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EFFECT OF GREEN ALGAE SPIRULINA EXTRACTION IN TREATMENT OF CRYPTOSPORIDIOSISI INFECTION IN EXPERIMENTAL INFECTED MICE

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Abstract

Cryptosporidiosis caused by Cryptosporidium spp. It is a zoonotic disease that is the most prevalent. Thus, the study was conducted to evaluate the anti-cryptosporidiosis efficacy of alcoholic and aqueous Spirulina algae extracts in comparison with azithromycin in laboratory mice. Stool samples were collected from patients attending in Al-Kut Hospital suffering from diarrhea from November 1, 2021 to February 29, 2022, and 124 samples were examined for both sexes microscopic by using the modified Ziehl-Nelson Stain to detect infected the parasite's oocysts , Isolation and purification by flotation with Scheither's sugar solution and preserved in potassium dichromate for the purpose of using it in causing infection in laboratory mice. The experimental study was conducted on groups of 57 mice by oral administration of parasite oocysts within the range of 104 oocyst / ml except for the negative control group that was drenched with physiological saline. The feces of mice inoculated with oocysts of parasites were microscopically examined using the modified Ziehl-Nelson Stain, as well as the molecular examination was conducted using Multiplex PCR technique.

After the mice were divided into five groups with the uninfected and untreated group kept as a healthy negative control. The first group which included 21 mice was treated after it was divided into three subgroups A, B, C for each secondary group 7 mice they were treated with alcoholic extract of Spirulina at different concentrations 50, 100, 150 mg/ml on the respectively, while the second group which included 21 mice on three groups A, B, and C was treated with aqueous extract of Spirulina at the previous concentrations for three consecutive days for each concentration. The third group was treated with azithromycin at a concentration of 500 ml, and the positive control group remained infected with the parasite and was not treated. After treatment a microscopic examination was performed by evaluating the excretion average of parasite oocysts using a hematocytometer slide.

There was a decrease in the shedding of fecal oocysts in the groups treated with alcoholic extract at different concentrations, reaching a complete stop when treating with the highest concentrations at the end of the treatment period. Where, the average of shedding of fecal parasite oocysts of groups of mice at concentrations 50, 100, 150 mg/ml reached 1.132, 487, 0 oocyst/ml respectively. The groups treated with the aqueous extract also showed a clear decrease in the shedding of fecal oocysts, as the average reached the three concentrations 50, 100, 150 mg/ml to 2.58, 1.45, 621 oocyst/ml respectively, while the avrage of shedding of oocysts in the group treated with

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azithromycin reached 0 oocyst/ml in comparison with the infected positive control group in which the number of oocysts continued to increase to 14.863 oocyst/ml at the end of the treatment period. The results of the therapeutic efficacy of spirulina alga extracts showed percentages of 92%, 97%, 100% for the alcoholic extract at concentrations 50, 100, 150 mg/ml, respectively, and percentages were recorded at 82%, 90%, 96% for the aqueous extract at concentrations 50, 100, 150 mg/ml, while the therapeutic efficacy of azithromycin reached 100% at the end of the treatment period.
Key words: Cryptosporidium spp., Spirulina Extract, Azithromycin

INTRODUCTION

Cryptosporidium spp. is a biological pathogen that infects many organs of the body and is a parasite that is endemic in the intestines of the host, and is widespread throughout the world more than 3000 million people are infected with one or more intestinal parasites during their lifetime (Al-Aboudi et al., 2015).Cryptosporidium spp. is one of the most common pathogens causing a disorder of autoimmune diarrhea in humans and animals, and may lead to death (Certad et al., 2017) especially in young children, the elderly, chronic disease and immunocompromised individuals, especially in Acquired Immunodeficiency Syndrome where they develop diarrhea Acute (Darlan et al., 2018). Humans and animals become infected when ingesting food and drink that contains the oicysts of this parasite due to food and water transmission, the incidence and prevalence of cryptosporidiosis is higher in developing and less developed countries where people do not have enough basic infrastructure or public facilities to avoid food and drinking water contaminated with infectious oocysts in their feces (Burnet et al., 2014). Oocytes also play a potential role in contributing to the spread of Cryptosporidium spp. because they tolerate many chemicals and disinfectants including chlorine that is commonly used in drinking water treatment, swimming pools and water gardens (Iqbal et al., 2019). Infection can be transmitted through single contact (Smith et al., 2021), milk (Karakavuk et al., 2021), infected animals and breathing (Ahmed and Karanis, 2018). Twenty-three species and 61 valid genetic species of Cryptosporidium spp. have been described from a wide variety of vertebrates including humans, mammals, fish, wildlife, domestic livestock, reptiles, birds, and amphibians and with causing asymptomatic or mild to severe gastrointestinal diseases in host species (Pumipuntu and Piratae, 2018). There are two types of Cryptosporidium spp. in humans, Cryptosporidium parvum and Cryptosporidium-like, which are responsible for more than 90% of Cryptosporidium spp. in humans (Xu et al., 2019).

