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RESEARCH ARTICLE 43

Antigiardial Activity of some *Cucurbita* Species and *Lagenaria Siceraria*

Ihsan Mohamed Elhadi [1], Waleed S. Koko[2]*, Mahmoud M. Dahab[2], Yahia Mohamed El Imam[3], Mona Abdu Elmonem Abdu El Mageed[1]

[1]Depart of pharmacognosy, Faculty of Pharmacy, Omdurman Islamic University, Sudan.
[2]Medicinal and Aromatic Plant Research Institute, National Center for Research, Khartoum, Sudan.
[3]Faculty of Pharmacy, The National Ribat University.

(**Received** April 09, 2013; **Accepted** July 04, 2013)

***Abstract*— This study was carried out to evaluate antigiardial activity of *Cucurbita maxima D, Cucurbita pepo L* and *Lagenaria siceraria.* Variety supreme court seeds petroleum ether and methanolic extracts *in vitro* tests were perform using three concentrations (1000 ppm, 500 ppm and 250 ppm). The highest activity against *Giaradia lambelia*, with respect to time, was obtained from *C. maxima* seeds petroleum ether extract which exhibited 100% mortality within 48 giving IC_{50} of 548.80 ppm (with a concentration of 1000 and 500 ppm) followed by *L. siceraria* petroleum ether extract which exhibited 100% mortality within 72 hours with IC_{50} of 95.65 ppm whereas Metronidazol, a pure compound, (positive control) showed 100% mortality within 96 hours. On the other hand the lowest antigiardial activity was recorded by *C. pepo* petroleum ether extract (83.67% mortality with 500 ppm concentration within 96 hours) giving IC_{50} of 60671.32ppm whereas *C. maxima* and *L. siceraria* methanol extract exhibited 100 % mortality within 96 hours with IC_{50} 35.6ppm and 120 hours with IC_{50} 8.9ppm respectively (with 1000ppm concentration). The best result was obtained by *C. maxima* petroleum ether extract at 250 ppm (up to 100% mortality within 72 hours) with IC_{50} 1 ppm. This result will approve that this species (*C. maxima*) is a promising species in treating *Giardia lambelia* and agree with traditional claims.**

Index Terms*— *Cucurbita maxima D, Cucurbita pepo L* and *Lagenaria siceraria, Gairdia lameblia

I. INTRODUCTION

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 [1and 2].

The treatment of giardiasis consists of the use of one or more drugs, with metronidazole being the first choice. Other nitroimidazolic derivatives (secnidazole, tinidazole, and ornidazole), benzimidazoles (albendazole, mebendazole), furazolin, quinacrine and paromomycin have also been employed in therapeutic regimens. However, these drugs have adverse effects including gastrointestinal disturbances, nausea, headache, leucopoenia, myopia, neuralgia, and allergic dermatitis and an unpleasant taste in the mouth. Furthermore, they can lead to neurotoxic effects, ataxia, convulsions and vertigo, bringing about the interruption of treatment. In addition, mutagenic and carcinogenic effects have been described in laboratory animals [3-8].

Thus the need of alternative drugs to reduce their burden of purchasing the synthetic drugs especially after the problem of getting resistant to many clinical patients against metronidazole [9 and10] and thus new antigiardial drugs are probably required.

Cucurbits are well –recognized source of secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tannins and cucurbitacins (tetracyclic triterpenoids) which impart a bitter flavor to many Cucurbits [11and 12]. Terpenoids which are rich in oxygen, are of potent antigiardial activity [13].

From ancient time the seeds of the genus *Cucurbita* and *Lagenaria* were used in treating intestinal parasites. Experimental research was carried out at the Parasitology and Chemistry laboratories of the Jorge Basadre Grohmann National University, in Tacna, for testing *Cucurbita maxima* as antiparasitic agent against canine tape worms *in vitro* and *in vivo* using albino mice, It was found that the MIC of 23 gr. of pumpkin seeds in 100 ml. of distilled water can produce an antihelminthic effect [14].

