thus developing new anti-malarial from indigenous plants. Natural products are important sources of biologically active compound and have potential for development of novel anti-malarial drugs. 20 There are series of synthetic new anti-malarial, that have been developed and undergoing different stages of drug trials. Attempts are being made to develop new drugs from medicinal plants. 21

The great antiquity of the malaria infection is confirmed by the fact that; over 100 parasite species similar to those of man are found in a wide range of vertebrate s from reptiles or birds to higher apes. None of the parasites, except for those found in some monkey, can be transmitted to man. 22

A historical Review and extensive compilation of plants, showing antimalarial activity has been published recently.23

The extract of the bark and leaves of *Azadirachta indica* is being used in Thailand and Nigeria. 24 In this study different extracts/fractions of the fruit coat of *Momordica charantia* (common name; bitter melon, Balsam. pear, sopropo, asorsossie, Ku gu foo, peria, Karela, balsamina and mara, were tested in vitro for its antiplasmodial activity. Karela had showed positive result in HIV which have inhibitory effects on in vitro culture.25

*Momordica charantia*, had been identified by belong to family *Cucurbitaceae*, and the Latin name: *Momordica charantia*, and it have the common names as balsm pear, balsm apple, cerasee bush, archuche, balsamina, achochilla, pepinillo and Karela.26 Bitter melon preparation shows a significant improves glucose tolerance without increasing blood insulin levels, and improves fasting glucose levels. 27 The plant had many document properties and chemicals.28 Bitter Gourd (Karela) is useful in treating disorders like scabies, itching, psoriasis, ringworm and other fungal diseases. A cup of fresh juice of bitter gourd mixed with a teaspoon of lime juice should be taken, sip by sip, on an empty stomach daily for 4 to 6 months for the treatment of this disease. Its regular use in endemic regions of leprosy acts as a preventive medicine.29 The aim of this study is to test the in vitro antiplasmodial effect of extract of different fractions of *Momordica charantia*, fruit coat. Using chloroquine sensitive *P. falciparum* isolate.

**II. MATERIALS AND METHODS**

Principally *P. falciparum* malaria parasite was collected and grown in vitro following the procedure of Trager& Jenson.30 The medium used to initiate the culture of *Plasmodium falciparum* was RPMI-1640, which was originally developed for the in vitro cultivation of leucocytes.

Basically we have to determine the in vitro antiplasmodial effect of Bitter melon fruit coat extract/fractions. The Bitter melon fruits were collected from the local vegetable market of Delhi . It was washed thoroughly with water and cut into small pieces after removing the seeds and spongy material inside. Then the fruit coat was dried under shade. The dried material was powderd and used for the extraction. Crude extraction was prepared using soxhlet apparatus and then this crude extract was fractionated using separating funnel, with different solvents (chloroform, hexane ,ethyl acetate and butanol).

Transfer 50 gm of plant powder to a Soxhlet apparatus and add 600ml of 50% ethanol to the powder and boil for about 48 hrs till the solvent become colourless in the siphon. The extract was filtered and concentrated and dried in a vacuum evaporator to get the crude extract.31 10 gm of the crude extract was dissolved in 200 ml of 50% ethanol and put it in separating funnel. 100 ml Hexane was added to above solution and shook thoroughly for five minutes and kept for one hour at room temperature the upper layer was taken as Hexane fraction. The same procedure was repeated to get chloroform, ethyl acetate and butanol fractions.

**In vitro screening of extract/fraction against Plasmodium falciparum** 32:

The culture was synchronized using 5% aqueous solution of sorbitol(1 portion of the pellet and 9 portion of sorbitol) and kept for five to seven minutes at room temperature. This ensure killing of all other stages except rings.

It was centrifuged for 5 minutes at 1500 rpm. The supernatant was discarded and the pellet was washed with incomplete media twice. Parasitaemia was adjusted to about 1% for assay by diluting with fresh washed RBCs. The material to be tested was dissolved in DMSO so that the concentration of DMSO never exceeded 0.1% in the experiment. The stock solution was diluted with RPMI-1640 to obtain different concentrations. The dilutions used were 0.1,0.3,0.5,1,3,5,10,30,50µg /100µl.
The test was performed in 96 well plates using chloroquine sensitive isolate (MRC-2). Different concentration of extract/fractions were dispensed in 96 well plate in triplicate. The first well in all the rows were without any drug and considered to be control. The synchronized parasites were inoculated to all the wells, to get a final concentration of 5% haematocrit.