Effective treatment for cryptosporidiosis has not already been demonstrated. Although more than 200 chemotherapeutic agents have evaluated their anti-Cryptosporidium spp. effects, the only FDA-approved drug therapy is nitazoxanide, and clinical studies (Rosinol et al., 2001). Those who suffer from diarrhea as a result of infection with cryptosporidiosis, there is a danger to their lives when they are treated with this medicine, and they need better treatment, especially those who suffer from other chronic diseases that lead to their immune deficiency, As it was found that the toxicity and side effects of these treatments persist even after modification the recommended dose and duration of treatment as (MNZ) has been shown to be a toxic substance that has an effect on living organisms (Ighalo et al., 2020).

Investigations to find an effective drug to completely eradicate Cryptosporidiosis are still ongoing and are much needed. The use of natural products, including algae extracts, for treatment is preferred instead of synthetic chemical drugs, the fact that natural medicinal extracts are cost-effective and have no side effects has made them favorable. In recent years, several medicinal formulations of algae with anti-parasitic activity have been identified, and suggested that it is safe and effective in the treatment and control of cryptosporidiosis (Jin et al., 2019).

Spirulina algae is one of the algae authorized to be eaten safely by the Food and Drug Administration, and therefore can be used as a food and drug without risks or side effects to human health (Pall and Bose, 2022). Spirulina is a major source of biological compounds that show potential as anti-cancer, anti-diabetic, anti-obesity, anti-hypertensive, anti-hyperlipidic, anti-coagulant, anti-inflammatory, anti-estrogen, thyroid-stimulating, anti-coagulant, and anti-inflammatory drug, antiviral and antibacterial (Khaled et al., 2018). It has also been used therapeutically against parasitic diseases, as a therapeutic study revealed that Spirulina isolate and control nematodes (Hamouda et al., 2019) and had an antibacterial effect on the parasite Schistosoma mansoni (Al-Otaibi et al., 2021) and malaria (Wulandari et al., 2018). Algae contain many physiologically important chemical compounds such as polypeptides, terpenes and polysaccharides (Kini et al., 2020). Spirulina also contains volatile compounds and, due to its biological activity, has been identified as phenols (Machado et al., 2019), alkaloids , unsaturated aliphatic alcohols, aldehydes, quinones, sulfur esters, terpene sulfides, acids Fatty, brominated hydroquinones and phycotin, VOCs provide anti-therapeutic efficacy (Ghirga et al., 2021) due to their content of sterols, including phytosterol,brassicasterol, ergostenolsterol, purevasterol, clinic acid which have a significant primary and physiological protective effect in it.

There are no realistic data reported regarding the effect of anti-cryptosporidium spp.spirulina extracts in vivo, among which laboratory animals, albino rats are the best candidate for experimental infection (Muawad et al., 2021) with the Cryptosporidium spp. parasite. Thereforethe current study was conducted to evaluate the efficacy of alcoholic and aqueous extract of Spirulina compared to azithromycin as a drug against cryptosporidiosis in an experimental albino mice specimen.

Materials and Methods

Samples collection

124 stool samples were collected from patients admitted to Al-Kut Hospital suffering from diarrhea for both sexes, for the period from November 1, 2021 to February 29, 2022, and kept in potassium dichromate solution for the purpose of conducting examinations.

Microscopic Examination of Stool

For the detection of cryptosporidium spp. oocysts, each sample was examined by smears stained with a by modified Ziehl- Nelson stain according to the method (AL-Ezzy and Kadhim et al., 2021) in which a portion of feces the size of the tip of a match was taken and mixed on a clean glass slide and mixed with a drop of distilled water, then distribute it over the entire area of the slide and leave

it in the open air to dry , For 10 minutes without using a flame, the swab was fixed by adding drops of 11% methyl alcohol for 5 minutes and left to dry at room temperature, after which carbol red concentrated fuchsin dye was added to the fixed swab and left for 3-5 minutes and passed over a quiet flame. The stain was washed off with a weak stream of tap water and left to air dry then shortened swab with acidified alcohol for 30 seconds, washed with tap water and left to dry. Then the swab was dyed with methylene blue dye for two minutes, washed with a weak stream of water, and dried. The stained specimens were examined with a light microscope under the objective lens of X40 and then the oil lens of X100 for the examination of Cryptosporidium spp. oocysts and to confirm its presence in the stool samples.

