

# Effect of Methanolic Extract of Green Algal *Enteromorpha Intestanalis* on the Bioactivity of *Giardia lamblia* Parasite

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## Abstract

The present study was conducted to detect the effect of *Enteromorpha intestanalis* methanol extract and study of its therapeutic effect in male *Mus musculus* experimentally infected with *Giardia lamblia* parasite, The mice infected with the parasite were dosed with the aforementioned extract at a concentration of (2, 2.5, 3) mg / ml at a dose rate per day for 25, 20, 15) days, and examining the faeces of the mice to monitor the change in the parasite preparation after the extract with the extract, and the results showed that the extract of the moss was Effective in reducing the numbers of encapsulated and feeding phases in mice infected with the parasite. The highest rate of killing of the feeding and encapsulated phases was recorded at a dose of (3) mg / ml during the twenty-five days' period. Specific disclosures were made of chemicals for secondary metabolism of the algae extract, which indicated the presence of chemically effective compounds such as alkaloids, phenols, flavonoids, cyclosides, turpenoids, tannins, soaps and carotenoids. During the experiment, the therapeutic efficacy of the extract was calculated (64.30%). The chemical compounds of the algae extract were diagnosed using the Gas chromatography mass spectroscopy (Gc-mass) technique.

**Keywords:** Methanolic extract, *Enteromorpha intestanalis*., *Giardia lambila* parasite.

## Introduction

Medicines cause a lot of unwanted side effects, in addition to their high cost and negative effects of chemotherapy, as well as the possibility of infection in some cases and the parasite's resistance to some therapeutic substances. Alternative treatments with fewer side effects and being inexpensive and available are therefore used in large parts of the world [1] as it used many types of algae that can be very important natural sources for new compounds with vital effectiveness, including large algae, which are medically important organisms because they contain highly valuable active substances and biologically active molecules in treating many diseases and may behave as antibiotics for a large number from microorganisms, including parasites [2]. Most of the algae isolated materials are due to the group of primary and secondary metabolites, and among these algae is *Enteromorpha intestanalis*, which is a green alga as it is one of the sources rich in natural materials that can be extracted by simple and easy methods [3]. The *Giardia lambila* parasite is one of the most prevalent diarrhea-causing innovations in the world, especially

in developing countries [4]. It is characterized by being one-celled organisms that live in the small intestine of humans and other mammals, which in turn represents one of the public health problems in the tropical regions [5]. This parasite infects many people in the world, causing Giardiasis disease. The infection rate for this parasite in developing countries reached (30%) while it reached (5%) in developed countries [6] as the infection of this parasite abounds in poor areas. That lacks general health conditions and personal hygiene [7]. The infection also occurs through eating food and drink contaminated with cysts or through direct contact, as well as animals a major role in transmitting the infection to humans. This is evident through the increase in the spread of the parasite in homes. In which pets live with humans, as this parasite causes losses significant economic result from its effects on domestic animals, cows Kalagnam [8]. This disease develops from carriers to acute and chronic infections, which are diarrhea and poor absorption, as this parasite has no ability to invade tissues [9]. In view of the medical importance of this parasite, vigorous efforts have been made to get to know more about the parasite and the

disease and how to treat it, and the increased interest in using algae extracts in treating parasite infection given some of the compounds that impede the growth of the parasite [10] as well as being less harmful and less toxic than chemical drugs.

## Materials and Methods

For the purpose of conducting the necessary tests to determine the effect of methanol extract on the parasite of the guardia, as the parasite trap was prepared for the fermentation of experimental animals using [11] and after obtaining faecal samples for patients it was confirmed that they were infected with the guardian parasite from the General Hospital in the Senate Market in the Senate District / Dhi Qar Governorate / Iraq for the period from August 2019 to December of the same year, and green moss was obtained ready from the Amazon American company in the form of a powder and the extract was prepared using the method [12]. As for laboratory animals, as they were obtained from the animal house of the Department of Life Sciences in the College of Education for Pure Sciences / University of Dhi Qar, which included (25) white mice, their weights ranged between (30-25) grams of males whose ages ranged between (10- 8) A week, after confirming its safety from infection with intestinal parasites through an examination , Stool microscopically, divided into five groups, each group contains (3) animals, the first three groups were dosed with the amoebic parasite by dosing its members with (3) ml of the parasite streak previously prepared for fifteen days at the rate of a dose per day, examining the excrement of the dose of rat mice periodically and after confirmation From infection with the parasite by watching feeding and encapsulated phases in feces were placed in clean and isolated cages, the first group of mice infected with the extract was concentrated at a concentration of 2 mg / ml and the second group at 2.5 mg / ml and the third group with 3 mg / ml once per day over a period of 25, 20,15 days, As for the group infected with the fourth parasite, it was left untreated as a positive control group (ve Control +). As for the fifth group, it was administered with a physiological salt solution only

and it was considered a negative control (ve Control) - next: Therapeutic efficacy (%)=The rate of preparation of the sac in the control group - The rate of preparation of the sac in the treated group / The rate of preparation of the sac in the control group\* 100. The active substances were detected in the algae extract under study according to [13]. Some chemical compounds were diagnosed in the extract using a gas chromatography mass model (G.C / M.S - QP 2010 Ultra) in the Dhi Qar Environment Directorate / Iraq.

