

**ANTI-MALARIAL ACTIVITY OF *MONECHMA CILIATUM* (BLACK MAHLAB)**

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**ABSTRACT**

Malaria is one of the most common major health problems all over the world. Pharmacotherapy is the most common treatment strategy for the disease. *Monechma ciliatum* (MC) belongs to the (family: *Acanthaceae*). *M. ciliatum* was used in remedy of general body pain, liver, cold, diarrhea and sterility. This study was carried out to evaluate anti-malarial activity (*Plasmodium falciparum*) of *M. ciliatum* extract of different solvent. The extracts of *M. ciliatum* were screened for it's anti-malarial activites (*Plasmodium falciparum*) with different concentrations (500, 250 and 125 ug/ml) and Artemether (the reference control) *in vitro*. The different solvent of *M. ciliatum* which exhibited

varied from (70.281% to 83.984%) mortality with in 72h, at concentration 500 ug/ml; this was compared with Artemisinin which gave 85.8% inhibition at the same time and the IC50 was found to be (4.670ug/ml to 65.784). In conclusion: These studies conducted for *M. ciliatum* (seeds) was proved to have potent anti-malarial activities against *Plasmodium falciparum in vitro*.

**KEYWORD:** Anti-malarial activity, *Plasmodium falciparum*, *M. ciliatum*, Artemether, Sudan.

## I. INTRODUCTION

Malaria is one of the most widespread infectious diseases, taking the lives of almost one million people a year, most of them in sub-Saharan Africa. Children under the age of 5 and, pregnant women are among the most vulnerable (WHO, 2010). Malaria is the 2nd leading cause of death from infectious diseases in Africa, after HIV/AIDS. According to the World Malaria Report 2011 (WHO, 2011), the demand for medicinal plants is increasing in Africa as the population grows and, pressure on medicinal plant resources will become greater than ever. Interest in plant derived medicines has also increased in the West, among the pharmaceutical companies (Pia, 2007).

Medicinal plants have been playing a vital role in the treatment of malaria for thousands of years (Van et al 2000; Rukunga and Simons 2006). Moreover, many drugs, e.g. quinine and, artemisinin were isolated from plants and hence wise because of the increased resistance of many pathogens, e.g. malaria parasites, towards established drugs, investigation of the chemical compounds within traditional plants is necessary (Rukunga and Simons 2006). *Plasmodium falciparum* is responsible for more than 95% of malaria cases in Sudan.

Medicinal plants are still invaluable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. People in Sudan and in other developing countries have relied on traditional herbal preparations to treat themselves. Therefore, it is useful to investigate the potential of local plants against these disabling diseases (Amaral *et al.*, 2006 & Koko *et al.*, 2008).

*Monechma ciliatum* (also known as black mahlab in Sudan), a member of family Acanthaceae, is an annual glabrous herb, 30 - 65 cm high. The seeds are used as flavoring material in different food products e.g. Kisra (traditional fermented sorghum flour sheets) and bread. The seeds are also used as an effective laxative, and contain a fixed oil which emits a sweet and pleasant odor. It is further used in traditional Sudanese fragrances, lotion and other cosmetics used for wedding preparation and childbirth (Sharief, 2001). *M. ciliatum* was used in remedy of general body pain, liver, cold, diarrhea and sterility in women and its leaves methanolic extract showed oxytocic property *in vivo* and *vitro* (Ayoub and Babiker, 1981 & Uguru, *et al.*, 1995).

*Monechma ciliatum* (MC) is a weed of the semi-arid areas of tropical Africa and belongs to the family *Acanthaceae*. It can grow up to about one meter in height making it possible to be grazed by domestic animals especially sheep and, goats. (Fadayomi *et al.* 1992) reported that *M. ciliatum* is the most dominant weed species in the Sudan Savannah ecological zone of Sokoto State of Nigeria. Hot Methanolic Extract (HME) of *M. ciliatum* had been reported to have potent oxytocic activity, while column chromatographic studies using silicon, accompanied by bioassay of the extract tested on isolated rat uterus enable partial isolation of the oxytocic principle (Uguru *et al.*, 1995).

With the purpose of searching for new anti-malarial agents, in the present work *M. ciliatum* which are used traditionally for treatment of clinical signs associated with malaria were selected to evaluate the activity of different solvent of crude extracts against *Plasmodium falciparum* in vitro.

## I. OBJECTIVES

### The objective

The detection of *Monechma ciliatum* seeds extracts anti-malarial activity against (*Plasmodium falciparum*) using artemisinin as stander.