Isolation and purification of Cryptosporidum spp. oocysts

Parasite oocysts were isolated from stool samples preserved in potassium dichromate solution in the first stage by flotation using Scheither's sugar solution according to (Al-Dahhan and Zghair, 2020) where stool samples were washed three times using phosphate-buffered saline (PBS) centrifugation at IO rpm for 5 minutes, Each time the filtrate was poured out and the precipitate was shaken until the yellow color of the potassium dichromate solution was removed. Then add 10ml of precipitated sugar sheather solution and mix well and then centrifuge at 700 rpm for 20 minutes, this process is known as flotation because the cysts float in a highly concentrated sugar solution. Then collect the floating portion containing the oval sacs using a Pasteur pipette and dilute it with distilled water in a volume ratio of 1:10 to prevent the sugar solution from affecting the oocysts. Then the diluted solution was precipitated in a centrifuge at 700 rpm for 15 minutes, then the liquid was poured out, the precipitate was re-washed three times with distilled water at the same speed and time. Precipitates containing oocysts were collected in conical tubes and a solution of l% sodium hypochlorite was added to it in a volume equal to the volume of the precipitate, then distilled water was gradually added to the wall of the conical tube. The Qasr product was washed several times with distilled water by rapid disposal (700 rpm for 15 min) and isolation steps were carried out at 4°C to prevent oocysts from breaking. After each separation process, a drop of sediment is taken on the glass slide; the sliding cover is placed on it and examined under the microscope to ensure the presence of the oocysts of the parasite. Then the oocysts were counted for each millimeter of the suspension using the counting slide scale. Finally, it was used in experimental injury.

Cryptospordium spp. oocysts Counting

The number of cysts of the parasite that were used in animal doses was calculated using a hemocytometer slide and based on the method (Kawan, 2018) where a drop of iodine solution was placed as a dilution agent to suspend the pure cysts to stain them and make them more visible under the microscope. The counting slide was washed with distilled water and covered with a lidcover slide, then put a drop stuck in the counting chamber to spread under the cover with the diffusion characteristic and put it under the microscope and adjust the power of the lenses to get a

clear view, as the bags were counted in the eight corners on both sides of the slide according to the equation:

Number of oocysts in 1 ml

$$= \frac{\textbf{calculated oocysts number}}{\textbf{8}} \textbf{x}\ 1000$$ (Kadhim and Al-Zubaidi, 2018)

Experimental Infection Animals

The experimental study was conducted in vivo (white mice), which included (57) male mice aged (8-10) weeks and weighing (28-30) gm, divided into (5) groups and dosed with 104oocyst/ml for each mice by the oral dosing syringe (Yuddhakaran and Veeraseatakul, 2002) except for the negative control group, which was dosed with physiological saline only, The stool was examined daily using M.Z.N stain to ensure that the parasite oocysts shed, as infection was confirmed on the seventh day by 100%.

Microscopic Examination of Experimental Animals Feces

Stools of mice inoculated with parasite oocysts were examined microscopically with M.Z.N stain on a daily basis for parasite detection and experimental infection was achieved (Khan et al., 2018) in which a portion of the stool sample was placed on a clean glass slide and mixed with a drop of distilled water and distributed over the entire area of the slide Leave in the air for 10 minutes to dry, taking into account the numbering of stool. A few drops of 95% methanol were then placed for 1 minute for the purpose of fixation. Then Carbol Fusion red dye was added to the fixed slide and left for 15 minutes. The sample was then washed with distilled water and left to dry. Acid alcohol was added for the purpose of default and washed with tap water. Then the slide was stained with methylene blue for two minutes. The slide was washed with light water and left to dry. Examination was performed under 40X and 100X optical microscopy.

DNA Extraction Multiplex PCR

Fecal samples DNA was extracted using the Presto™ Stool DNA Extraction Kit and performed according to the company's instructions, all samples were treated with heat shock for 5 cycles and boiled in a water bath each for 5 min, then incubated at 56°C for 10 min, extended for 1 h. at 95°C. DNA was extracted and amplified by multiplex PCR targeting the heat shock protein 70 (hsp70) gene. The Multiplex PCR primers for detection Cryptosporidium parvum ,Cryptosporidium hominisbased on heat shock protein 70 (hsp70) gene were designed in this study using NCBI-Genbank (KM116517.1 and EF591787.1) and primer 3 plus design. These primers were provided from Scientific Resercher. Co.Ltd, Iraq as following Table (1).

