

Therapeutic Comparison Between Alcoholic and Aqueous Plant Extract of Tannins with Metronidazole in Experimentally Infected Laboratory Mice *Cryptosporidium parvum* Oocysts

Luma Abdullatef S.N.¹ and Firas M.B. Al-Khashab²

¹Department of Biology, Collage of Education for Girls, University of Mosul, IRAQ.

²Department of Biology, Collage of Education for Girls, University of Mosul, IRAQ.

¹Corresponding Author: luma.20gep58@student.uomosul.edu.iq



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ABSTRACT

The current study was conducted during the period between from the beginning of October 2021 until the end of March 2022. The aim of the study was to measure the therapeutic efficacy of alcoholic and aqueous extracts from the local tannins *Cupressus sempervirens* for the treatment of experimental infected laboratory mice with Cryptosporidiosis.

Through a significant decrease in the number of Oocyst of the parasite that causes infection *Cryptosporidium parvum* after oral administration of the aqueous and alcoholic extracts with three concentrations (2, 1.3, 1) mg/ml compared to MTZ drug, as the alcoholic extract proved its efficacy by stopping the shedding of parasite Oocyst at the ninth day of treatment with its total absence on the eleventh day of treatment, while the shedding of parasite Oocysts in the group treated with aqueous extract stopped on the eleventh day and completely absent on the thirteenth day of infection.

Keywords- *Cryptosporidium parvum*, *Cupressus sempervirens*, alcohol extract, aqueous extract.

I. INTRODUCTION

Cryptosporidium parvum (*C. Parvum*) is a pathogenic protozoan parasite belonging to the phylum Apicomplexan that causes Cryptosporidiosis characterized by severe watery diarrhea[1]. It is a parasite that infects intestinal epithelial cells and causes malabsorption leading to severe diarrhea. It is considered the second leading cause of diarrheal diseases transmitted by water and food worldwide and ranks second after rotavirus [2]

Due to the breadth of the host and environmental persistence of this parasite, 44 species have been identified.

Among these species, *Cryptosporidium hominis* and *Cryptosporidium parvum* is responsible for most human infections, [3] and is the main cause of death for children with diarrhea, as in 2016 an estimated 48,000 deaths were recorded due to acute infection[4][5]

The immunity of the host body, whether it is natural immunity or acquired immunity, has a significant impact on the severity of the disease and its prognosis, as the amount of pathogenicity depends on the immune status of the host [6]as individuals usually suffer Those with normal immunocompetence may present with diarrhea, transient gastroenteritis for up to two weeks, accompanied by a slight rise in temperature, intestinal colic, nausea and vomiting, and then spontaneously clears, indicating an effective immune response to the host parasites.

As for the treatment of *C. parvum* infection, it does not have an effective treatment directly, except for (NTZ) Nitazoxanide which is proven by the World Health Organization, but it is not effective for people with immunodeficiency, and many anti-Cryptosporidiosis compounds have been used, but they did not give positive results. Effective and did not kill the Oocyst permanently in a record time, including Metronidazole, Azithromycin, Paromomycin [7]

From recent studies, Nullscript was identified as a compound that has a growth inhibitory effect, and was less toxic to host cells, Nullscript was able to significantly reduce the release of Oocyst in mice infected with *C. parvum* [8]

Pathogenicity

Intestinal diseases caused by microorganisms, including protozoa parasites, are of public health importance, with a child mortality rate of 8-10% worldwide. The epithelial layer of the intestine represents an important site for nutrient absorption and a Barrier barrier against microorganisms. However, due to its exposure to virus invasion Parasites and bacteria that infect the digestive system can disrupt the function of this barrier. Changing the normal state of the intestinal epithelium is one of the determinants of the outcome of the disease[9]. This barrier consists of a single layer of epithelial cells. The cells are fixed within the barrier by means of junctions. Intracellular and as a result of the pathological condition resulting from infection with *C. parvum* parasite, this leads to the production of large amounts of signals such as phosphoinositide 3-kinase (PI3K), Proto-oncogene tyrosine-protein kinase (Src), cell division control protein 42 homolog (Cdc42).) and GTPase stimulating the process of epithelial cell division and its peak presence is in the area of interaction of the parasite with the cells of the host body, which leads to the formation of large numbers of epithelial cells causing the formation of tumors Or if studies showed a close relationship between cryptosporidiosis and gastrointestinal cancer [10], where China reported a significant association between colorectal cancer and cryptosporidiosis[11]

The severity of infection varies from one organism to another according to several factors, including those related to the host in terms of type, body immunity, age, and the environment in which it lives, as the environment helps the growth and continued survival of the parasite[12] either Concerning the parasite, it depends on the parasite's ability to cause infection and its ability to settle within the tissues of the host's body and secrete intestinal toxins, as it has the ability to secrete toxic genes, making it one of the most virulent sporozoites.

