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## Study the Efficiency of Two Concentrations from Algae *Cladophora glomerata* Extract on the *Giardia lamblia* parasite

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### Abstract:

*Giardia lamblia* parasite was isolated from the diarrhea samples of patients with Giardiasis dysentery and was developed in HSP media, four mice groups have been used to find in vivo efficacy of two concentrations (128,256) mg/ml of chlorophorm extracts from *Cladophora glomerata* algae against *Giardia lamblia* parasite as compared with (Flagyl) by measuring several biochemical markers as ( GPT and GOT) enzymes ,sodium ,potassium and iron concentration as well as counting the number of parasitic cysts in each mice groups. The results demonstrate that levels of GPTA GOT enzymes have been decreased in mice treated with algal extract. As for the concentration of the Sodium, Potassium and Iron increased in mice treated with algal extract. The number of the Giardia cyst is also reduced in orally inoculated mice with both concentrations of algal extract as compared with positive control and the Flagyl treated group. In terms of bioactive compounds, GC-Mass results indicate the presence of many phytochemicals with different biologically active properties. This study represents the first attempt to use *Cladophora glomerata* derived from phytochemicals to treat giardiasis in vivo.

**Keywords:** Bioactivity, Chlorophorm extract, *Cladophora*, Giardiasis, In vivo

### Introduction:

Intestinal parasitic infection is a potential communal health problem distributed around the world mostly in developing countries. These parasite cause many severe symptoms<sup>1</sup>. One of the most prominent intestinal parasite is Giardia Giardia which is the first unicellular parasite discovered by Leevenhok through his simple microscope while examining feces at 1681<sup>2</sup>. A primitive parasite has two stages: active trophozoite and cyst. The trophozoite is flattened pear shaped which have two nuclei, two slender median rods (axostyles), and eight flagella arising from the anterior end. The cyst is an oval which contains two or four nuclei surrounded by a thick wall<sup>3</sup>. This parasite infects the upper part of the intestine duodenum and the jejunum trophozoite feed on the intestinal secretions after it sticks to its wall and form a dense cover of flagellates. This leads to molting villi of the small intestine and thus produces little food absorption and fatty diarrhea<sup>4</sup>.

Seaweed is a huge and diverse group of algae that are rich in bioactive metabolites and a source of innovative constituents for functional foods. Many seaweed types have been consumed as diet in some countries of the world. Some seaweed is usually used as a traditional medication because they are rich in biologically compounds, i.e., proteins, lipids, polysaccharides and polyphenols, which authenticated as antibacterial, antiviral and antifungal agents. As well as important vitamins and minerals<sup>5</sup>. These secondary metabolites have been used in new pharmaceutical agents' progress<sup>6</sup>.

*Cladophora* is a macro-algae which belongs to chlorophyte. It's characterized by the branched filamentous appearance that forms a net like construction, it is usually located in benthic region, attached on submersed wood or stones and in extreme circumstances will grow on plants also. Widespread in freshwater and marine habitats, this algal thread is diminutive and very hard mostly it is

stable in one spot, which makes it easy to reach and remove<sup>7</sup>.

Since many investigators have focused on antiparasitic compounds derived from algae, there are studies of biologically active constituents from green algae that have led to the discovery of many phytochemicals used as antiparasitic agents<sup>8</sup>.

Because of the importance of the locally isolated Giardia parasite, several researches have been carried out to find the treatment of patients, so in this study in vivo efficacy of the *C. glomerata* algae to treat mice infected with this parasite by determining several blood parameters and calculating the number of parasite cysts in feces of infected mice.

## Material and Methods:

### Preparation of algal extract:

Samples of locally isolated *C. glomerata* have been kindly obtained from Asst. Prof. A Sahi/Department of Biology/Mustansiriyah University. As a fine powder Soxhlet extraction was used to prepare extract as explained in<sup>9</sup>. Dried powder form of *Cladophora glomerata* material has been extracted by using chloroform. The traces of chloroform have been removed using a rotary evaporator at 50 °C. After calculating the weight of dry extracts, two concentrations (128, 256) mg/ml have been prepared by dissolving extract dry weight in sterilized distilled water, then kept in refrigerator for further examination.