With the purpose of searching for new antigiardial agents, in the present work *Cucurbita pepo, Cucurbita maxima* and *lagenaria siceraria* which are used traditionally for treatment of clinical signs associated with giardiases were selected to evaluate the activity of their petroleum ether and methanolic crude extracts against *Giardia lamblia* trophozoites *in vitro*.

* Corresponding author: wasyko2002@yahoo.com

II. MATERIALS AND METHODS

Plant materials

The seeds of *Cucurbita pepo L, Cucurbita maxima D,* and *Lagenaria siceraria* variety supreme court were collected between April 2008 and August 2008. The seeds of *Cucurbita pepo, Cucurbita maxima* were collected from Khartoum state whereas *Lagenaria siceraria* variety supreme court was gathered from Saudi Arabia. The plants were identified and authenticated by the taxonomists Dr, Abdu Elgabar Nasir Gumaa, Department of Biology, Faculty of Education, Khartoum University. The seeds were air-dried and coarsely ground to poweder.

Preparation of Crude extracts

30 grams of the coarsely ground material of the seeds were successively extracted by Soxhlet apparatus using petroleum ether, and methanol.

The extracts were then filtered and evaporated under reduced pressure using rotatory evaporator.

Parasite isolate

G.lambelia used in all experiments were taken from patient. All positive samples were examined by wet mount preparation. Trophozoites of *G. lambelia* were performed at 37 ± 1°C in RPMI 1640 medium containing 5% bovine serum. The trophozoites were maintained for the assays and were employed in the log phase of growth. Parasites were counted under the microscope by haemocytometer chamber.

In vitro susceptibility assays

In vitro susceptibility assays used the sub- culture method of Cedilla *et al.,* [15]. This is highly stringent and sensitive method for assessing the anti-protozoal effects (gold standard) particularly in *Entamoba histolytica*, *Gairdia intestinalis* and *T. vaginalis* [16].

5 mg from each extract 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 (5000ppm). The concentrates were stored at -20 ℃ 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 of an extract solution (5 mg/ml) were added to the first column wells C-1: On the other hand , 20 µl of complete RPMI medium were added to the other wells of` the second column and third column (C-2 and C-3) . Serial dilutions of the extract were obtained by taking 20 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.

Each test included metronidazole pure compound [(1-(2-hydroxyethl)-2-methyl-5 nitroimidazole], a drug was used as positive control in concentration 312.5 µg/ml, whereas untreated cells were used as a negative controls (culture medium plus trophozoites). Samples were taken for counting at 0, 24, 48, 72, 96, and 120 hours.

For counting, the samples were mixed with Trypan blue in equal volumes The final number of parasites was determined with haemocytometer in triplicate.

The mortality % of parasite for each extract activity was carried out according to the following formula:

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

Statistical analysis

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

III. RESULTS AND DISCUSSION

Out of six extract investigated, 5 extracts (83.33) exhibited 100% mortality of parasite within 120 hours or less (Figure 1, 2, 3, 4, 5 and 6).

Out of 5 active extracts, one showed 100% mortality within 48 hours, one within 72 hours whereas one within 96 hours and two within 120 hours.

Three extracts (60%) attained 100% mortality by concentrations: (1000, 500 and 250ppm) and two extracts (40%) attained 100% mortality only at 1000ppm.

