WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 5, 192-199. Research Article ISSN 2277– 7105

# ANTI-AMOEBA ACTIVITY OF MONECHMA CILIATUM (BLACK MAHLAB)

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Article Received on
09 March 2016,

Revised on 28 March 2016,
Accepted on 18 April 2016

DOI: 10.20959/wjpr20165-6147

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## ABSTRACT

Protozoan disease now a days is become more propagation and one of the important cause of deaths specially in developing countries.form long time Herbal plants are source for many drugs used in treatment this protozoan diseases like Emetin, Artemether, quinine. *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-amoebic activity (*Entamoeba histolytica*) of *M. ciliatum* extract of different solvent. The extracts of *M. ciliatum* were screened for it's anti-malarial activites (*Entamoeba histolytica*) 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 (93.65% to95.96%) mortality with in 72h, at concentration 500 ug/ml; this was compared with metronidazole which gave 92.4% inhibition at the same time and the $IC_{50}$ was found to be (55.36ug/ml to158.68ug/ml). In conclusion: These studies conducted for *M. ciliatum* (seeds) was proved to have potent anti-amobic activities against *Entamoeba histolytica in vitro*.

**KEYWARD:** Anti-amoebic activity, *Plasmodium falciparum*, *M. ciliatum*, metronidazole, Sudan.

## METHOD

### 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.

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.

### 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.

### 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 $80C^0$ was used for 2hr, A Buchner Funnel was used for filtration. This process was repeated two times. 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.

**In vitro susceptibility assays**

*In vitro* susceptibility assays used the sub- culture method of (Cedilla et al. 2002), which is being described as a highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoeba histolytica*, *Gairdia intestinalis* and *Trichomonas vaginalis* (Arguello *et al*., 2004). 5 mg from each extract and compound was dissolved in 50 µl of dimethyl sulfoxzide (DMSO) at Eppendorf tube containing 950 µl D.W in order to reach concentration of 5 mg/ml (5000 ppm). The concentrates were stored at -20°C for further analysis. Sterile 96- well microtite plate was used for different plant extracts, positive control and negative control.

Three out of 8 columns of microtitre plate wells (8 columns × 12 rows) were chosen for each extract, 40 µl (micro-liters) of an extract solution (5 mg/ml) were added to the first column wells C-1: On the other hand, Twenty µl of complete RPMI medium were added to the other wells the second column and third column (C-2 and C-3) . Serial dilutions of the extract were obtained by taking twenty µl of extract to the second column wells and taking 20 µl out of the complete solution in C-2 wells to C- 3 wells and discarding 20 µl from the total solution of C-3 to the remaining 20 µl serial solutions in the successive columns. 80 µl of culture medium was complemented with parasite and added to all wells. The final volume in the wells was 100 µl.

$$\text{Mortality of parasite (\%)} = \frac{(\text{Control negative} - \text{tested sample})}{\text{Control negative}} \times 100$$

In each test metronidazole (a trichomonocide) pure compound[(1-(2-hydroxyethl)-2- methyl-5 nitroimidazole], a was used as positive control in concentration 312.5 µg/ml, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). For counting, the samples were mixed with Trypan blue in equal volume. The final number of parasites was determined with haemocytometer three times for counting after 0, 24, 48 and 72 h. The mortality % of parasite for each extracts activity was carried out according to the following formula.

Only 100% inhibition of the parasite considered, when there was no motile parasite observed.[54]

### 4.2.3. Statistical analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results were done using 2007 Microsoft Excel program. Student t test was used to determine significant difference between control and plant extracts at level of $P < 0.05$.

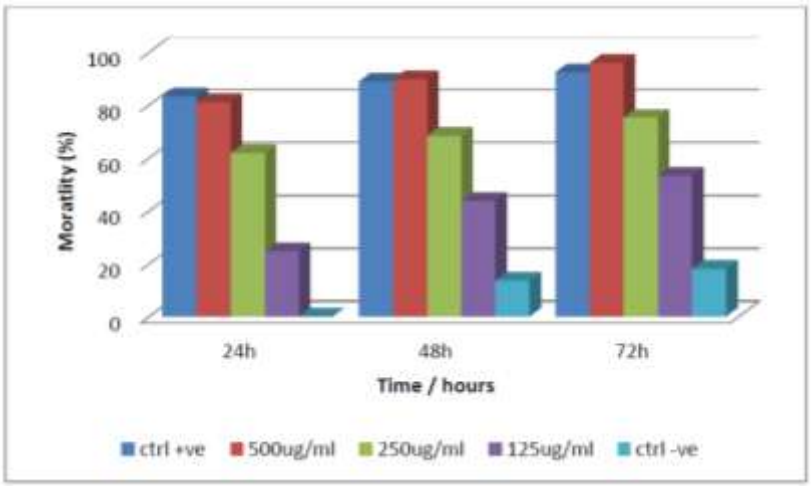
## RESULT

The Anti –amoebic culture readings effect for *M. Ciliatum* extracts using different extractions methods by different solvents were found to be.

a- Ethanol 70%.