Cupressus sempervirens

Cupressus sempervirens (*C.sempervirens*) is one of the trees of the coniferous family that has an ecological, physical and aesthetic role at the same time. It is a member of the family Cupressaceae, which is represented by 25 species divided into three main groups, including Mediterranean trees, Asian cypresses and North American trees.

Mediterranean trees of *Cupressus sempervirens* L., *Cupressus atlantica* Gaussen and *Cupressus dupreziana* A. Camus[13]

Cypress trees are generally represented by medium-sized trees and shrubs with a height of 30-40 meters, and *Cupressus sempervirens* In particular, its

subspecies differ in terms of variety according to the type of branching and branching of trees, to include *C. horizontalis*, , *C. pyrmedalis*

It is the type on which the current study was carried out, in addition to the fact that the horizontal type contains larger pollen grains and a thicker wall than the vertical type[14]

Active Compounds in Cypress

Chemical analyzes revealed the primary components of the plant, as it contains major components represented by saponins 1.9% - alkaloids 0.7% - flavonoids 0.22% - tannins 0.31% and phenolic compounds 0.067% in addition to essential oils[15] .

II. MATERIAL AND METHOD

Samples collection

(220) faeces samples were collected from children under the age of 5 years of both sexes who suffer from intestinal colic and persistent diarrhea, who have been admitted to Ibn Al-Atheer Hospital, Mosul General Hospital and Al-Salam Hospital in Nineveh Governorate in the period from the beginning of October 2021 - At the end of March 2022, the families of the children were given clean plastic bottles, on which were recorded the sample data (date of sample collection, gender, age, area of residence).

The first group: samples were preserved in a potassium dichromate solution at a concentration of 2.5%. Two volumes of the solution were added to each volume of the fecal sample, and the samples were kept in a refrigerator at a temperature of 4 C. [16]

The second group: samples kept in the refrigerator without any addition at a temperature of 4 ° C until the isolation process for the parasite.

Examination of stool samples

The stool samples were examined after taking the sample using the Modified Zeihl Neelsen Stain - or the so-called Acid fast stain [16] and the steps shown in Figure (1).



Figure 1: Scheme showing the dyeing with the modified Zihle Nelseen

Laboratory animals

The laboratory mice used Swiss Albino Mice, Balb-C strain, as they were dosed with a suspension containing *Cryptosporidium parvum* cysts in order to induce the experimental infection. 120 male mice of 1-2 months of age, weighing between 20-30 g, were used. Mice were obtained and bred in the animal house of the College of Veterinary Medicine / University of Mosul, where the room was equipped with drawers, lighting, and means of cooling and heating, as the temperature was recorded between 20-30 degrees Celsius, and the mice were placed in special cages with 11 plastic cages provided With an iron cover that contains a place to put the feed and a place to put a special water bottle containing a thin tube at the end of it to drink water, the feed ration consists of 10% animal protein, 20% soybean meal, 45% yellow corn fodder, 24% wheat bran, 0.5% lime 0.5% table salt, and rat stool samples were examined before use to ensure that they were free of any other parasitic infestation.

Determination of the lethal dose of the parasite *Cryptosporidium parvum*

By dosing mice with different amounts of the suspension containing parasite Oocyst, the lethal dose was known, as 6 mice were used, dosed with a quantity of (0.25 - 0.50 - 0.75 - 1 -1.5 - 2) ml respectively, and the condition of mice was followed up after infection, and it was determined the lethal dose is 1.5 ml of a suspension of parasite Oocyst.

Collection of plants used for study

The nut of the local tannins *Cupressus sempervirens* was used in the current study

III. RESULTS AND DISCUSSION

Examination and diagnosis of oocyst isolated from humans

The results of microscopic examination of faeces samples collected from children under 5 years of age and of both sexes using Modified Ziehl Nelsen dye showed that the Oocyst of *C. parvum* appear spherical to oval with red to pink color containing four elongated sporozoit surrounded by A distinctive transparent halo as in the figure (2) The results came in the results with what was also found by [17][18] [19] that the parasite Oocyst are spherical in shape, red and pink, surrounded by transparent.