## II. MATERIALS AND METHODS

### Plant material

The *M. ciliatum* were collected from central Sudan between January 2014 and, February 2014. The plant was identified and, authenticated by the taxonomists of Medicinal and, Aromatic Plants and, Traditional Medicine Research Institute (MAPTMRI), Khartoum, Sudan.



Picture (3) *Monechma Ciliatum* seed

**Preparation of crude extracts**

Extraction was carried out for the seeds of *M. ciliatum* plant, by using different extraction method with different solvent.

**1- Maceration method using two different solvent system**

a- 50 g from crushed seeds were taken and, macerated in 500ml of acetone 36%, water 64% for 72 hr and, then filter using Buchner apparatus. After that the acetone was evaporate using Rotor –evaporator. Then the residue extract was dried using Freeze dryer and, residue was kept in dry closed bottles at 4°C until it was used.



**Picture (2) *M. Ciliatum* macerated in acetone36%**

b - 50 g from crushed seeds were taken and macerated in 500ml of ethanol 70% for 72 hr and then filtered using Buchner apparatus . After that the ethanol was evaporate using Rotor – evaporator . Then the residue extract was dried using Freeze dryer and, residue was kept in dry closed bottles at 4°C until it was used.



**Picture (3) *M. Ciliatum* macerated in ethanol70%**

## 2- Soxhelt extraction method

- 50 g from crushed seeds were taken and, then extracted with 250ml of methanol 80% by using soxhelt apparatus for 8 hr. After that the fats were separated from methanolic extract with n-hexane using separating funnel. The defatted methanolic extract evaporated by Rotor-evaporator. Then the extraction residue was fractionated with chloroform, water system (3:1 ratio) using separating funnel.

Finally the chloroform fractions were evaporated using Rotor- evaporator, while water fractions were dried using freeze dryer. Then these residues extracts were kept in dry closed bottles at 4°C until they were used.



**Picture (4) of methanolic extract after de fatting**



**Picture (5) evaporating process**



**Picture (6) extract after evaporate**



**Picture(7) fractionation process**



**Picture (8) chloroform fraction****Picture (9) water fractions**

### 3- Decoction method

The plants Mac from soxhelt method were allowed to dry over night, after that the dried remained Mac was collected in a conical flask and, 100ml of distilled water was added. A water bath in 80°C for 2hr was used Buchner Funnel after that was used for filtration. This process was repeated two times, after that the all filtrates were collected together and, dried using the Freeze dryer apparatus. The dried extracts were kept in dry place at 4°C until they were used.

**Picture(10) decoction process****Picture(11) filtration process****Picture (12) *M. Ciliatum* decoction extract**

### Parasite culture and *in vitro* assessment of anti-malarial activity

*Plasmodium falciparum* parasite strain was cultured using candle jar method by (Trager and Jensen, 1975). For the *in vitro* anti-malarial assessment of the plant extracts, selected for this study, and the level of parasitemia in the cultures was maintained between 3 to 5 %. Initial screening of anti-malarial activities of different solvent of extracts of the *M. ciliatum* was performed in 96-well micro-titer plates using SYBR Green-I based assay (Bennett *et al*, 2004). All assays were performed using 5 % parasitemi, and 7 % hematocrit. Each extract was initially screened for anti-malarial activity against K1 strain of *P. falciparum* at the concentration of 50µg/ml. The potential candidates which resulted in parasite survival of less than 50 % were further assessed for their IC<sub>50</sub>. The concentration range of the plant extracts used was 100µl. Artemisinin (14.455ug/ml) were used as standard anti-malarial drugs.

### Statistical analysis

All data were presented as mortality%. Statistical analysis for all the assays results were done using Microsoft Excel program (2007).

## III. RESULTS

The anti malaria activities for The *M. ciliatum* (family: Acanthaceae) screening results against (*P. falciparum*) *in vitro* studies were carried out for different extracts, in different concentrations (500, 250 and 125ug/ml) taking Artemisinin in concentration (14.455ug/ml) as a reference control.

## RESULTS

The Anti –malaria culture readings effect for *M. Ciliatum* extracts using different extractions methods by different solvents.