**Table (1): Mortility % of E. histolytica.**

| E. Conc. | 24h | 48h | 72h | average | SD | IC50 ug/ml |
|---|---|---|---|---|---|---|
| ctrl +ve | 83.22304 | 88.90879 | 92.43522 | 88.18902 | 4.648079 | 158.68288 |
| 500ug/ml | 81.07773 | 89.70256 | 95.96394 | 88.91474 | 7.474307 | |
| 250ug/ml | 61.79622 | 68.37885 | 75.18838 | 68.45448 | 6.696399 | |
| 125ug/ml | 24.68312 | 43.74037 | 53.27206 | 40.56518 | 14.55655 | |
| ctrl –ve | 0 | 13.59419 | 18.05707 | 10.55042 | 9.40547 | |




**Figure(1): Mortality percent of E.histolytica by ethanolic extract *M. Ciliatum*.**

**a- Acetone 36% with water 64%**

**Table (2): Mortility % of *E.histolytica***

| E. Conc. | 24h | 48h | 72h | average | SD | IC50 ug/ml |
|---|---|---|---|---|---|---|
| ctrl +ve | 83.22304 | 88.90879 | 92.43522 | 88.18902 | 4.648079 | 61.470963 |
| 500ug/ml | 84.67437 | 89.69363 | 95.4403 | 89.9361 | 5.38706 | |
| 250ug/ml | 77.92419 | 81.66054 | 85.51383 | 81.69952 | 3.794968 | |
| 125ug/ml | 55.5576 | 65.30917 | 68.93908 | 63.26862 | 6.92018 | |
| ctrl -ve | 0 | 13.59419 | 18.05707 | 10.55042 | 9.40547 | |

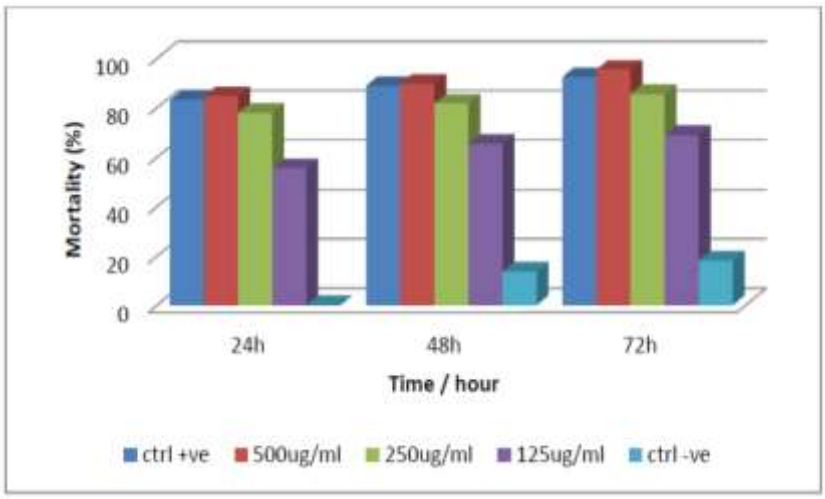



**Figure (2): Mortality% of *E.histolytica* by acetone 36% extract of *M. Ciliatum*.**

**2- Soxhelt extractions**

**a- *Monechma Ciliatum* chloroform fraction of methanolic extract.**

**Table (3): Mortility percent of E.histolytica**

| E. Conc. | 24h | 48h | 72h | average | SD | IC50ug/ml |
|---|---|---|---|---|---|---|
| ctrl +ve | 83.22304 | 88.90879 | 92.43522 | 88.18902 | 4.648079 | |
| 500ug/ml | 76.25088 | 79.78904 | 89.6903 | 81.91007 | 6.966249 | |
| 250ug/ml | 58.50245 | 67.93592 | 77.72146 | 68.05328 | 9.610044 | 55.360449 |
| 125ug/ml | 40.5611 | 48.04534 | 56.3638 | 48.32341 | 7.905017 | |
| ctrl -ve | 0 | 13.59419 | 18.05707 | 10.55042 | 9.40547 | |