Table 1. Primer sequences used in this study

Primers	Sequence 5'-3'		Product size
C. parvum hsp	F	GCTGTTGCTTATGGTGCTGC	625 bp

70 gene	R	CCTTGATCTTCTTCTCAGCCTCA	
C.hominis hsp 70 gene	F	TCTGCGCTGATTACTTCCGT	310 bp
	R	CCACCAGCAGTTTCTAAACCG	

Preparation of Aqueous and Ethanolic Extracts of Spirulina

A sample of Spirulina in powder form was obtained from Kuching Company, Sarawak, Malaysia. Where 100 grams of dry Spirulina powder were weighed and placed in a glass beaker, then 1000 ml of distilled water was added to it, then placed in an electric mixer for 15 minutes and then the mixture was left for 24 hours. The next day, the mixture was filtered using several layers of medical gauze and placed in a centrifuge at 300 rpm for 10 min. Then the solution was placed in clean and sterile metal dishes and dried in the oven at a temperature of 40 ° C, and the dried product was placed in sterile opaque vials and kept at a temperature of 4 ° C until use, and the ethanolic extract was placed, and prepared in the same way as an aqueous extract that replaces distilled water with ethanol. Prepared according to the method (El-Hamed et al., 2021).

Preparation of The Concentrations Used in The Study (50%, 100%, 150% mg/L) of Raw Spirulina Extract

The different concentrations used in the study were prepared from the crude Spirulina extract, according to the method (Okechukwu et al., 2019), three concentrations (50%, 100%, 150% mg) mg/ml were prepared according to the following equation:

$$\text{concentration} = \frac{\boldsymbol{wt\ (mg)}}{\boldsymbol{v\ (ml)}} \times 100$$

Wt (mg) = Extract weight

V (ml) = distilled water or ethanol.

Azithromycin

Azithromycin (500 mg/5 ml syrup, Iraq. Al Kut) was purchased and used as the control standard drug.

Evaluation of therapeutic efficacy

The therapeutic efficacy of the aqueous and ethanolic extract of Spirulina and azithromycin was calculated according to (Ganai et al., 2021) according to the following equation:

$$\text{Therapeutic efficacy} = \frac{\text{The average number of oocysts in the control group} - \text{The average number of oocysts in the treatment group}}{\text{The average number of in the c oocysts ontrol group}} \times 100$$

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Statistical Analysis

Statistical significance was determined by entering the obtained data into a computer database, the Statistical Package for Social Sciences (SPSS) program was used for statistical analysis, data were recorded in numbers and percentages, numbers were compared using chi-square test, and $P \leq 0.05$ was considered significant (Nuchjangreed, 2018; Gharban, 2022).

RESULTS

Detection of Cryptospordium spp. by Using Modified Ziehl-Nelson Stain

As expected, the results of microscopic examination of 124 stool samples from the patients showed that Cryptosporidium spp. oocysts were spherical in shape, red in color with a blue background, containing four spores , using an oil lens after staining it with a modified Ziehl Nelson stain as shown in Figure (1)

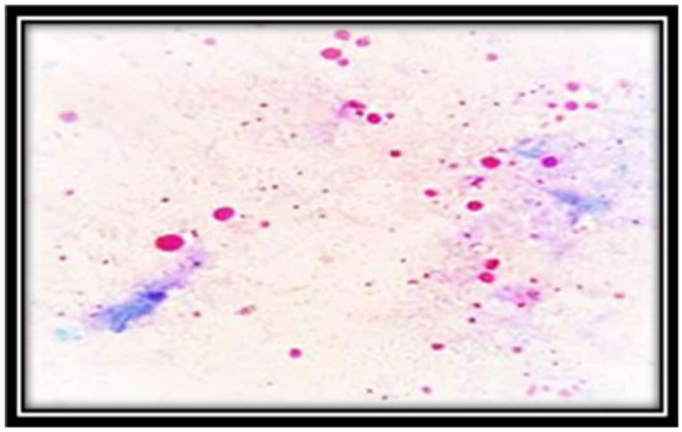


Figure (3.1): Oocyst of Cryptosporidium spp. stained by modified Ziehl Nelson stain with magnification 40X

