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EFFECT OF AQUEOUS EXTRACT OF *CHLORELLA SP.* ON *ENTAMOEBEA HISTOLYTICA* PARASITE IN VIVO

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Abstract:

The objective of this study was to conduct in vivo to detect the effect of *Chlorella* extract on *Entamoeba histolytica*. and to examine its therapeutic effect in male laboratory rats type *Mus musculus*. These rats were dosed with a concentration of (1, 1.5 and 2) mg/ml of the aforesaid extract at a daily dose of (10, 15 and 20) days and then, the rats' faeces were tested to observe the change in parasite numbers after dosing the extract. The results showed that the aqueous extract of *Chlorella sp.* was effective in reducing the vesicated and fed stages *Entamoeba histolytica*-infected rats, where the rate of killing those stages had recorded the highest percentage at dose of 2mg/ml during a period of 20 days. Moreover, qualitative detections of chemicals for secondary metabolism of aqueous extract of *Chlorella sp.* were carried out, which indicated the presence of chemically active compounds. The therapeutic efficiency of the extract was also calculated, which has recorded 62.70%.

Chemical compounds were diagnosed using the gas chromatography technique - gas chromatography mass spectrometry (Gc -mass). These compounds were represented by 1- (5-Bicyclo [2.2.1] heptyl) ethylamine, 1,4-Bis [5- [3-trifluoromethylphenyl]. 1, 2,4-oxadiazol-3-yl] benzene Pentadeoanoic, acid, Estra-1,3,5 (10) -trin-17. beta. -0l, 1-Heneicosanol, 1,2,4-Triazol, 4 - [N- (2-hydroxyethyl) -N-nitro] amino-, 9,12-Octadecadienoic acid (Z, Z), 6-octadecadienoic acid, (Z) -, 9-Oxa-bicyclo [3.3.1] Noonan-2,6-dione, cyclopentanocarboxylic acid, 2-acetyl-5-methyl-).

Keywords: Aqueous extract, Gc – mass, *Chlorella*, *Entamoeba histolytica*.

Introduction:

Searching for new and common compounds from publications such as antibiotics and wintering again scientific research on the emergence of many antibodies after testing, active and knowledge of their composition in medical fields [1], economics of traditional medical practices known in a certain geographical area [2] , And thus increased interest in algae as a source of bioactive compounds in the last four decades, as well as an increase in studies on the importance of algae in the medical and pharmaceutical fields, such as the use of medicinal plants rich in these compounds. Aquatic life during the time period between 1977 and 1987 was from algae alone [3].

These compounds are chemically classified into Carbohydrates, Proteins, Alkaloids, Phenols, Terpens, and Fatty Acids [4] and have been used as Antivirus, Antibacterial, Antifungal, Anti-inflammatory [5] and anti-inflammatory [5].

Entamoeba histolytica is one of the intestinal primary that infects humans and causes amoebic dysentery, and it represents a public health problem in tropical regions 8,7,6. According to the World Health Organization (WHO), it is responsible for (100,000 - 40,000) deaths annually [9]. The parasite passes through its life cycle two main stages, namely the trophozoite phase and the cyst phase, and the last represents the infective stage, where the parasite is infected by eating And the contaminated drink at that stage [10] The nutritive phase is the harmful phase, and it lives in the lumen of the large intestine of the cleanser and feeds on the intestinal mucosa and red blood cells and secretes enzymes that break down the mucous membrane and go deep into the intestinal wall, damaging its cells, causing painful ulcers, thus causing amebic dysentery [12,11].

Due to the medical importance of this parasite, vigorous efforts have been made to know more about the parasite and disease and how to treat it, and the increased interest in using plant extracts in treating the parasite infection due to the compounds contained in some of them hinder the growth of the parasite as well as help in the elimination and healing of intestinal ulcers [13] as well as Being less harmful and less toxic than chemical drugs.

Materials and Methods:

The samples of faeces for patients infected with amoebic dysentery were collected from Al-Hussein Educational Hospital, Al-Mousawi Hospital for Children and Bint Al-Huda Hospital in Nasiriyah district for the period from November 2018 to February 2019. The green mosses were obtained from the American company Amazon in the form of powder. The animals of the experiment were obtained from the Animal House of the Department of Life Sciences at Faculty of Science-University of Dhi- Qar, which is 25 white rats. The weights ranged from 25-30 grams of males, which ranged in age from 8-10 weeks. The first, second, third group was dosed by aqueoueous extract with a concentration of 1, 1.5 and 2 mg/ml once a day over 10. 15. 20 days, whereas the fourth group infected with the parasite and fifth group was fed with 0.5% of the normal saline solution.