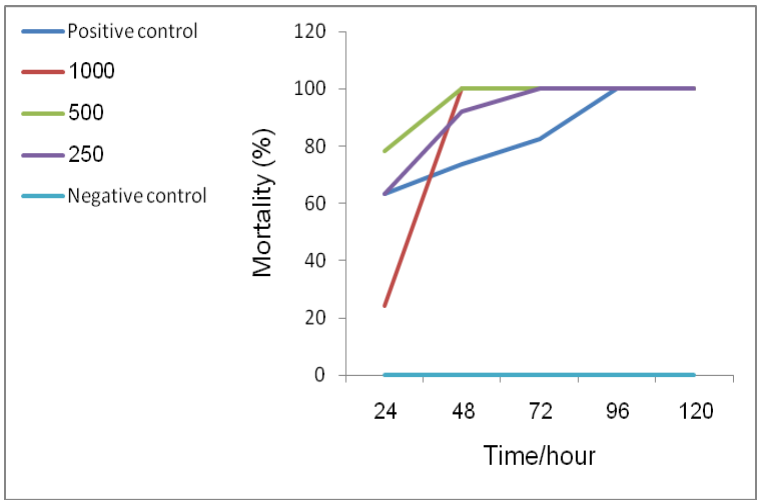

Figure 1. *In vitro* activity of *Cucurbita maxima* seeds petroleum ether extract against *Giardia lambelia*; mortality percentage in relation to time

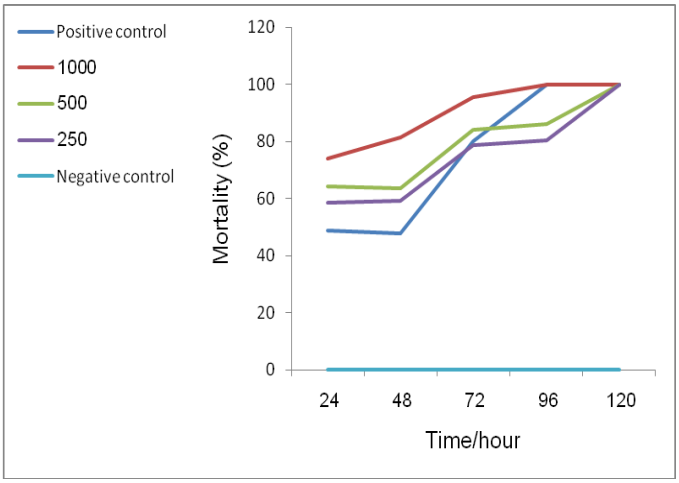

Figure 2. *In vitro* activity of *Cucurbita maxima* seeds methanolic extract against *Giaradia lamblia*

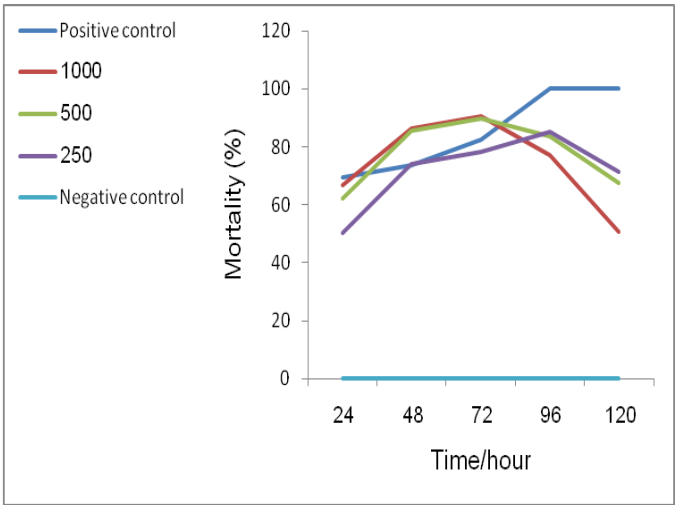

Figure 3. *In vitro* activity of *Cucurbita pepo* seeds petroleum ether extract against *Giaradia lambelia*: ; mortality percentage in relation to time