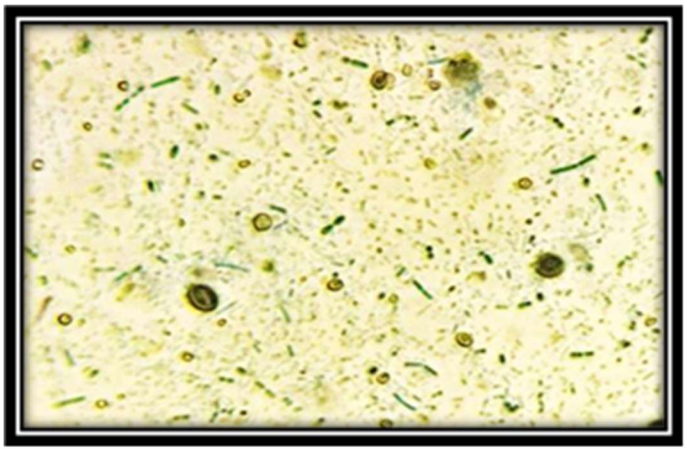
The result in Table (2) showed that the percentage of those infected with the disease amounted to 53.2% as the number of positive samples reached (66) samples.

Table 2) distribution of Cryptosporidium spp. according to microscopic examination

Microscope examination of *Cryptisporidium spp.*	**Results**	
	No.	**%**
Specimen Positive for *Cryptosporidium* spp.	66	53.2 %
Specimen Negative for *Cryptosporidium* spp.	58	46.8 %
Total	124	100

Isolation and purification of Cryptosporidium spp. oocyst by using floatation method

The distinctive morphological shape of Cryptosporidium spp. oocysts was observed, as the ovules appeared in a circular to oval shape of green color surrounded by a thin membrane and containing uncharacteristic spores as in Figure (2).



Figure(2)Cryptosporidium spp. oocyst of Isolated by using floatation method 100X

Experimental Infection

After using the oral dosing method to induce experimental infection in groups of mice, the current study showed that a dose of 104 oocyst/ml of the parasite severely infected mice within 7 days.

Experimental Infection Detection of Cryptosporidium spp. oocysts in Experimental Animals by Using Modified Ziehl-Nelson Stain

The results of the examination of (52) stool samples isolated from mice after conducting the experimental infection by M.Z.N stain showed that Cryptosporidium spp. oocysts are spherical in shape with red to pink color with a blue background.

Detection of C. Parvum and C. hominis in Experimental animals by Using Multiplex PCR Technique

The PCR multiplex results are shown in Figure 4, where amplification of the (hsp70) gene yielded a clear (2000–100) bp range, confirming the presence of C. parvum and C. hominis in stool samples from lab mice infected with the parasite Cryptosporidium spp.

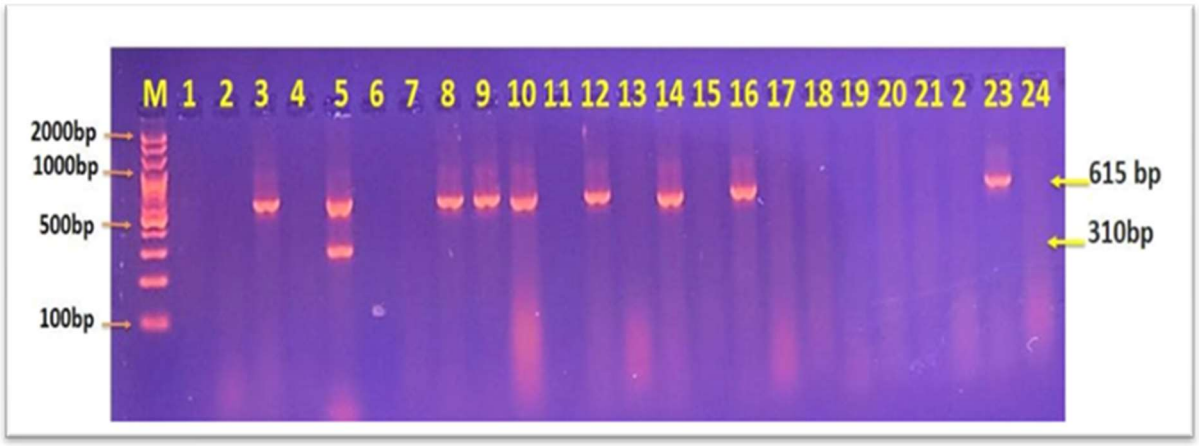


Figure (4): Agarose gel electrophoresis image that showed the Multiplex PCR product analysis of hsp70 gene in C. parvum and C. hominis from Rats feces samples. Where, the Lane (M): DNA marker ladder (2000-100 bp) and the Lane (1-24) were showed some positive hsp70 gene in C. parvum at 615 bp and C. hominis at 310 bp PCR product size.

