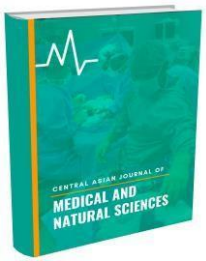

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Article

In Vivo Evaluation of Pumpkin Seed Extract on Entamoeba histolytica Activity

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Abstract: In regions like Iraq, where amoebiasis is prevalent, finding effective treatments against Entamoeba histolytica is crucial due to the emergence of drug-resistant strains. This study investigates the potential antiamebic properties of the ethanolic extract of pumpkin seeds (Cucurbita pepo) as an alternative medication. Using in vitro and in vivo assays, the efficacy of C. pepo extracts against E. histolytica was evaluated, with metronidazole as a comparative control. GC-MS analysis identified compounds with known antimicrobial and anti-inflammatory properties in the extract. Results showed significant anti-amoebic activity of C. pepo extracts, particularly at a concentration of 500 µg/ml, with mortality rates ranging from 58.13% to 86.95%. Additionally, previous studies corroborate the anthelmintic properties of C. pepo against various parasites. These findings suggest the potential of C. pepo as a natural treatment for amoebic dysentery, highlighting its broader implications in combating parasitic infections and addressing the limitations of current therapies.

Keywords: Amebiasis, Dysentery, Entamoeba histolytica, Plant extract, Cucurbita pepo, C. pepo.

1. Introduction

Whether or not symptoms of the disease manifest, one of the intestinal protozoan parasites that causes amoebiasis is Entamoeba histolytica. It is the third most deadly parasite in the world, behind schistosomiasis and malaria. It affects about 50 million people globally each year, infects 100,000 people, and spreads throughout the world, posing a threat to human health in both developed and developing tropical and sub-tropical regions as well as overseas [1].

According to an epidemiological study, 10% of individuals infected with the parasite E. histolytica experience symptoms and go on to develop the disease, while 90% do not experience any symptoms and remain carriers of the infection without developing an invasive disease [2]. The ability of the parasite E. histolytica to secrete enzymes—the most significant of which are Active cystein proteinase and phospholipase—as well as lectin-binding factors (Gal/Gal Nac lectin) Galactose/N-acetyl-D-galactosamine lectin and the creation of Amoebapore are among its many virulence factors that aid in tissue penetration and invasion and increase pathogenicity [3].

One of the most popular edible plants is the pumpkin (Cucurbita pepo), which has excellent medicinal qualities because it contains certain natural edible compounds. Alkaloids, flavonoids, palmitic, oleic, and linoleic acids are just a few of the plant

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components it contains. Other minerals and salts include calcium, iron, magnesium, phosphorus, potassium, and others. Numerous significant health benefits, such as antidiabetic, antioxidant, anticancer, and anti-inflammatory properties, have been reported [4]. Since pumpkin seeds have shown to have strong immune-modulating properties, they can be used as immune-boosting nutrients to strengthen the body's defenses against infectious illnesses [5]. The anti-inflammatory, antioxidant, antidiabetic, and anticarcinogenic properties of pumpkin have attracted a lot of attention. Consuming pumpkin fruit can provide a variety of health-promoting bioactives [6].

2. Materials and Methods

Sample collection

The samples were obtained from patients in Tikrit City who were suspected of having parasaite E. histolytica infection due to their mucous, bloody diarrhea, and abdominal pain. Stool samples were collected in plastic containers, which were then used for direct wet mount preparation, infection confirmation, and examination and diagnosis using an ELISA test to find the E.histolytica parasite.

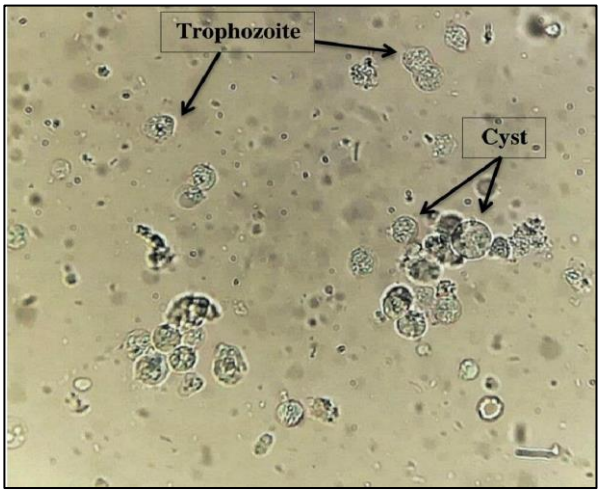

Figure 1. Shows the cyst and Trophpzoite of E.histolytica.

Infection of experimental animals BALB/c mice

Through the use of an ELISA test on stool samples taken from patients whose parasite presence had been verified, the parasite was isolated. The injection dose was found to reach 104 cysts per cm3 after counting the number of cysts in a single drop. Mice were injected with this concentration. verbally [7].It was determined that the mice were parasite-infected by finding parasite cysts in their feces 10–15 days after the dose.