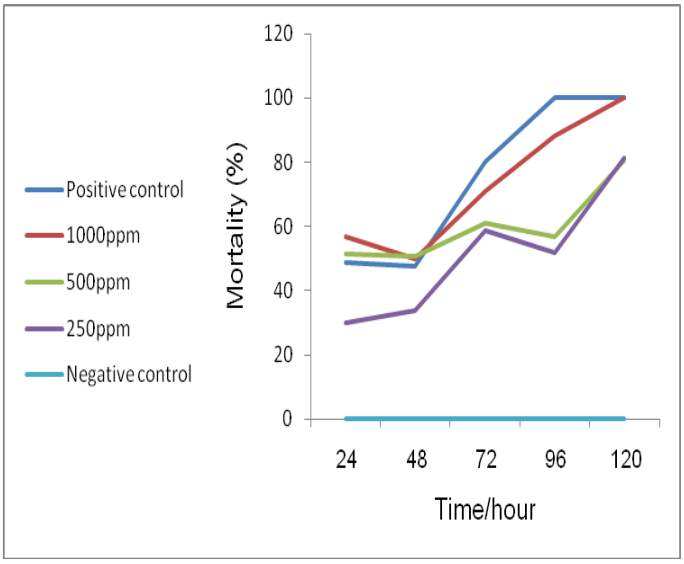

Figure 4. *In vitro* activity of *Cucurbita pepo* seeds methanolic extract against *Giaradia lambelia*

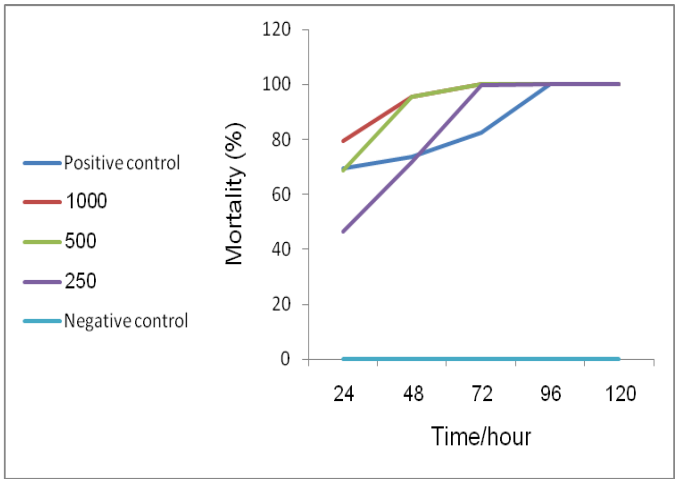

Figure 5. *In vitro* activity of *Lagenaria siceraria* seeds pet ether extract against *Giaradia lambelia*: ; mortality percentage in relation to time

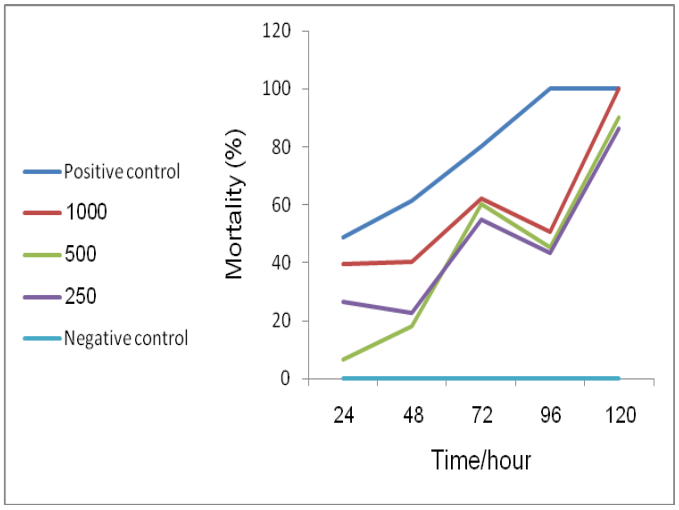

Figure 6. *In vitro* activity of *Lagenaria siceraria* seeds methanol extract against *Giaradia lambelia*: ; mortality percentage in relation to time

Giardia lamblia is an important cause of acute and chronic gastrointestinal disease throughout the world and has been identified as the etiologic agent in numerous waterborne outbreaks of diarrheal disease. Although *G. lamblia* is among the most prevalent enteric protozoal infections in humans, it is relatively recently that improvements in t he *in vitro* cultivation of this organism have allowed reliable, reproducible tests to assess the *in vitro* activity of therapeutic agents against *G. lambelia* [17].