This study included the examination of (52) stool samples taken from mice inoculated with the parasite. The result using Multiplex PCR technology in Table (5) showed that the infection rate was 38.4% with a distribution rate of (20) samples.

Table (5): Confirmation percentage of infection with Cryptosporidium spp. according to Multiplex PCR Technique

Multiplex PCR for *Cryptosporidium Spp*.	**Results**	
	.No	**%**
Specimen Positive for *Cryptosporidium Spp*.	20	38.4 %
Specimen Negative for *Cryptosporidium Spp*.	32	61.5 %
Total	52	100 %
Chi-Square P Value at p≤0.05	0.144	

Evaluation of the Effect of Spirulina Extracts Compared with Azithromycin on Cryptosporidium in Vivo (white mice)

The results of the therapeutic study with alcoholic Spirulina extract showed a decrease in the excretion means of parasite oocysts starting from the first day of treatment to the twentieth day of the treatment period that reached the final cessation of oocyst shedding at the highest concentration of 150 mg/ml at a mean of 0 oocyst/ml, while the concentrations of 50 and 100 mg/ml recorded a very low mean of 1.132, 487 oocyst/ml respectively, compared to the group treated with azithromycin with a decrease that reached to stop at a mean of 0 oocyst/ml, the positive control group continued to shed oocysts in the last day at a very high mean of 14,863 oocyst/ml compared to the negative control group as shown in Table (6).

Table (6) Effect of alcoholic extract of Spirulina compared to azithromycin on infection with parasite C. parvum, C. hominis

		Average number of oocysts /ml during the treatment period						
Groups	**Concentration Used mg/ml**	**Immediately after confirming the injury**	**3**	**6**	**10**	**13**	**17**	**20**
Treatment with alcoholic *spirulina* extract	**50 %**	**11.417**	**10.648**	**.785**	**7. 821**	**5. 178**	**2.442**	**1. 132**
	P Value* 0.144 **1							
	100 %	**11. 231**	**10. 185**	**.648**	**5.455**	**3.114**	**1. 692**	**487**
	P Value*0.688 **0.465							
	150 %	**10.183**	**9.655**	**6.387**	**4. 976**	**2.331**	**842**	**0**
	P Value*0.055 **0. 583							
Azithromycin drug-treated mg/ml 500		**11. 539**	**10. 776**	**8.645**	**5.183**	**2.34**	**301**	**0**
positive control		**11.223**	**11.624**	**12. 996**	**13.263**	**13. 923**	**14.697**	**14.863**
Negative control		**0**	**0**	**0**	**0**	**0**	**0**	**0**

* Represents the value of the statistical significance of the concentration of the extract compared with azithromycin at level p≤0.05

** Represents the value of the statistical significance of the concentration of the extract compared with control group at level p≤0.05

The results of the therapeutic study of Spirulina aqueous extract on the last day of the treated groups showed a decrease in the shedding of oocysts in the treated group at a concentration of 150 and 100 mg/ml, which amounted to 621 and 1.45 oocyst/ml, respectively and the highest mean at concentration 50 reached 2.58 oocyst/ml compared to the group treated with azithromycin in which oocytes shedding stopped it reached a mean of 0 oocyst/ml, while the positive control group reached its peak in shedding oocysts at a mean of 14 .863 oocyst/ml as shown in Table (7).

Table (7) Effect of aqueous extract of spirulina compared with azithromycin on infection with C. parvum C. hominis

		Average number of oocysts /ml during the treatment period						
Groups	**Concentration Used mg/ml**	**Immediately after confirming the injury**	**3**	**6**	**10**	**13**	**17**	**20**

Treatment with aqueous *spirulina* extract	**50 %**	**10.417**	**10. 148**	**9. 585**	**8. 821**	**6. 178**	**4.418**	**2.58**
		P Value *0.189 **1						
	100 %	**12. 231**	**11. 185**	**10. 787**	**8. 267**	**5 .114**	**3. 213**	**1. 45**
		P Value* 0.204 **1						
	150 %	**13. 107**	**11.325**	**9.798**	**7. 421**	**4. 176**	**2.442**	**621**
		P Value *0.676 **0. 465						
Azithromycin drug-treated mg/ml 500		**11.539**	**10.776**	**8.645**	**5.183**	**2.34**	**301**	**0**
positive control		**11.223**	**11.624**	**12. 996**	**13. 263**	**13. 923**	**14.697**	**1 4.863**
		P Value 0.538						
Negative control		**0**	**0**	**0**	**0**	**0**	**0**	**0**