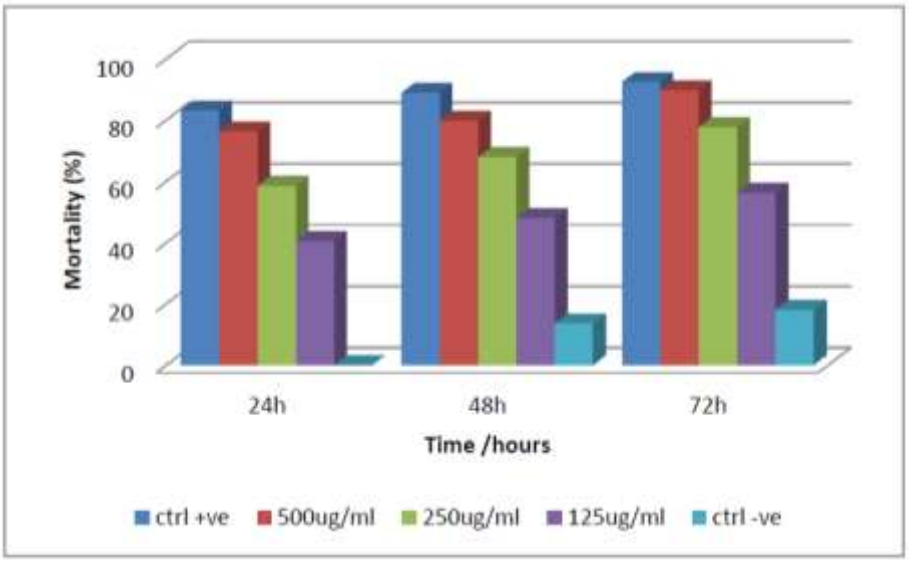



**Figure (3): Mortality percent of *E.histolytica* by *Monechma Ciliatum* chloroform fraction of methanolic extract.**

**b- *Monechma Ciliatum* water fraction of methanolic extract.**

**Table (4) : Mortility percent of *E.histolytica.***

| E. Conc.. | 24h | 48h | 72h | average | SD | IC50ug/ml |
|---|---|---|---|---|---|---|
| ctrl +ve | 83.22304 | 88.90879 | 92.43522 | 88.18902 | 4.648079 | 100.36571 |
| 500ug/ml | 76.16159 | 89.69363 | 93.86064 | 86.57195 | 9.253257 | |
| 250ug/ml | 52.96936 | 76.16159 | 89.69363 | 72.94153 | 18.57268 | |
| 125ug/ml | 34.18242 | 52.96936 | 76.16159 | 54.43779 | 21.02807 | |
| ctrl -ve | 0 | 13.59419 | 18.05707 | 10.55042 | 9.40547 | |