**Table (1) Anti-malarial activity of different solvent of *M. ciliatum* (seeds)**

| S. NO | Solvent of extracts | Concentration (µg/ml) |          |          | IC50 (µg/ml) |
|-------|---------------------|-----------------------|----------|----------|--------------|
|       |                     | Mortality (%)         |          |          |              |
|       |                     | 500                   | 250      | 125      |              |
| 1     | Methanol            | 70.28112              | 61.11111 | 50.62241 | 48.23027     |
| 2     | Ethanol 70%         | 81.36201              | 78.98551 | 77.77778 | 4.670333     |
| 3     | Acetone 36%         | 83.98438              | 79.98551 | 77.82101 | 63.5185      |
| 4     | Chloroform          | 72.41379              | 68.64111 | 65.30612 | 65.74847     |
| 5     | Water               | 77.9661               | 72.43816 | 64.72868 | 58.1133      |
| 6     | *Control            | 85.85859              |          |          |              |

**Key:** \*Control (Artemisinin) used Concentration (14.455ug/ml)

### 1- *M. Ciliatum* water fraction of methanolic extract.

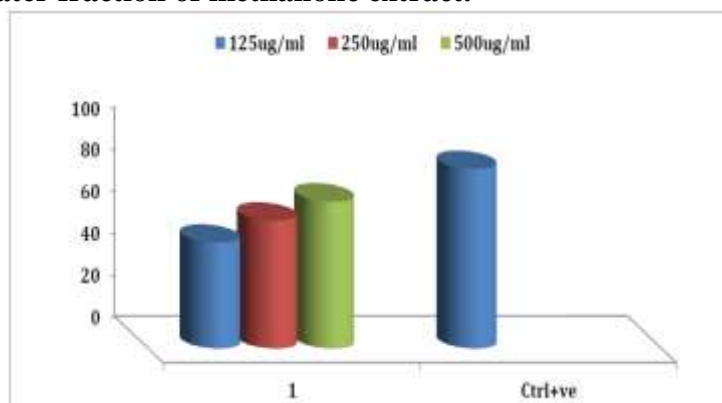


Fig (4) b -Anti Malaria culture reading for *M. Ciliatum* of *M. Ciliatum* water fraction of methanolic extract in comparison within Control (Artemisinin) Concentration (14.455ug/ml)

### 2- Ethanol 70%.

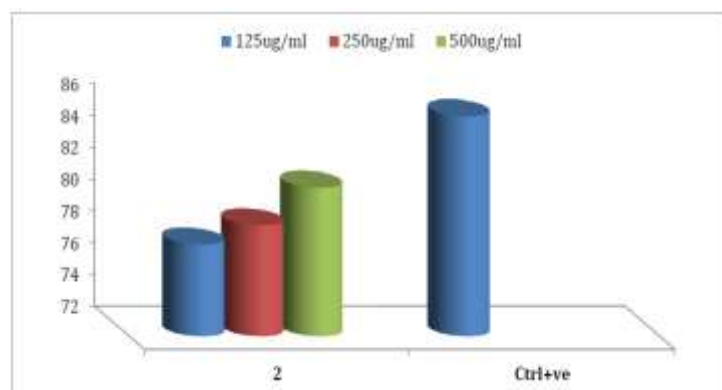


Fig (2) Anti Malaria culture reading for *M. Ciliatum* of Ethanol 70% extract in comparison within Control (Artemisinin) Concentration (14.455ug/ml).

### 3- *M. Ciliatum* chloroform fraction of methanolic extract.

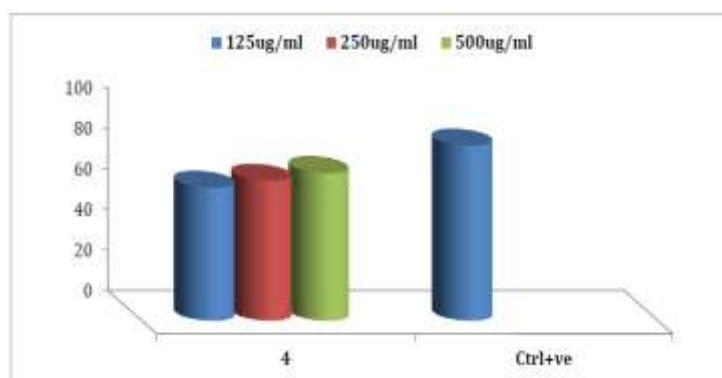
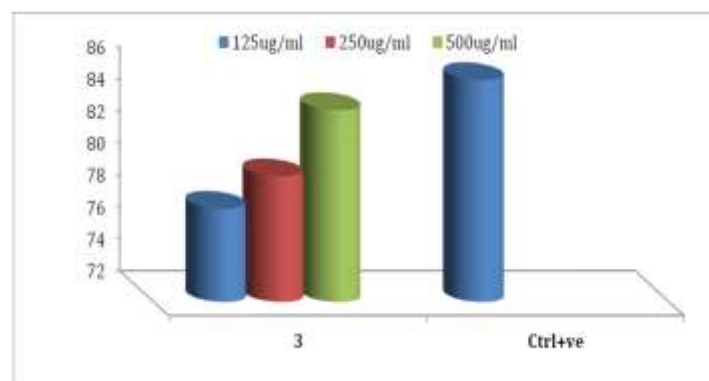


Fig (3)Anti-Malaria culture reading for *M. Ciliatum* of chloroform fraction from methanolic extract in comparison within Control (Artemisinin) Concentration (14.455ug/ml).