Some chemical compounds were diagnosed in the extract using a Gas chromatography mass model (G.C / M.S - QP 2010 Ultra) at the College of Agriculture / University of Basra.

Statistical Analysis:

Statistical analysis of the results was performed by one-way ANOVA using SPSS ver.23. Significant differences ($P < 0.05$) among the concentrations were analyzed by Duncan test.

Results:

The rats that have been orally dosed by suspend contain the parasite showed a highly recorded Susceptibility to the infection of cyst phases to *E. histolytica*, this was proved through direct microscopic examination of infected rats after ten days of the oral dosing, where the cysts of *E. histolytica* parasite were marked through their spherical shape, which contain four nuclei, while the vegetative phase has irregular shape with one nucleus and food gaps containing red blood cells. The results of the ethanol extract of moss showed a significant disincentive effect on the parasite, using concentrations of 1, 1.5 and 2 mg/ml for the period of 10, 15 and 20 days. Table 1 shows the rate of cystic phases in the parasitic faeces of a single microscopic field during the days when laboratory animals infected with the parasite were treated, treated

with extract and +ve control. It was observed that the parasite was completely eliminated at the concentration of 2 mg/ml of day 20 when aqueous extract was used, this compares with the +ve control, in which the number of cystic phases continued to increase over time, with the therapeutic efficiency of the extract at 62.70%.

Table 1: Rate of cysts in the faeces of rats infected with *E.histolytica* parasite

Time (day)	Concentration mg/mL			
	1	1.5	2	Control
10	13	10	8	14
15	8	5	3	20
20	6	2	0	27

^{a-c} Different letters within each column indicate significant difference (P< 0.05)

Table 2: Effect of *chlorella* sp. on *E. histolytica* cysts after 10, 15 and 20 days after infection

Time (day)	Concentration mg/mL			
	1	1.5	2	Control
10	9.80 ± 0.83 a	7.60 ± 1.14 a	5.00 ± 1.00 a	14.00 ± 2.07b
15	7.40 ± 1.34a	1.40 ± 4.40 a	3.00 ± 1.00 a	19.80 ± 6.89 b
20	5.40 ± 1.40 b	1.60 ± 1.14 c	0.00 ± 0.00 c	27.80 ± 1.14 a

^{a-c} Different letters within each column indicate significant difference (P< 0.05).

The results showed that diagnosing compounds using GC-mass technology, the presence of 10 compounds in the algae ethanol extract, (Table 5 and Fig. 1).

(Table 5) The chemical compounds diagnosed with GC-mass

Percentage of all (%)	Retention time	Molecular weight Gram / mol	Molecular formula	The name of the chemical compound
0.44	3.021	139	C9H17N	1-(5-Bicyclo[2.2.1]heptyl)ethylamine
2.00	17.060	502	C24H12F6N4O4	1,4-Bis[5-[3-trifluoromethylphenyl]-1,2,4-oxadiazol-3-yl]benzene
30.27	17.272	242	C15H30O2	Pentadecanoic acid
0.93	17.500	256	C18H34O	Estra-1,3,5(10)-trin-17.beta.-01
4.91	19.342	312	C21H44O	1-Heneicosanol
1.19	19.500	173	C4H7N5O3	1,2,4-Triazol, 4-[N-(2-hydroxyethyl)-N-nitro]amino-
14.79	20.400	280	C18H32O2	9,12-Octadecadienoic acid (Z,Z).
44.22	20.527	282	C18H34O2	6-Octadecenoic acid, (Z)-
0.77	20.800	154	C8H10O3	9-Oxa-bicyclo[3.3.1]nonane-2,6-dione
0.49	20.847	170	C9H14O3	Cyclopentanecarboxylic acid, 2-acetyl-5-methyl-