Plant collection:

Seeds of pumpkins C. pepo were fresh and dry when they were purchased from agricultural offices. Subsequently, the seed coat was eliminated through crushing, and the seed pulp was taken and finely ground in a laboratory electric grinder. Getting the ethanolic extract ready:

Riose et al. 1987 [8] provided the method used to prepare the plant's ethanolic extract. modified from the basic procedure used by the researcher (Verpoorte et al., 1982). Next, a stock solution containing 200 mg/ml of Dimethyl sulfoxide, an organic solvent,

was formed from a portion of the dry alcoholic extract, and the extracts were sterilized using filters by pasteurization at 62°C for 20 minutes [9].

GC-MS analysis

Utilizing gas chromatography-mass spectrometry (GC-MS) for qualitative phytochemical analysis, the compounds present in the alcoholic extract of C. pepo seeds were identified, analyzed, and scrutinized.

For the GC-MS analysis, 2 µl of the Cucurbita pepo alcoholic extract was used. Alcoholic extract's phytochemical analysis was carried out using Thermo Scientific Co.'s Thermo GC-TRACE ultra ver. 5.0 Thermo MS DSQ II GC-MS apparatus. The following were the GC-MS system's experimental conditions: Standard non-polar column dimensions for TR 5-MS capillary: 30 meters, ID of 0.25 mm, and film thickness of 0.25 µm. In the gas chromatography section, the mobile phase flow rate (carrier gas: He) was set at 1.0 ml/min. The temperature program involved raising the oven temperature from 40°C to 250°C at a rate of 5°C per minute, with an injection volume of 1 µl. The sample dissolved in chloroform was tested extensively within the 50–650 m/z range, and the outcomes were compared using the Wiley Spectral library search program.

Antiparasitic activity and mortality rate

Following confirmation of the mice's E. histolytica infection, six groups were identified: one untreated positive group and one treated with the ethanolic extract. The concentrations of the ethanolic extract of pumpkin seeds (C. pepo) were determined to be (200, 300, 400, and 500) micrograms/ml. at various concentrations (200, 300, 400, and 500) and a comparator group that received ten days of treatment with metronidazole at a dose of 250 mg/kg.

Optical microscopy was used to assess the efficacy of the C. pepo ethanolic extract and calculate the parasite's mortality rate using a white blood cell counting chamber. The following formula was used to calculate the mortality rate [10]. Parasite activity % = (number of cysts or Trophozoites after treatment / number of cysts or Trophozoites before treatment) x 100.

3. Results and Discussion

Chemical tests on the plant seed showed that ethanolic - based extract of C.pepo contained many chemical compounds, as the highest percentage was recorded for the compound hexadecanoic acid (31.55%), followed by the compound 9-Octadecenal (26.82%), then the compound Glycidyl palmitate (11.62%), and the compound delta.7,25-Stigmastadienol (5.49%) compared to the rest of the other compounds whose peak area was: hexadecanoic acid (5.06%), Dodecanoic acid (4.51%), Hydromethyl-siloxane (1.55), Rhodopin (1.55%), Allocholesterol (%) 1.52), Squalene (1.19%). shown in Figure 1 and Table 1.

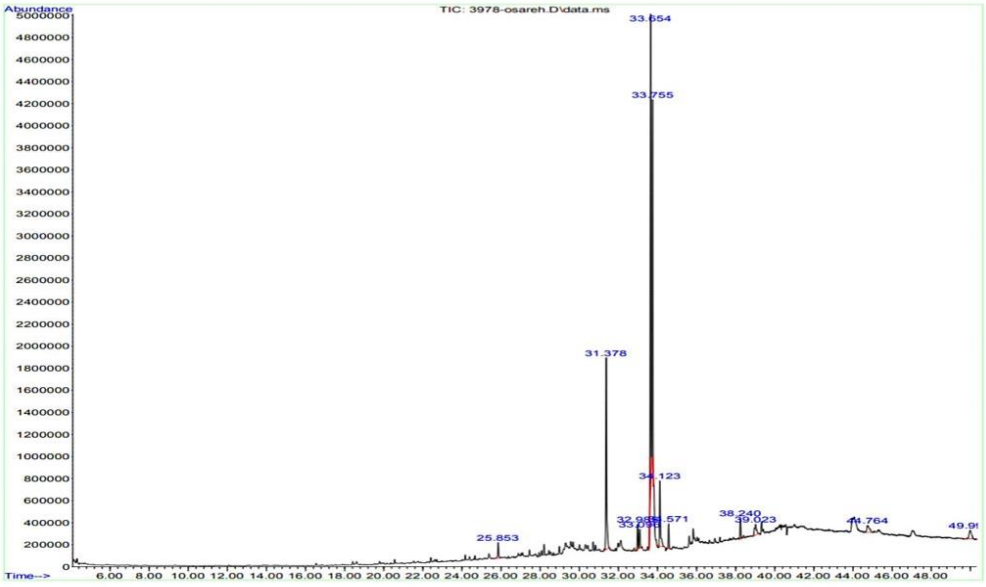

Figure 2. Graphical curve of chemical compounds separated from the ethanolic extract of seeds C. pepo using (GC Mass)