Determination of the lethal median dose of *C. parvum* ovarian cysts

6 mice were used and by giving fixed doses in increasing volume (0.25 ml) for each time, the results showed that the dose of 1.5 ml and the number of oocyst as (15×10^3) was a lethal dose for mice within 24 hours

Diagnosis of the active substances in the tannins plant

The results of chemical analysis of alcoholic and aqueous extracts figure(4,5) of tannins using high-performance chromatography (HPLC) technique showed that the plant contains 5 compounds of the active

substances that are considered phenolic compounds [15], which are (Gallic acid, Tannic acid, Ferulic acid, Hydrobenzoic acid, Chlorogenic acid). In both alcoholic and aqueous extract with a difference in the concentration of the active substances that were calculated according to the Behbahani equation [20]

The results of the therapeutic study of alcoholic and aqueous extracts of tannins showed their efficiency in treating Cryptosporidia infection resulting from the experimental infection of laboratory mice with *C. parvum* sacs. The extracts worked to stop the oocyst put out.

Healing cases appeared in the group treated with alcoholic extract at a concentration of 2 mg/ml on the ninth day of treatment and thus it was therapeutically superior to in the group treated with metronidazole at a concentration of 2 mg/ml, in which cysts were stopped on the eleventh day of infection, while the group treated with aqueous extract at a concentration of 2 mg/ml, cysts were no longer excreted on the eleventh day of treatment.

The results we have reached through the therapeutic study with plant extracts motivate us to a promising future and encourage more laboratory experiments in vivo, especially as we are facing great progress in the field of plant extracts and the use of medicinal plants in treatment, and support the optimal exploitation of cypress trees rich in substances The potent properties it possesses, which has a clear pharmaceutical role in fighting many diseases.

Histopathological effects

The mucosal layer of the lining of the small intestine is the most important site for the parasite *C. parvum* to invade the host cell in humans. The degree of infection of the intestine itself varies, as it was found that the middle part of the small intestine is more affected than the rest of the parts.

Among the histological tomographic examination of intestine samples in laboratory mice experimentally infected with *C. parvum* parasite in the positive control group, which showed the effect of the parasite on intestinal tissues, which was severe necrosis and desquamation of epithelial cells in the tops of the intestinal villi with the observation of mucous degeneration and mucositis in the intestine Infiltration of inflammatory cells and the presence of parasite egg cysts inside the villi of the intestinal glands.

While the histopathological tomography of the intestine samples in laboratory mice belonging to the negative control group showed the normal structure of the intestinal tissue represented by the presence of the villi in its normal shape and the thin lamina pallidum, no pathological changes appeared.

The group treated with the Alcohol extract at a concentration of 2 mg/ml showed an improvement in histological picture compared to the rest of the treatments on the ninth day of treatment, as it showed the beginning of the formation of new villi to compensate

for the lost villi due to the influence of the parasite. The group treated with aqueous extract at a concentration of 2 mg/ml showed a slight improvement in the tissue layer and the development of the intestinal glands on the eleventh day of treatment compared to the group treated with Metronidazole (MTZ) drug, which showed a delay in the state of recovery. Property MTZ the eleventh day of treatment in comparison with the group treated with MTZ drug, which showed a delay in the state of recovery and the following figures show the histological effects in the intestine of groups treated with alcohol extract, aqueous extract and MTZ drug and addition to the negative control group (without infection) and the positive control group (infected without treatment).

Figure (6) The normal structure of the intestinal tissue is represented by the villi (A), intestinal glands (B), and the epithelial tissue lining the intestines(C). (Hematoxylin and eosin stain 40x).

Figure (7) A cross-section of the intestines of mice of the positive control group infected with *C. parvum* Oocyst, in which severe necrosis and desquamation of the tops of the intestinal villi(A) and intense infiltration of inflammatory cells are observed with the presence of Oocyst of parasite inside the villi

and intestinal glands(B). (Hematoxylin and eosin stain 10x and 40x).

Figure (8) A cross section of the intestines of mice from the group infected with *C. parvum* Oocyst and treated with alcoholic extract of tannins, in which hyperplasia of the epithelial cells lining the villi and intestinal glands(A) was observed with thickening of the muscular layer and the epithelial lamina and infiltration of inflammatory cells(B) (hematoxylin and eosin stain 40X).