Calzada *et al*.[18] reported that methanolic extracts of nineteen plant species of Mexican origin, distributed among thirteen families, and described potent giardicidal activity in six species (*Acalypha phleoides*, *Cnidoscolus tehuacanensis*, *Geranium nievum*, *Hellianthella quinquenervis*, *Heliopsis longipes* and *Teloxys graveolens*), with IC_{50} values less than or equal to 20.64 µg/mL.

The results represented in **Figures 1, 2, 3, 4. 5 and 6**, revealed that, the highest activity against *Giaradia lambelia*, with respect

to time, was obtained from *C. ma*. seeds petroleum ether extract which exhibited 100% mortality within 48 hrs giving IC_{50} of 548.80ppm (with a concentration of 1000 and 500ppm) followed by *L. s*. petroleum ether extract which exhibited 100 % mortality within 72 hours with IC_{50} of 95.65ppm whereas Metronidazol, a pure compound, (positive control) showed 100% mortality within 96 hours. On the other hand the lowest antigiardial activity was recorded by *C. p*. petroleum ether extract (83.67% mortality with 500 ppm concentration within 96 hours) giving IC_{50} of 60671.32ppm whereas *C. ma*. and *L. s*. methanol extract exhibited 100 % mortality within 96 hours with IC_{50} 35.6ppm and 120 hours with IC_{50} 8.9ppm respectively (with 1000ppm concentration).

It had been clearly noticed that all studied extracts reached 100 % mortality except *C. p*. petroleum ether extract. The best result was obtained by *C. ma*. petroleum ether extract at 250 ppm (exhibited 100% mortality within 72 hours) with IC_{50} 1ppm. This result will approve that this species (*C. ma*) is a promising species in treating *Giaradia lambelia* better than synthetic antigiardial drugs.

The antigiardial activity of *C. ma*. and other studied species could be due to the presence of triterpene (Cucurbitacins) as has been demonstrated by Loiy *et al*., [19] who investigated the antigiard [20] ial activities of *Citrullus lanatus* var. *citroides* (wild watermelon) fruits petroleum ether, ethyl acetate, butanol crude extracts as well as Cucurbitacin E and Cucurbitacin L 2-O-β-glucoside pure isolated compounds from *C. lanatus* var. *citroides*. Cucurbitacin E and Cucurbitacin L 2-O-β-glucoside were revealed to have strong potent antigiardial activity against *Giardia lamblia in vitro* with IC_{50}= 2 and 5 µg/ml after 5 days respectively. It could be due to the presence of essential oil of the seeds as had been demonstrated by Marisa *et al*., who evaluated the anti-Giardia activity of phenolic-rich essential oils obtained from *Thymbra capitata*, *Origanum virens*, *Thymus zygis* subsp. *Sylvestris chemotype thymol*, and *Lippia graveolens* aromatic plants. The tested essential oils inhibited the growth of *Giardia lamblia* at IC_{50} (71– 257) µg/ml since the first hour of incubation and were able to kill almost 50% of the parasites population in a time-dependent manner.

The phytochemical screening of *C. ma*. and the other studied species revealed the presence of triterpene in the seeds petroleum ether extract as well as methanolic extract, and GC-Ms analysis of seeds oil declared the presence of Myristic, Stearic, Palmitic, Linolenic, ω6, Arachidic acid and other fatty acids [21]. All these constituents could be the causative factors of antigiardial activity for the above investigated species.

IV. CONCLUSION

It has been concluded that the seeds of the studied species can solved the problem of diarrhea that caused by *Giardia intestinalis* instead of Metronidazol which has been demonstrated to have side effects and they can be used traditionally or can be formulated

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