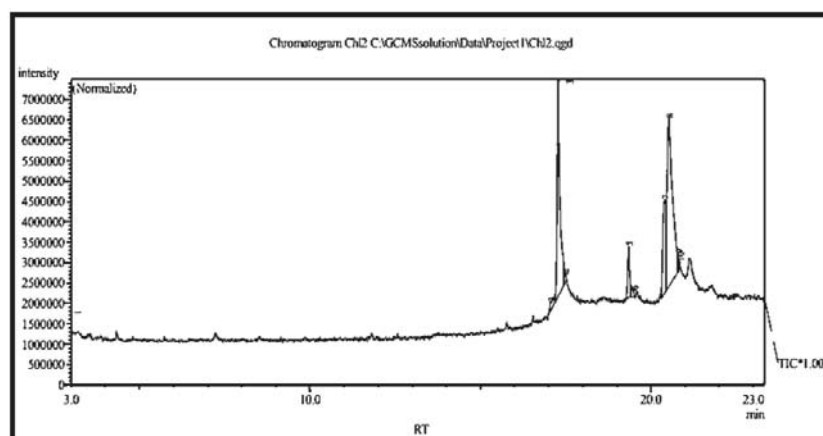


Figure (1) Compounds of *Chlorella* specific by GC-mass technique

Discussion:

Effective chemical compounds extracted from natural sources such as plants are widely used in the treatment of many diseases because they are safe compared to common manufactured chemical treatments. They may have genetically mutagenic properties, which makes these natural alternatives required as they contain effective molecules in treatment with the least chance of The development of resistance against it and its mutagenic effects on the host are few [17], because algae are a large and diverse group that produces many secondary metabolites that have vital efficacy as they are included in the field of medicine industry and in the treatment of many cancers, AIDS and arthritis, as well as antibacterial, fungal and viral [18], The type of extraction and the type of solvent used determines the success in isolating the bioactive compounds [19]. Therefore, water was used as an internationally known solvent and as the most hygienic solvent to prepare the extracts [20].

The results of the current study showed that the extract had a clear inhibitory effect, to varying degrees (according to the concentration) on the appearance of cysts in the feces of experimental animals, which was caused by the effect of the extract on the parasite in the intestine compared to the positive control group that persisted. The infection increased in it throughout the period of the experiment, and the qualitative disclosures of the algae showed that the presence of alkaloids in the extract showed its inhibitory ability to microorganisms through their interference in a series of protein metabolism reactions necessary for the continuation of its vitality and its ability to break down the cell wall and the proteins and fats it contains, and then its destruction [21].

Berberine sulphate was found to be highly effective against *E. histolytica*, *Giardia lamblia*, *Trichomonas vaginalis* by causing chromatin clumping in the vegetative phase of *E. histolytica* with autophagic vacuoles forming and small vacuoles in the cytoplasm [22]. It leads to the loss of the parasite's cell membrane to the permeability property and thus the entry and exit of substances to and from the parasite without regulation and then the death of the parasite. Its functional structure and then the death of the living cell [24], and the effect may be due to the presence of other active substances present in the algae, such as flavonoids that have the ability to denature proteins. And stopping the action of enzymes associated with the process of glycolysis, the most important of which is the enzyme Hexokinase, and thus the microorganism loses its ability to continue life [25].

As for the results of the chromatography technique, it showed the presence of a number of active chemical compounds in the algae ethanolic extract, and among these compounds that occupied the largest

area of the total area of the diagnosed compounds represented by the compound 6-Octadecenoic acid, (Z) - which occupied an area of (44.22%) which is It is one of the monounsaturated fatty acids and has a role in stimulating the immune system and improving its ability to combat infectious agents and infections and may be attributed to it the vital activity being the largest part of the total area [27,26], followed by the compound Pentadecanoic acid, which occupied an area of 30.27%) It is a carboxylic acid that has the ability to be soluble in organic solvents and does not dissolve in water.

As for 9,12-Octadecadienoic acid (Z, Z). It is called linoleic acid and one of the essential fatty acids that the body cannot manufacture. It is an unsaturated fatty acid that occupies an area of 14.79%, as studies conducted on humans indicated that this compound works as an anti-inflammatory and reduces oxidative stress and treating many diseases. [29,28].

Conclusion:

A marked anti- parasite activity of *Chlorella* extract was observed which may be attributed to the presence of bioactive compounds and other phytochemicals. The *Chlorella* can be used to control infectious diseases and prevent *Entamoeba histolytica* parasite.

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