Figure (9) A cross-section of the intestines of mice from the group infected with *C. parvum* Oocyst and treated with aqueous extract of tannins, in which hyperplasia of the epithelial cells lining the villi and intestinal glands(A), infiltration of inflammatory cells and congestion in the blood vessels(B) (Hematoxylin and Eosin stain, 10X and 40X)

Figure (10) A cross-section of the intestines of a Mice from the group infected with Oocyst of parasite *C. parvum* and treated with MTZ drug, in which necrosis of epithelial cells lining the villi and intestinal glands(A) and the proliferation of goblet cells(B) (hematoxylin and eosin stain 40X).

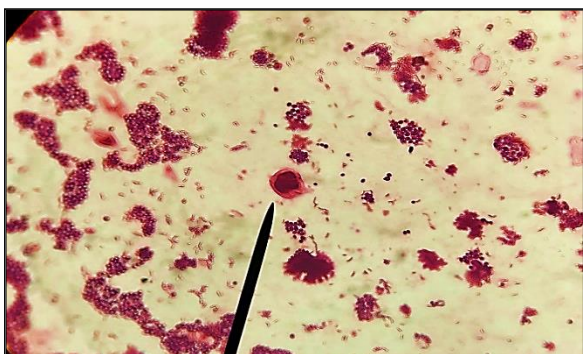


Figure 2: Oocyst *C. parvum*



Figure 3: Laboratory mice



Figure 4: Crude aqueous extract of tannins



Figure 5: Crude alcoholic extract of tannins

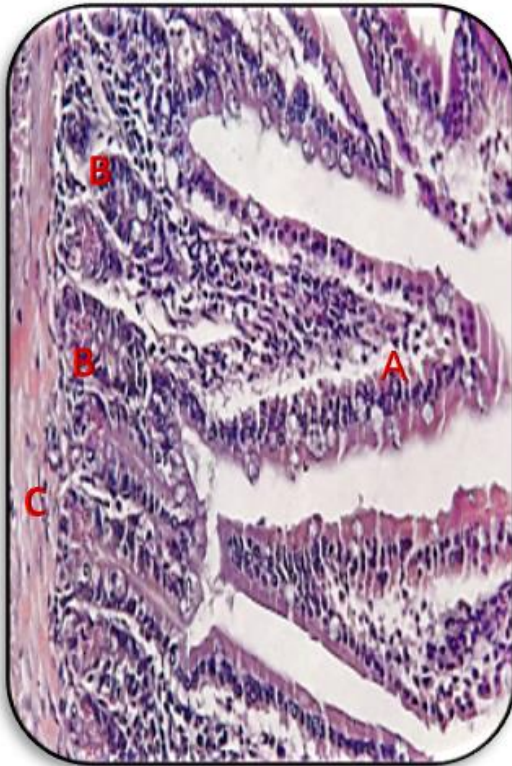


Figure 6: A section of the intestine of a mouse showing the normal structure of the intestine from the negative control group

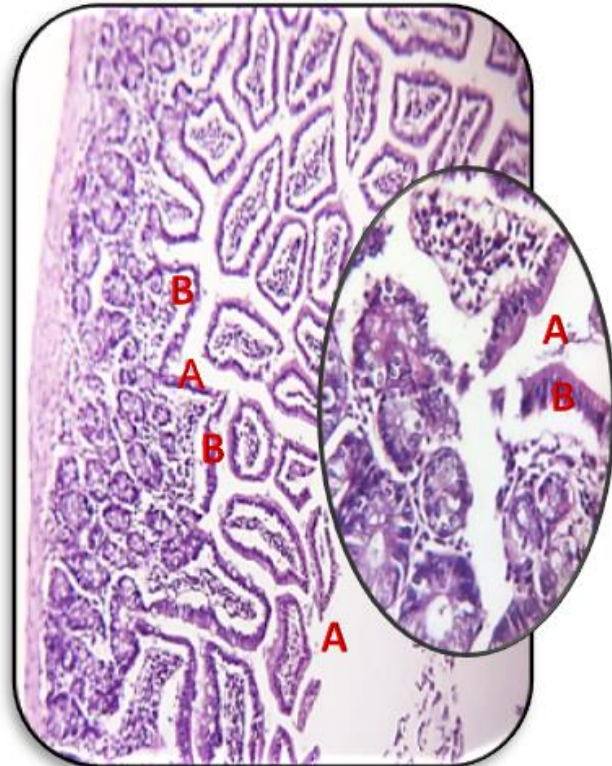


Figure 7: A cross section of the intestine of a mouse infected with a parasite *C. parvum*