### Collection of parasite samples:

This study includes the collection of 100 stool samples from children patients, adult males and females suffering from diarrhea. Samples have been collected from Al-Kadhimiya Teaching Hospital in Baghdad/Iraq from September 2016 to January 2017. Notes have been taken during diagnosis such as appearance, color and presence of mucus and blood in the stool sample then the exam has been done by direct microscopic exam of stool smear by putting a drop of normal saline on a slide then taking a small amount of stool by a wood stick and mix it well with the drop of normal saline after that it has been covered with cover slide and examined under microscope with power magnification 40x. The other way of staining has been by using Lugol's Iodine because it has been suitable for staining both trophozoite and cyst cytoplasm in yellow color and nucleus with dark brown color.<sup>10</sup>

### Purification of cyst

Method of<sup>11</sup> has been adopted with a simple modification to isolate the parasite cysts.

1-Dilution of stool sample at the rate of 0.1-0.15 ml with distilled water.

2- The sample has been filtered by a piece of nylon cloth, then filtered out by metal filter with 0.9-1.2 micron hole diameter.

3- about 0.4-0.5 ml has been centrifuged by 1800 cycle for 5 min.

4- The deposit has been diluted at the rate of 1:10 with distilled water and then centrifuged 1800 cycle for 5 min.

5- a volume (0.4 ml) of distilled water has been added to the deposit.

6- This has been repeated three times.

7-Then it has been refrigerated in 4°C till used.

8- The number of cysts have been counted using Haemocytometer 10<sup>5</sup> cell/ml.

### Laboratory growth

After preparing the HSP-1 media according to<sup>12</sup>:

1- 0.1ml of the stock that has 10<sup>5</sup> cell of cyst and 0.3 ml of human serum has been added, also penicillin and streptomycin have been added to avoid pollution.

2- Tubes have been incubated in 37°C for 24hrs and determine less concentration for extract (inhibited parasite).

3- Routine examination has been done in the third day of culturing by taking a drop from the culture and put it on a slide and put a cover slip on it then examine it by microscope using 400x and 1000x objectives, the trophozoite has been observed in a little amount in addition to the cysts.

4- Secondary culturing have been done after ten days then the trophozoite has been counted in the first days of culturing to insure the existence of trophozoites after taking 0.3 ml from the grown culture and 0.1 ml from new culture that is free from antibiotics.

### Experimental Design

Using 25 mice from white albino Balb/c (males and females) weighted (23-25) gm have been inoculated orally (except negative control group) with 0.1 ml contain 1x10<sup>5</sup> cell from Giardia after (4-8) hrs., the stool of all mice has been examined and after sure infection by Giardia divided into four groups; each group contains 5 mice, then inoculated as follows:

Group 1: 0.1 ml from 256mg/ml algae extract orally

Group 2: 0.1 ml from 128 mg/ml algae extract.

Group 3: 0.1 ml from metronidazole 30 mg/ml.

Group 4: 0.1 ml of normal saline and consider as positive control.

Group 5: un infected mice consider as negative control

The two enzymes glutamate pyruvate Transminase (Gpt) and glutamate oxaloacetic transminase (Got) have been measured by Randox kit according to instructions of manufacturer.

Iron (Fe) level in serum mmole/L has been measured by using kit from Biomaghreb Company according to instructions of manufacturer .

Sodium ion has been measured according to<sup>13</sup> by Flame photometer device

Sodium concentration= $\frac{150 \times \text{sample reading}}{100}$   
150=Concentration of standard solution  
100=Number when the device read the standard solution

### Gas Chromatography-Mass Spectrometry

This technique has been used to find the active compounds found in chloroform extract of Macro algae by employing a high-temperature column according to<sup>14</sup> using (SHIMADZU—Japan) and postrun software. Phytochemical compounds have been identified by comparison of their mass with NIST library search and authentic standards

### Results and Discussion:

Results show high levels of liver function enzymes Gpt, Got in serums of mice infected with the parasite, Table 1.