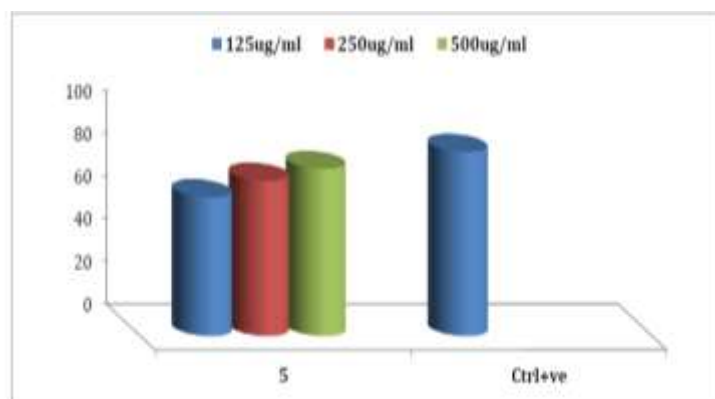


#### 4- Aceton 36%



**Fig (1) Anti Malaria culture reading for *M. Ciliatum* of acetone 36% extract in comparison within Control (Artemisinin) Concentration (14.455ug/ml).**

#### 5- Water decoction extract.



**Fig (5) Anti Malaria culture reading for water decoction of *M. Ciliatum* extract in comparison within Control (Artemisinin) Concentration (14.455ug/ml )**

#### Attached Results Tables

1The Anti –malaria culture readings effect for *M. Ciliatum* extracts using different extractions methods by different solvents.

##### 1- Maceration method.

###### A -Acetone 36%.

| RBC       | 125ug/m |    |    | average  | 250ug/ml |    |    | average  | 500ug/ml |    |    | average  |
|-----------|---------|----|----|----------|----------|----|----|----------|----------|----|----|----------|
| Infected  | 60      | 65 | 75 | 66.66667 | 67       | 65 | 78 | 70       | 70       | 77 | 68 | 71.66667 |
| noninfect | 17      | 20 | 20 | 19       | 13       | 18 | 22 | 17.66667 | 15       | 12 | 14 | 13.66667 |

###### B -Ethanol 70%.

| RBC       | 125ug/ml |    |    | average | 250ug/ml |    |    | average  | 500ug/ml |    |    | average  |
|-----------|----------|----|----|---------|----------|----|----|----------|----------|----|----|----------|
| Infected  | 70       | 70 | 70 | 70      | 75       | 68 | 75 | 72.66667 | 78       | 80 | 69 | 75.66667 |
| noninfect | 20       | 21 | 19 | 20      | 23       | 15 | 20 | 19.33333 | 15       | 19 | 18 | 17.33333 |

## 2- Soxhelt extractions.

### *a-M. Ciliatum* chloroform fraction of methanolic extract.

| RBC       | 125ug/ml |    |    | average | 250ug/ml |    |    | average  | 500ug/ml |    |    | average  |
|-----------|----------|----|----|---------|----------|----|----|----------|----------|----|----|----------|
| Infected  | 65       | 67 | 60 | 64      | 67       | 60 | 70 | 65.66667 | 71       | 69 | 70 | 70       |
| noninfect | 33       | 39 | 30 | 34      | 29       | 33 | 28 | 30       | 29       | 28 | 23 | 26.66667 |

### *b-M. Ciliatum* water fraction of methanolic extract.

| RBC       | 125ug/ml |    |    | average  | 250ug/ml |    |    | average  | 500ug/ml |    |    | average  |
|-----------|----------|----|----|----------|----------|----|----|----------|----------|----|----|----------|
| Infected  | 40       | 42 | 40 | 40.66667 | 46       | 48 | 49 | 47.66667 | 57       | 58 | 60 | 58.33333 |
| noninfect | 31       | 35 | 53 | 39.66667 | 28       | 33 | 30 | 30.33333 | 25       | 27 | 22 | 24.66667 |