Figure :8 A cross section of the intestine of a rat treated with alcoholic extract



Figure 9: A cross section of the intestine of a mouse from the aqueous extract group

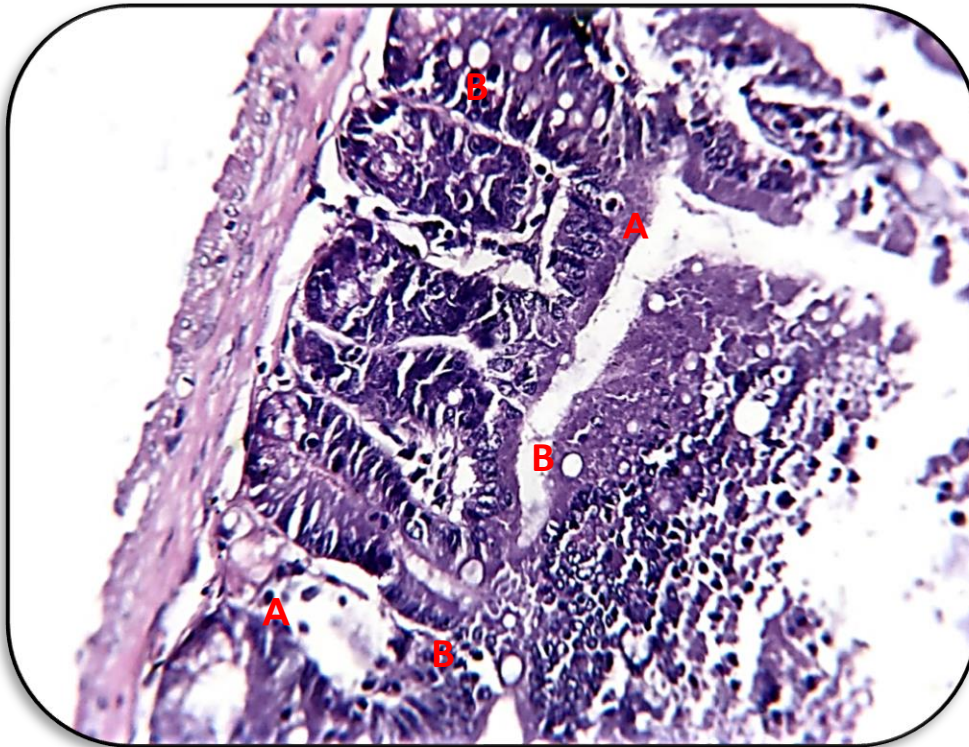


Figure 10: A cross section of the intestine of a mice from the group treated with Metronidazole