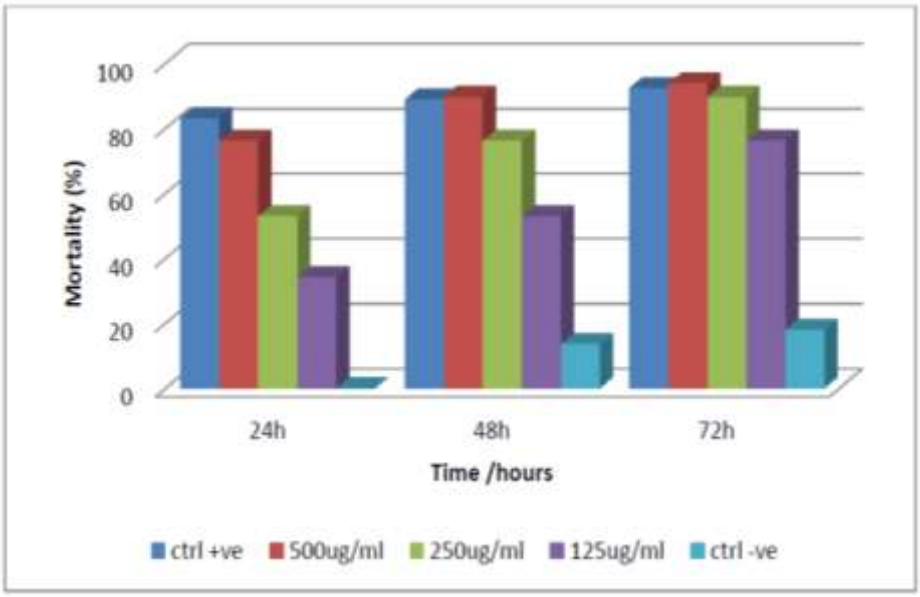



**Figure (4): Mortality percent of *E.histolytica* by *M. Ciliatum* water fraction of methanolic extract.**

**3- Decoction extractions.**

**Table (5): Mortality percent of *M. Ciliatum Water decoction extract.***

| E. Conc. | 24h | 48h | 72h | average | SD | IC50ug/ml |
|---|---|---|---|---|---|---|
| ctrl +ve | 83.22304 | 88.90879 | 92.43522 | 88.18902 | 4.648079 | 99.723272 |
| 500ug/ml | 75.20921 | 89.06863 | 93.65231 | 85.97672 | 9.602445 | |
| 250ug/ml | 52.96936 | 76.16159 | 89.69363 | 72.94153 | 18.57268 | |
| 125ug/ml | 34.18242 | 52.96936 | 76.16159 | 54.43779 | 21.02807 | |
| ctrl -ve | 0 | 13.59419 | 18.05707 | 10.55042 | 9.40547 | |

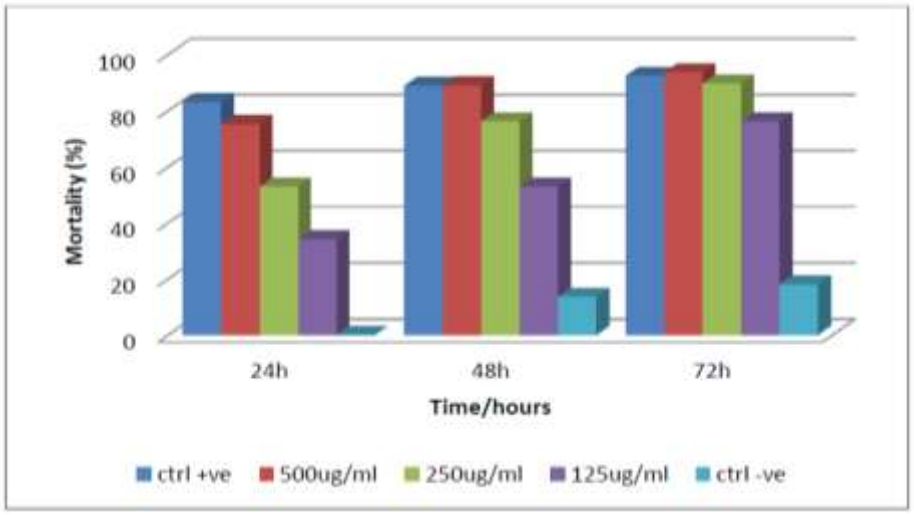



**Figure (5): Mortality % of *E.histolytica* by *M. Ciliatum water decoction extract.***

## DISCUSSION AND CONCLUSION

Amoebiasis is present all over the world About 480 million people are infected with *E. histiolytica* and this results in the death of between 40,000–110,000 people every year(*John Wiley & Sons, 2014*). And it is one of the most popular infectious diseases in Sudan(M.A. Babiker,M.S.M. Ali2 and E.S. Ahmed, 2009). The most popular drug used in different protocols to treat is Metronidazol, which is used as an standard drug in this study.

Sudan flora is rich in Herbal effective medicinal plants. A lot of researches were worked in these days to confirm the native's, & domestic's medicinal uses of these plants. One of these plants is *M. ciliatum* (family: Acanthaceae) which is used in local perfumes, other cosmetics (Sharief, 2001) &, also to treat many diseases especially, infectious diseases, diarrhea ,& fevers(Ayoub and Babiker, 1981& Uguru, *et al*., 1995). In this study *M. ciliatum* was screened for it's anti-amoebic activity against, (*E. histolytica*) *in vitro, by using it's different extracts* with different concentrations (500, 250 and 125 ug/ml) and, Metronidazole (the reference control) with concentration (312.5 ug/ml).

The anti- amoebic activity of *M. ciliatum,* carried out , by using water fraction of methanol extract, ethanol 70%, extract, acetone 36%, chloroform fraction of methanol and ,water decoction extract, 93.65, 95.96, 95.44, 89.69 and 93.65% respectively in the higher concentration (500 ug/ml)with in 72hr, while that detected from Metronidazle control was 92.44% (mortality %). This indicates a reasonable anti-malaria activity for the plant *M. ciliatum,* different types extracts in comparison with controlled used standard. The $IC_{50}$ for these extracts against *E.histolytica* was found to be; 100.37, 158.68, 61.47, 55.36 and 99.72

ug/ml respectively. The lower concentrations of these solvent extracts also increased within time (72hr) &these might indicate sustained action of *M. ciliatum against E.histolytica.* 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 *Amoebiosis* treatments; causing outbreak in *E. histolytica* parasite eradication,

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