## Results

Orally administered laboratory animals with the parasite trap showed a high sensitivity of susceptibility to infection of the encapsulated phases of the *G. lamblia* parasite after fifteen days of oral dosage through direct microscopic examination of infected rat stools, as the parasite cysts were distinguished by their pear shape and containing two nuclei as well as flagella whose number is Four front pairs in addition to the adhesion disc. The vegetative phase does not contain mitochondria and oxidative phosphorylation components. The results of the methanolic extract of algae showed a clear inhibitory effect on the parasitic phases of the parasite using concentrations (2, 2.5 (3 mg / mL for the period (15, 20, 25) days), as shown in Table 1). During the days in which laboratory animals infected with the parasite were treated and treated with the extract and the positive control group, it was noted that the parasite was completely eliminated at concentration (3) mg / ml on the twenty-fifth day when using the methanol extract compared to the control group that continued to prepare the increasing phases Where with m Over time, the therapeutic efficacy of the extract reached (64.30%). The results of the current study also showed that the extract of the algae extract *E.intestanalisis* contains a group of effective secondary metabolism compounds, as it was observed from the specific disclosures of the algae extract containing alkaloids, phenols, flavonoids, tannins, turpenoids and saponins.

**Table 1: Rate of cysts in the faeces of rats infected with *G.lamblia* parasite**

Time (day)	Concentration mg/mL			
	2	2.5	3	Control
15	12	9	7	13
20	7	4	2	19
25	5	1	0	26

<sup>a-c</sup> Different letters within each column indicate significant difference (P< 0.05)

**Table 2: Effect of *E. intestinalis* on *G. lamblia* cysts after 15, 20 and 25 days after infection**

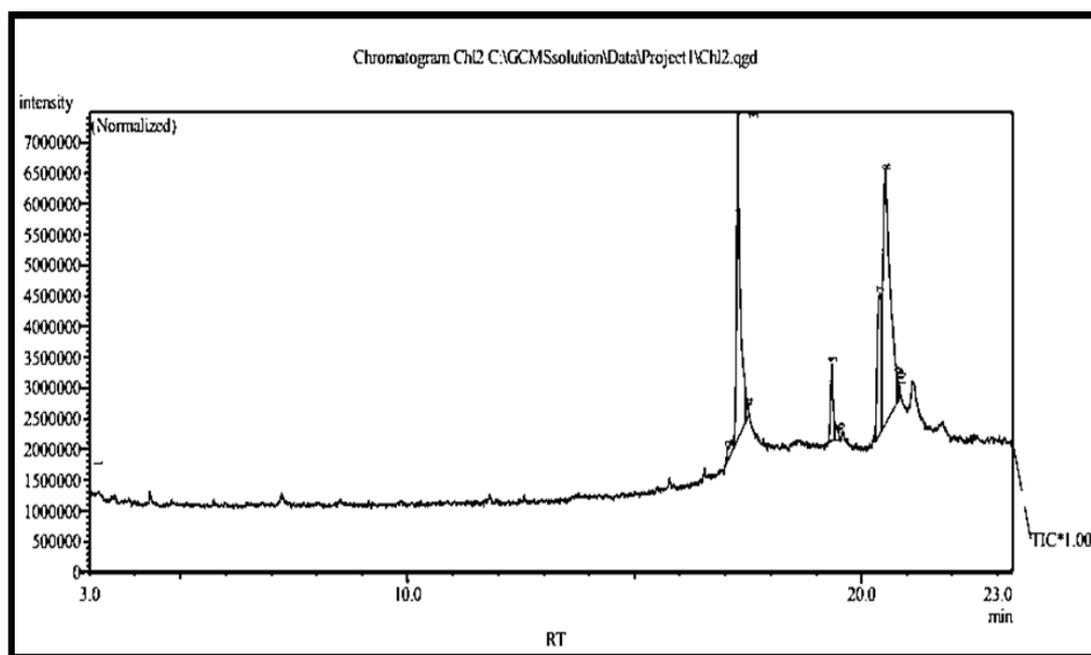
Time (day)	Concentration mg/mL			
	2	2.5	3	Control
15	8.82 $\pm$ 0.83 <sub>a</sub>	6.62 $\pm$ 1.16 <sub>a</sub>	4.95 $\pm$ 1.00 <sub>a</sub>	13.00 $\pm$ 2.05 <sub>b</sub>
20	6.37 $\pm$ 1.33 <sub>a</sub>	1.40 $\pm$ 4.42 <sub>a</sub>	2.88 $\pm$ 1.00 <sub>a</sub>	18.82 $\pm$ 6.87 <sub>b</sub>
25	4.41 $\pm$ 1.42 <sub>b</sub>	1.67 $\pm$ 1.16 <sub>c</sub>	0.00 $\pm$ 0.00 <sub>c</sub>	26.81 $\pm$ 1.17 <sub>a</sub>

<sup>a-c</sup> Different letters within each column indicate significant difference (P< 0.05)

The results showed that the diagnosis of compounds using the GC-mass gas chromatography technique was the presence of (8) compounds in the methanolic algae extract as shown in Table 3 and Figure 1.