**Table 1. Levels of Gpt,Got compared with positive and negative controls .Result represent (mean± standard deviation)**

Groups	Gpt Unit/L	Got Unit/L
Negative control	2.3±10.9	3.8±1
Positive control	25.5±43.0	32.5±53.0
Flagyl	7.2±20.2	7.8±25.0
128 mg/ml	8.5±6.2	7.4±144.1
256 mg/ml	5.9±138.0	11.6±13.8

**Gpt:** glutamate pyruvate Transminase; **Got:** glutamate oxaloacetic transaminase

The increase may be attributed to the parasitic effect of the parasite in the liver and spleen cells. This damage results in leaks of enzymes from damaged liver cells to the bloodstream and increases its level in serum and lowering level of Gpt. Got means reduction of damage caused to liver cells and that liver tissue returns back gradually to its normal state<sup>15</sup>.

The results in Table 2 indicate a slight increase in the concentration of sodium but remains within the natural limits (136-155 mmol / L). Sodium remains within normal range until renal function decreases and renal filtration become (5-10%) of the normal function of the kidneys, there is an increase in serum sodium concentration while potassium concentration is significantly higher at

the control value<sup>16</sup>. Decrease of Iron concentration has been observed in patients infected with Giardia. This maybe due to chronic inflammation that occurs in the body and leads to loss of iron representation and changes include decreasing in the concentration of iron in the serum as well as a decrease in iron absorption by the intestine leading to loss of blood<sup>17</sup>.

**Table 2. Concentration of Na,K,Fe in blood serum of infected and control groups Results represent (mean± standard deviation)**

Groups	Na (mmole/L)	K (mmole/L)	Fe (mmole/L)
Negative control	5.9±138.1	0.3±3.8	6.0±23.0
Positive control	7.4±144.2	0.8±5.0	32.5±53.0
Flagyl	4.9±136.3	0.5±4.0	7.6±15.0
128 mg/ml	8.5±6.2	0.9±1.1	10.0±0.8)
256 mg/ml	7.8±25	0.5±0.1	11.6±13.8

The number of parasite cyst in the infected group, treated group and treatment group with two concentrations of algae extract are shown in Table 3, a decrease in the number of parasite cysts when using the two concentrations of algae compared to the positive control.

**Table 3. Number of parasite cyst in infected group, treated group and exposed group with two macro algae concentrations .Result represent (mean± standard deviation)**

Groups	10 <sup>5</sup> cell/ml No. parasite cyst
Negative control	No cyst
Positive control	18.9±0.17
flagyl	6.1±0.2
128mg/ml	11.5±0.08
256mg/ml	8.3±0.21

Infections with Intestinal parasites are one of 17th ignored tropical disease listed by world health organization and being the 4th top causative agent that leads to community distributed disease<sup>18</sup>.

There is a developing interest in marine biological resources, particularly seaweeds and microalgae as sources of therapeutic substances. There has been a lot of attentiveness to natural phytochemicals derived from marine algae to discover their medicinal properties<sup>19</sup>.

Since the mechanism by which the *Cladophora* extract causes this decline in Giardia number during this study is difficult to speculate,<sup>8</sup> suggesting that there are several mechanisms of antiparasitic activity of *Cladophora* extract; one of these

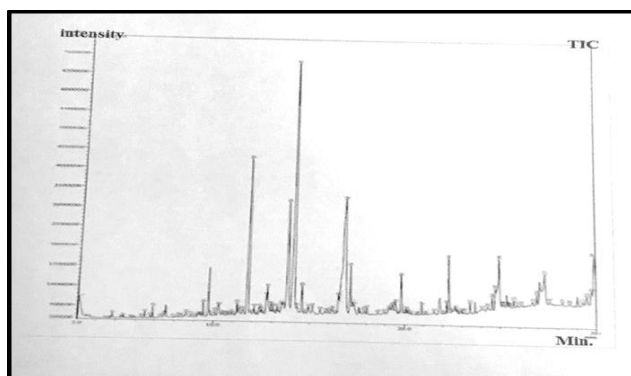
mechanism is the interference of bioactive constituents with the reduce balance of the parasitic organism. Another mechanism that phytochemicals are known to bind to DNA of the parasite.