* Represents the value of the statistical significance of the concentration of the extract compared with azithromycinat level $p \leq 0.05$

** Represents the value of the statistical significance of the concentration of the extract compared with control group at level $p \leq 0.05$

Therapeutic Efficacy of Alcoholic and Aqueous Spirulina Extract and Azithromycin against Cryptosporidiosis

After applying the therapeutic efficacy equation on the third day of treatment, the highest percentages of therapeutic efficacy were recorded for the alcoholic extract with the highest concentration of it , Where the efficiency of the alcoholic extract concentrations reached 50, 100, 150, azithromycin mg/ml different percentages reached 8%, 12%, 16%, and 7% respectively until today the last day of treatment the highest therapeutic efficacy Percentages were recorded for concentrations 50, 100, 150 and azithromycin, reaching 92%, 97%, 100%, 100%, respectively as shown in Table (8).

Table (8) Therapeutic efficacy percentages of alcoholic Spirulina extract compared with azithromycin against cryptosporidiosis

Treatment days	**The therapeutic efficacy percentages of alcoholic *spirulina* extract**			**Azithromycin**
	50 % mg/ml	**100 %** mg/ml	**150 %** mg/ml	**500 %** mg/ml
3	**8%**	**12%**	**16%**	**7%**
6	**24%**	**33%**	**50%**	**33%**
10	**41%**	**58%**	**62%**	**60%**
13	**62%**	**77%**	**83%**	**83%**
17	**83%**	**88%**	**94%**	**97%**

20	**92%**	**97%**	**100%**	**100%**
P Value at p≤0.05	0.152	0.207	0.061	

The results after applying the therapeutic efficacy equation at the beginning of the treatment period showed a slight superiority of the aqueous extract over the drug azithromycin ,where varying percentages were recorded for each of 50, 100, 150, azithromycin mg/ml amounted to 12%, 3%, 2%, 7% respectively until today twentieth day of treatment, the study recorded increase in the therapeutic efficacy Percentages for all concentrations with a marked superiority of azithromycin and the highest concentration of the aqueous extract at percentages of 82%, 90%, 96%, 100% for each of the concentrations 50, 100, 150, and azithromycin respectively as in the Table (9).

Table (9) Therapeutic efficacy percentages of aqueous Spirulina extract compared with azithromycin against cryptosporidiosis

Treatment days	**The therapeutic efficacy percentagesof aqueous *Spirulina* extract**			**Azithromycin**
	50 % mg/ml	**100 %** mg/ml	**150 %** mg/ml	**500 %** mg/ml
3	**12%**	**3%**	**2%**	**7%**
6	**26%**	**16%**	**24%**	**33%**
10	**33%**	**37%**	**44%**	**60%**
13	**55%**	**63%**	**70%**	**83%**
17	**71%**	**78%**	**83%**	**97%**
20	**82%**	**90%**	**96%**	**100%**
P Value at p≤0.05	0.215	0.161	0.152	

DISCUSSION

Regarding detection of Cryptospordium spp. by using modified ziehl-nelson stain from the patients the current study agreed with the results obtained by Rahi and Alwan (2021) which recorded 60% in the children of Wasit governorate, where 100 samples of both sexes with diarrhea were collected using M.Z.N. In Najaf Sayal (2019) 50 stool samples were collected for people with diarrhea, where 58% of the positive cases were recorded with Cryptosporidium spp. using M.Z.N distributed between males and females with ages above 11 years, 55.2 % and 44.8 % respectively. The percentages of the current study are higher than Alkhanaq and Thamer (2022) which recorded 40.4 % for both sexes and for different ages, where 109 samples were collected using M.Z.N in Wasit city. As well as Al-Saeed et al. (2020) in the city of Dohuk in the Kurdistan region, which collected 122 random samples of different ages, where it recorded 22.2 % in a group of patients of both sexes with immune competence and 44 % in patients with immunodeficiency using M.Z.N.

As for the detection of C. Parvum and C. hominis in expermintal animals by using multiplex pcr techniqul the current study agreed with the results obtained by Galán-Puchades et al. (2021) which recorded 37% in feces of 100 mice using Multiplex PCR in Spain for molecular investigation of intestinal parasites, including Cryptosporidium spp. The current study was higher than Horcickova

et al. (2019), which amounted to 22% during the examination of 19 positive stool samples of 74 male and female mice of origin by multiplex pcr in the Republic of El Jik.