**Table 3: Chemical compounds diagnosed with GC-mass gas algae technique under study**

Percentage of the whole (%)	Detention time	Molecular weight (g / mol)	Molecular formula	The name of chemical compound
3,296	19.131	196.242	C11H16O3	Loliolide
1.07	19.972	312.530	C20H40O2	Ethyl stearate
3.538	20.274	256.424	C16H32O2	Palmitic Acid
23.744	20.536	284.477	C18H36O2	Ethyl palmitate
2.8413	21.646	296.531	C20H40O	Phytol
4.916	22.102	310.514	C20H38O2	Ethyl oleate
21.855	24.541	258.3538	C14H26O4	Ethylhexyladipate
4.91	25.886	410.718	C30H50	Squalene



**Figure 1:** *E.intestinalis* algae compounds defined by GC-mass technique .

## Discussion

Effective chemical compounds extracted from natural sources, such as plants, were widely used in treating many diseases because they are safe compared to common manufactured chemical treatments that may have genetically mutagenic properties, which makes these natural alternatives required, as they contain effective molecules in treatment with the lowest chance of Developing resistance against it and its mutagenic effects on the host are few [14]. Algae is a large and diverse group that produces many secondary metabolism compounds that have vital effectiveness as they enter the pharmaceutical industry and in the treatment of many cancerous diseases, AIDS, arthritis, and as anti-bacterial, fungal, and viral antigens [15]. The extraction pattern and type of solvent used determine the degree of success in isolating the active compounds vitally so water was used as a solvent known globally and for being the safest health solvent to prepare the extracts. Algae is a large and diverse group that produces many secondary metabolism compounds that have vital effectiveness as they enter the pharmaceutical industry and in the treatment of many cancerous diseases, AIDS, arthritis, and as anti-bacterial, fungal, and viral antigen [15]. The extraction pattern and type of solvent used determine the degree of success in isolating the active compounds vitally so

water was used as a solvent known globally and for being the safest health solvent to prepare the extracts. It was found that Berberine sulphate showed high efficacy against *E. hstolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, as it caused swelling of the vegetative phase of the *G. lamblia* parasite, and for this alkaloid, it leads to the emergence of large numbers of autopharyngeal gaps with the emergence of one large gap or it may be due to the presence of phenolic compounds that lead to the loss of the cellular membrane of the parasite due to the permeability property and consequently the entry and exit of materials to and from the parasite without regulation and then the death of the parasite [16]. It was found that Berberine sulphate showed high efficacy against *E. hstolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, as it caused swelling of the vegetative phase of the *G. lamblia* parasite, and for this alkaloid, it leads to the emergence of large numbers of auto pharyngeal gaps with the emergence of one large gap, Or it may be because it contains phenolic compounds that lead to the loss of the cellular membrane of the parasite of the permeability property and consequently the entry and exit of materials to and from the parasite without regulation and then the death of the parasite [16] or perhaps the reason for that is due to the containment of the algae extract On active substances that inhibit the metabolism of carbohydrates

by their effect on mitochondria and then the breathing process and thus lead to the death of the parasite [17]. There are studies that indicate that phenolic compounds in algae extracts have the ability to bind with fats in the cytoplasm, cell membrane and cell membranes of membranes and change their functional composition and then the death of the living cell [18] and the effect may return. The presence of other active substances present in the algae, such as flavonoids that have the ability to denature of proteins and stop the action of enzymes accompanying the process of glycolysis, the most important of which is the enzyme Hexokinase, and thus the microorganism loses its ability to continue life [19], and its reason may be attributed to Phenols have an effect on the cholinesterase-style enzyme that controls the permeability and elasticity of the cell membrane. Thus, the membrane lost its permeability property, which led to the entry of various substances, including toxic nitrogenous substances, into the parasite and consequently its death [16]. As for the results of the chromatography technique, it showed the presence of a number of effective chemical compounds in the algae ethanol extract, and among these compounds occupied the largest area of the total area of the diagnosed compounds represented by the ethyle palmitate which occupied an area of 744 (23%) which is one of the fatty acids that possess effective against infections and has a role in stimulating the immune system and improving its ability to combat infectious agents and may be attributed to its vital activity being the largest portion of the total area [20] (followed by the compound Ethylhexyladipate which occupied an area of 21.855 (%). As for the ethyl oleate compound, which occupied an area of 4.916 (%), as studies conducted on humans indicated that this compound works as an anti-inflammatory, reducing oxidative stress and treating many diseases [21].

### Conclusion

Based on these results, we can conclude that the green algae extract contains many vitally active chemicals to make it to be included in the algae of medicinal importance. The algae extract also showed inhibitory efficacy in affecting and eliminating the parasite.

**Conflict of Interest:** None

**Funding:** Self

**Ethical Clearance:** Not required

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