The previous author was used alcoholic extract of *Cladophora* to treat hydatid disease caused by *Echinococcus* parasite. Another researcher<sup>20</sup> also used *Cladophora* extract to treat infected mice with *Entamoeba histolytica* parasite. Also<sup>21</sup> results reveal that this algal species extract has successfully treated *Trichomonas vaginalis* infected mice. The current study considers the first study investigate of the impact of crude extract of this alga against *Giardia* parasite in vivo.

### Gas Chromatography-Mass Spectrometry

The data obtained from this analysis shows that *Cladophora* extract contain many phytochemical constituents since the main six phytochemicals are located in largest area as shown in Fig.1. In the active fraction, Salicylic acid has been found to be a major compound (16.09%) followed by Octadecenoic acid (14.22%) then Heptadecenoic acid (8.68%). Also followed by Dodecan (10.18%), Tetradecan (8.29%) and 9-octadecenyloxy has been found in less area (6.41%). Salicylic acid is a phenolic nature component and is widely present in plants where it has a crucial role to protect the plants against pathogenic agents<sup>22</sup>.

Octadecenoic acid is a Stearic acid authenticated to exhibit antimicrobial and antitumor effects<sup>23</sup>. The previous author mentioned that Heptadecenoic acid and Dodecan are alkane compounds possess Antioxidant, Antimicrobial and cytotoxic activity, respectively.<sup>24</sup> reported that Tetradecan also exhibited antimicrobial activity.



**Figure 1. GC-Mass spectrophotometer chromatogram displayed the chloroform extract of *Cladophora glomerata*.**

### Conclusions:

The findings of this study represents a supporting factor to use green alga *Cladophora*

*glomerata* in the development of a new natural drug for Giardiasis disease medication. The authors recommend that further investigations are needed to determine the exact phytochemicals responsible for anti-parasitic activity within this alga and purify it.

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### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Mustansiriyah.

### Authors' contributions statement:

- SN Lefta Conception and design, R G Fayyad drafting the MS and revision and proofreading, D Y Mohammed analysis and interpretation, S AJ Almaliki acquisition of data and analysis.

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## دراسة كفاءة تركيزين من مستخلص الطحالب الخضراء *Cladophora glomerata* ضد طفيلي *Giardia lamblia*

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### الخلاصة:

خلال الدراسة الحالية تم عزل طفيلي *Giardia lamblia* من عينات البراز لمرضى مصابين باسهال *Giardiasis* حيث تم عزل الطفيلي و تتميته باستخدام وسط HSP . استعان الباحثون بفئران تجريبية بواقع اربع مجاميع من الفئران وذلك لتقييم فعالية تركيزين (128,256) ملغم /مل من مستخلص كلوروفورم للطحالب الخضراء *Cladophora glomerata* ضد الطفيلي المعزول ومقارنة بالعلاج التجاري للطفيلي (Flagyl) وذلك بقياس بعض المؤشرات مثل انزيمات الكبد (GPT and GOT ) ، مستوى تراكيز الصوديوم والبوتاسيوم والحديد بالدم اضافة الى تعداد اكياس الطفيلي لبراز كل مجموعة من مجاميع الفئران المستخدمة خلال التجربة ، اظهرت النتائج انحدار في مستويات انزيمات الكبد بعد معالجة الفئران المصابة بالطفيلي بمستخلص الطحالب. بينما اشرت قياسات مستوى الصوديوم والبوتاسيوم و الحديد زيادة بعد العلاج بمستخلص الطحلب. وبالنسبة لتعداد اكياس الطفيلي فقد قل تعدادها في براز الفئران المصابة بعد تجربتها فموبا بمستخلص الطحلب مقارنة بالعلاج التجاري. واخيرا تم الكشف عن المركبات الفعالة في مستخلص الطحالب المدروسة باجراء فحص GC-Mass حيث اظهرت نتائج الفحص وجود العديد من المركبات ذات فعالية بايولوجية متنوعة . تعتبر هذه الدراسة الاولى على مستوى العالم لبيان امكانية استخدام المركبات الفعالة بايولوجيا الموجوده في طحلب *Cladophora glomerata* كعلاج مناسب وبديل عن العلاج المصنع للقضاء على اصابات الطفيلي *Giardia*.

الكلمات المفتاحية : داخل الجسم الحي، داء الجيارديا فعالية بايولوجية، كلادوفورا، مستخلص كلوروفورم