## 3-Water decoction extract.

| RBC       | 125ug/ml |    |    | average  | 250ug/ml |    |    | average  | 500ug/ml |    |    | average  |
|-----------|----------|----|----|----------|----------|----|----|----------|----------|----|----|----------|
| Infected  | 68       | 70 | 29 | 55.66667 | 66       | 69 | 70 | 68.33333 | 75       | 73 | 82 | 76.66667 |
| noninfect | 30       | 28 | 33 | 30.33333 | 26       | 25 | 27 | 26       | 24       | 22 | 19 | 21.66667 |

**Table (1): Anti-malarial activity of different solvent of *M. ciliatum* (seeds).**

| E. NO | RBC        | Average  |          |          | Mortality % |         |         |
|-------|------------|----------|----------|----------|-------------|---------|---------|
|       |            | 125ppm   | 250ppm   | 500 ppm  | 125ppm      | 250ppm  | 500ppm  |
| 1     | Infect     | 40.66667 | 47.66667 | 58.33333 | 50.6224     | 61.1111 | 70.2811 |
|       | Non infect | 39.66667 | 30.33333 | 24.66667 |             |         |         |
| 2     | Infect     | 70       | 72.66667 | 75.66667 | 77.7778     | 78.9855 | 81.362  |
|       | Non infect | 20       | 19.33333 | 17.33333 |             |         |         |
| 3     | Infect     | 66.66667 | 70       | 71.66667 | 77.821      | 79.8479 | 83.9844 |
|       | Non infect | 19       | 17.66667 | 13.66667 |             |         |         |
| 4     | Infect     | 64       | 65.66667 | 70       | 65.3061     | 68.6411 | 72.4138 |
|       | Non infect | 34       | 30       | 26.66667 |             |         |         |
| 5     | Infect     | 55.66667 | 68.33333 | 76.66667 | 64.7287     | 72.4382 | 77.9661 |
|       | Non infect | 30.33333 | 26       | 21.66667 |             |         |         |

## IV. DISCUSSION

The remarkable activity of quinine and related drugs and the success of artemisinin have stimulated the search for new plant-derived anti-malarials. A large number of plants have been screened for anti-plasmodial activity (Potterat O, Hamburger M, 2008), (Krettli AU, 2009). Unfortunately treatment with the usual anti-malarial drugs, have induced parasite resistance, reinforcing the need to finding natural anti-malarial components that would be found on plants from the forest. Therefore, we decided to look for these components in Sudanese local plants used in traditionally in malaria treatment (*M. Ciliatum*).

To ensure the activity of *M. ciliatum* (family: Acanthaceae) against (*P. falciparum*) which is the most problematic parasite in treatment for Sudanese patients (treatment resistance).

In this study *M. ciliatum* was screened for its anti-malarial activity against, (*P. falciparum*) *in vitro*, by different extracts with different concentrations (500, 250 and 125 ug/ml) and, Artemisinin (the reference control) with concentration (14.455ug/ml).

The activity of *M. ciliatum*, by using water fraction of methanol extract, ethanol70%, extract acetone 36%, chloroform fraction of methanol and water decoction extract, 70.28, 81.36, 83.98, 72.41 and 77.97% respectively in the higher concentration (500 ug/ml), while that detected from Artemisinin control was 85.98 % ( mortality % ). This indicates a reasonable anti-malaria activity for the plant *M. ciliatum*, different types extracts in comparison with controlled used standard. The IC<sub>50</sub> for these extracts against *P. falciparum* was found to be; 48.23027, 4.670333, 63.51865, 65.74847, 58.12133 respectively. This study can confirm & will support the domestic traditional uses of this Herbal Medicine. This might in lightened the way for discovery of new molecules for malaria treatments; causing outbreak in malaria parasite eradication, and protection.

## V. CONCLUSION

*M. ciliatum* seeds different extracts show anti-malarial activity against, (*P. falciparum*). Their IC<sub>50</sub> was found to be < 65ug/ml and ethanol70% the lowest one which has IC<sub>50</sub> equal 4.67ug/ml this study verify the use of the seeds extracts of *Monechma ciliatum* in traditional medicine.

## RECOMMENDATION

- 1- Photochemical screening must be carried out to identify and elucidate structures of these anti-malarial compounds and predict drug's structural activity relationship.
- 2- More in-vivo and in-vitro studies must be done to have more information about the mechanism of killing different type malaria parasite of different stage.
- 3- The effects of anti-malarial drug's combination with *M. ciliatum* and identification of their effects on washing drug's resistance.
- 4- Further clinical pharmacological, pharmacokinetics & pharmacodynamics studies must be carried out to support their medicinal uses in malaria treatment.
- 5- Suitable pharmaceutical dosage forms are going to be designed, formulated and evaluated for their uses and administration.

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