REFERENCES

- [1] R. Xu *et al.*, 'Characterization of INS-15, a metalloprotease potentially involved in the invasion of *Cryptosporidium parvum*', *Microorganisms*, vol. 7, no. 10, 2019, doi: 10.3390/microorganisms7100452.
- [2] F. Yu *et al.*, 'CRISPR/Cas12a-based on-site diagnostics of *Cryptosporidium parvum* IId-subtype-family from human and cattle fecal samples', *Parasites and Vectors*, vol. 14, no. 1, pp. 1–11, 2021, doi: 10.1186/s13071-021-04709-2.
- [3] F. S. Cunha, J. M. Peralta, and R. H. S. Peralta, 'New insights into the detection and molecular characterization of *Cryptosporidium* with emphasis in Brazilian studies: A review', *Rev. Inst. Med. Trop. Sao Paulo*, vol. 61, no. April, pp. 1–12, 2019, doi: 10.1590/s1678-9946201961028.
- [4] G. Certad, 'Is *Cryptosporidium* a hijacker able to drive cancer cell proliferation?', *Food Waterborne Parasitol.*, vol. 27, no. March, p. e00153, 2022, doi: 10.1016/j.fawpar.2022.e00153.
- [5] R. A. Guy *et al.*, 'Molecular characterization of *Cryptosporidium* isolates from humans in Ontario, Canada', *Parasites and Vectors*, vol. 14, no. 1, pp. 1–14, 2021, doi: 10.1186/s13071-020-04546-9.
- [6] O. Article *et al.*, *Intestinal Parasitic Infections among the Pediatric Patients in a Metropolitan City of Bangladesh with Emphasis on Cryptosporidiosis*, no. March. 2022.
- [7] E. Tomczak, A. N. McDougal, and A. Clinton White, 'Resolution of *Cryptosporidiosis* in Transplant Recipients: Review of the Literature and Presentation of a Renal Transplant Patient Treated with Nitazoxanide, Azithromycin, and Rifaximin', *Open Forum Infect. Dis.*, vol. 9, no. 1, pp. 1–5, 2022, doi: 10.1093/ofid/ofab610.
- [8] F. Murakoshi *et al.*, 'Nullscript inhibits *Cryptosporidium* and *Toxoplasma* growth', *Int. J. Parasitol. Drugs Drug Resist.*, vol. 14, no. October, pp. 159–166, 2020, doi: 10.1016/j.ijpdr.2020.10.004.
- [9] J. A. Gullicksrud *et al.*, 'Enterocyte–innate lymphoid cell crosstalk drives early IFN- γ -mediated control of *Cryptosporidium*', *Mucosal Immunol.*, vol. 15, no. 2, pp. 362–372, 2022, doi: 10.1038/s41385-021-00468-6.
- [10] G. Certad, 'Food and Waterborne Parasitology Is *Cryptosporidium* a hijacker able to drive cancer cell proliferation?', *Food Waterborne Parasitol.*, vol. 27, no. April, p. e00153, 2022, doi: 10.1016/j.fawpar.2022.e00153.
- [11] N. Zhang *et al.*, 'Prevalence and genotyping of *cryptosporidium parvum* in gastrointestinal cancer patients', *J. Cancer*, vol. 11, no. 11, pp. 3334–3339, 2020, doi: 10.7150/jca.42393.
- [12] S. P. Sherchand, D. R. Joshi, N. Adhikari, K. P. Pant, and R. Pun, 'Cyclosporiasis Among School Going Children of Kathmandu Valley', *Nepal J. Sci. Technol.*, vol. 11, pp. 193–198, 2010, doi: 10.3126/njst.v11i0.4145.
- [13] I. E. Orhan and I. Tumen, 'Potential of *Cupressus sempervirens* (Mediterranean Cypress) in Health',

Mediterr. Diet An Evidence-Based Approach, no. January, pp. 639–647, 2015, doi: 10.1016/B978-0-12-407849-9.00057-9.

[14] M. A. Farag, F. M. Harraz, H. M. Hammoda, E. Shawky, A. Mahana, and A. El-Hawiet, 'Quantitative Nuclear Magnetic Resonance and High-Performance Thin-Layer Chromatography of Cupressuflavone as a Marker for Cupressus Species', *Rev. Bras. Farmacogn.*, vol. 30, no. 4, pp. 488–493, 2020, doi: 10.1007/s43450-020-00060-2.

[15] B. G. Patgar, S. Satish, and A. R. Shabaraya, 'Essential Oil of Cupressus Sempervirens: A Brief Review', *World J. Pharm. Pharm. Sci.*, vol. 10, no. 4, pp. 864–872, 2021, doi: 10.20959/wjpps20214-18713.

[16] M. Khudhair and N. Al-Niaeemi, 'Experimental study of heat-killed oocysts of *Cryptosporidium Parvum* in Balb/ c Mice', *J. Educ. Sci.*, vol. 29, no. 2, pp. 158–173, 2020, doi: 10.33899/edusj.2020.165305.

[17] S. Abada, 'Effect of garlic and pomegranate extract

on rats experimentally infected with *Cryptosporidium parvum* and its comparison with metronidazole', 2015.

[18] M. Gaber, L. A. A. Galal, H. M. M. Farrag, D. M. Badary, S. S. Alkhalil, and N. Elossily, 'The Effects of Commercially Available *Syzygium aromaticum*, *Anethum graveolens*, *Lactobacillus acidophilus* LB, and Zinc as Alternatives Therapy in Experimental Mice Challenged with *Cryptosporidium parvum*', *Infect. Drug Resist.*, vol. 15, no. January, pp. 171–182, 2022, doi: 10.2147/IDR.S345789.

[19] M. Al-Khalil, 'An epidemiological and molecular study to diagnose *Cryptosporidium* ssp in some areas of Dohuk Governorate', 2017.

[20] M. Behbahani, M. Shanehsazzadeh, and M. J. Hessami, 'Optimization of callus and cell suspension cultures of *Barringtonia racemosa* (Lecythidaceae family) for lycopene production', *Sci. Agric.*, vol. 68, no. 1, pp. 69–76, 2011, doi: 10.1590/s0103-90162011000100011.