The plates were incubated at 37°C for 24-30 hrs depending on the maturation of the schizont. After confirmation of schizont maturation, smears were prepared from all the wells. If the schizont maturation in control is below 10%, the experiment is considered to be invalid. The smears were stained in Giemsa stain and numbers of schizonts were counted per 200 asexual stage parasites.

The values were compared between control and test wells. The inhibition percentage of schizont for each concentration of extract/fraction was calculated as:

\[ \text{Inhibition} = 100 - \frac{a}{c} \times 100 \]

Where \( a \) is percent of schizont in the test wells, which was determined by the following formula:

\[ A = \frac{Z}{m} \times 100 \]

Where \( Z \) is the number of schizont per 200 asexual parasites in the test wells, \( m \) is number of schizonts in control wells. From dose-response curves, IC\(_{50}\) values (concentration at which the inhibition of the parasite growth represent 50%) were derived for each extraction/fraction by plotting % inhibition against concentration of the test material.

### III. RESULTS AND DISCUSSION

When the crude extract was tested in vitro it did not show much antiplasmodial effect; the IC\(_{50}\) value of the crude extract being 52.0 ± 0.265 µg/ml. Compared to the IC\(_{50}\) value of chloroquine where the IC\(_{50}\) value is 0.025 µg/ml.

Hexane, Ethyl acetate and Butanol fraction, have shown less effects with IC\(_{50}\) 15.07 ± 0.351, 19.0 ± 0.173, > 500 /µg/ml, respectively.(see Figure and Table [1])

Only chloroform fraction from the crude extract of Momordica charantia had shown good antiplasmodial activity with IC\(_{50}\) of 1.83 ± 0.029 µg/ml. IC\(_{50}\) value of 5 µg/ml or less is considered to be effective.

The result of this preliminary study is encouraging. This is the first report on the in vitro antiplasmodial activity of Momordica charantia, Even though tribal population is using Momordica charantia for treating malaria /fever.

Bitter melon has been used as an excellent medical virtue and as a folk medicine for respiratory disorders from ancient times. A teaspoon of root paste mixed with equal amount of tulsi leaf juice taken for a month acts as an excellent medicine for asthmatics. Still there is a need for continued efforts to discover new antimalarial template molecules from herbal sources. When we compare our result with that obtain from other medicinal plants, that from the extracts prepared from the combination of four plants (C. papaya, C. aurantifolia, P. guajava and M. indica) and the extraction with methanol and hot water solvents from 100 g of Cassia alata leaves, which demonstrated the best antimalarial activities with IC\(_{50}\) values of 15.07±1.74µg/ml and 13.23% respectively and with the result of chloroform fraction from the crude extract of Momordica charantia had show promising antiplasmodial activity with IC\(_{50}\) of 1.83 ± 0.029 µg/ml.

![Figure 1](image-url)

Figure [1] Inhibition percentage of different extraction of Crude, Hexane, chloroform, Butanol, Ethyl Acetate and Ethanol
Table [1] In vitro antiplasmodial effect of the extract and fraction of *Momordica charantia* L.

<table>
<thead>
<tr>
<th>Material Tested</th>
<th>IC50 (µg/mL)</th>
<th>IC90 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract 50% Ethanol</td>
<td>52.0 ± 0.265</td>
<td>122.67 ± 2.267</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>1.83 ± 0.029</td>
<td>17.33 ± 0.230</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>15.67 ± 0.351</td>
<td>43.33 ± 0.289</td>
</tr>
<tr>
<td>Ethylacetate fraction</td>
<td>19.0 ± 0.173</td>
<td>42.67 ± 0.404</td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>&gt;500</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Choloroquine (CQ)</td>
<td>0.025</td>
<td></td>
</tr>
</tbody>
</table>

IV. CONCLUSION

The *in vitro* antiplasmodial activity of *Momordica charantia* has been demonstrated and further therapeutic assessment of fruit coat extracts and their combinations would assist in developing combinations with optimum efficacy for further *in vivo* analyses and exploitation towards a rational antimalarial phyotherapeutic drug discovery. Additionally, it is expected that the antiplasmodial components of *Momordica charantia* would interact positively with conventional antimalarial compounds, thereby potentiating their activity in resistant parasite strains.

V. REFERENCES


20. Wright, C. W. and Phillipson, J. D. 1990. Natural products and development of selective antiprotozoal drugs. phytotherapy Research .4.127-139