The apparent decrease in the shedding average of parasite oocysts with increasing concentration could be explained by the chemical content of alcoholic Spirulina extract which could be attributed to this effect due to its high content of long chain unsaturated fatty acids (Li et al., 2019). In addition the alcoholic extract of Spirulina contains phenolic compounds which are considered one of the most important classes of natural antioxidants, and include medicinal compounds produced by these microalgae, such as caffeic acid, ferulic acid, pcoumaric acid (Kapoor et al., 2021). The halogenated compounds in Spirulina have been shown to be organic chlorides and are among the most widely used organohalides in the pharmaceutical industry , activity is due to the presence of fatty acids, acrylic acid, aliphatic halogens, terpenes and other cyclic compounds of antimicrobial nature (Mohammed et al., 2021). The mechanism of the great inhibitory effect of alcoholic Spirulina extract to varying degrees on the shedding of parasite oocysts can be explained by its effect on the vegetative stages of the intestine, and the inhibitory mechanism may be attributed to its ability to weaken the enzymatic system of the parasite including enzymes involved in energy production as well as its effect on the integrity and structure of the parasite membrane (Kusmardi et al., 2022).

The effect of the aqueous extract of Spirulina may be due to the presence of alkaloids, which have a great inhibition capacity on microorganisms through their interference in the metabolic chain reactions of proteins necessary to maintain their vitality, as well as their ability to destroy the cell wall and its contents of proteins and fats (Kata et al., 2018). Where the alkaloids showed high efficacy against the parasites E. hstolytica, T. vaginalis, Giardia lamblia, as it causes chromatin clumping in the nucleus with the formation of self-vacuoles and small vacuoles that collect in the cytoplasm (Akbari et al., 2018). The phenolic compounds led to the parasite's cell membrane losing its selective permeability and consequently the unregulated entry and exit of substances the parasite and then the parasite's death (Karampetsou et al., 2021). And the phenolic compounds in algal extracts have the ability to bind with lipids found in the cell membrane and the membranes of cellular organelles and direct their functional structure and then the death of the living cell (Martins et al., 2022). The flavonoids which have the ability to denature proteins of denature and stop the action of enzymes accompanying the glycolysis process, the most important of which is Hexokinase, thus losing the microorganism's ability to continue life (Ayal and Wahab, 2021) .The amino acids that are essential in building cell membranes, which are important in the continuation of microorganisms or because of their ability to unite with proteins which leads to the occurrence of trends in the chemical characteristics of the cell wall or the shape of the entire cell and death (Torres et al., 2021).

The reason for the efficiency of the alcoholic extract of Spirulina can be attributed to the ability of ethanol to dissolve alkaloids better than water (Takla, 2018). This is due to the active secondary metabolites in many therapeutic applications dissolved in ethanolic extracts of different types of algae against parasites including Cryptosporidium spp. (Saikia et al., 2020). In addition to its content of phenolic compounds, volatile compounds, sterols, proteins, amino acids and peptides

(Proestos et al., 2018). The reason for the therapeutic efficiency of the aqueous Spirulina extract is due to the presence of therapeutically active compounds against microbes the most important of which are alkaloids, flavonoids, glycosides, phenols, sterols, tannins, anthraquinones and volatile oils (Mane et al., 2019). These compounds have an inhibitory ability as a result of their interference in the chain of protein metabolism reactions and their ability to destroy cell wall, proteins and lipids, and then destroy them (Hamad, 2021). Also, the difference in the Percentages of therapeutic efficacy of Spirulina extracts against parasites is due to the nature of the compounds extracted from each solvent (Karthikaidevie et al., 2009).

Conclusions

The alcoholic and aqueous extract of Spirulina algae showed an effective and safe therapeutic effect for cryptosporidiosis, and the alcoholic extract had a greater effect than the aqueous extract. Increasing the therapeutic efficacy of the alcoholic and aqueous extract of Spirulina algae by increasing the concentration, as high concentrations showed greater therapeutic efficacy than low concentrations of both extracts. The extracts of Spirulina algae have an important effect on the parasite, and when compared with the drug azithromycin it was found that the alcoholic extract of Spirulina algae with the highest concentration it has a therapeutic efficacy comparable to that of azithromycin, while the efficiency of the aqueous extract of Spirulina algae less effective than efficacy of azithromycin.

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