Table 1. Phytochemicals detected in the ethanolic extract of seed C. pepo by GC-MS

Number	RT (min)	Area%	Hit Name	Quality	CAS Number
1	18.413	0.25	Phenol, 2,4-di-tert-butyl-	81	**000096-76-4**
2	18.61	0.19	Pentadecane	96	**000629-62-9**
3	19.788	0.16	Spathulenol	64	**000000-00-0**
4	20.556	0.37	Hexadecane	95	**000544-76-3**
5	22.408	0.26	Heptadecane	97	**000629-78-7**
6	24.172	0.25	Octadecane	96	**000593-45-3**
7	24.38	0.23	Pentadecane	60	**000629-62-9**
8	24.665	0.19	Tetradecane	60	**000629-59-4**
9	25.386	0.37	1-Heptadecene	89	**006765-39-5**
10	25.853	1.05	Nonadecane	97	**000629-92-5**
11	28.199	0.56	1,6-Cyclodecadiene	72	**007049-13-0**
12	31.379	11.62	Glycidyl palmitate	27	**000999-59-7**
13	32.988	1.42	.beta.-Monolinolein	87	**003443-82-1**
14	33.097	1.15	.beta.-Monoolein	83	**003443-84-3**
15	33.654	31.55	9,12-Octadecadienoic acid(Z,Z)	89	**000060-33-3**
16	33.755	26.82	9-Octadecenal,	96	**002423-10-1**
17	33.813	5.06	Hexanedioic acid, dicyclohexyl ester	35	**000849-99-0**
18	34.124	4.51	Dodecanoic acid	38	**000143-07-7**
19	34.57	1.21	Isooctyl phthalate	86	**027554-26-3**
20	35.623	0.65	p-Menth-8(10)-en-9-ol, cis-	80	**015714-13-3**
21	35.826	0.85	Ethyl iso-allocholate	90	**054594-42-2**
22	38.239	1.19	Squalene	87	**007683-64-9**
23	39.022	1.55	Rhodopin	95	**020175-84-2**
24	44.75	5.49	.delta.7,25-Stigmastadienol	90	**006785-58-6**
25	47.059	1.55	Hydromethylsiloxane	64	**001873-88-7**
26	49.991	1.52	Allocholesterol	55	**104461-29-2**

After conducting a GS-MS examination, the highest value was found for the compound 9,12-Octadecadienoic acid (Z,Z). It is an unsaturated fatty acid, which is important as an anthelmintic, anti-inflammatory and analgesic, anti-stomach ulcer, anti-prostate, atherosclerosis, anti-inflammatory. For eczema and arthritis, cancer prevention, liver protection, and anti-hypocholesterolemia [11.12]. The compound 9-Octadecenal, It is one of the specialized compounds that have inhibitory effects on polymerase activation in prokaryotes, which has a functional role in biological activities and has antimicrobial activity against pathogenic microbes [13]. This compound is considered one of the important compounds as an anti-inflammatory, anti-cancer, anti-hypocholesterolemic, nematicide, antihistamine, antieczemic, anti-acne, 5-alpha reductase inhibitor, antiandrogenic. Insectifuge [11]. It is also considered an antimicrobial[14]. the compound Glycidyl palmitate is a fatty acid that has multiple properties and is considered an antifungal [15].

The compound Dodecanoic acid has an antimicrobial effect, works to disperse the agar against some Gram-negative and positive bacteria, and has a high inhibitory effect against Escherichia coli, Pseudomonas, aeruginosa, Aspergillus niger, and Aspergillus[16]. It has a role in preventing the colonization of pathogenic microorganisms, as it acts as a layer to reduce the surface tension of the target cell, forming a layer that envelops the cell and hinders the entry of parasites and microbes into it, or killing microbes inside the cell after encapsulation [17].

As for the compound delta.7,25-Stigmastadienol, it is classified as an alcohol because it contains a hydroxyl group (-OH). It is a compound that works to reduce cholesterol levels in the blood [18]. It has a role in preventing the colonization of pathogenic microorganisms, as it acts as a layer to reduce the surface tension of the target cell, forming a layer that envelops the cell and hinders the entry of parasites and microbes into it, or killing microbes inside the cell after encapsulation [17].

anti-amebic activity

In this work we investigate the anti-amebic activity of ethanolic extracts derived from seed C. pepo. Table 2 summarizes the anti-amoebic activity of C. pepo extracts on E. histolytica. the plant seed ethanolic extracts , showed significant anti-amoebic activity on E. histolytica with a high mortality rate, in the Alcoholic extract . The results of the study showed that the highest killing rate at a concentration of 500 μg/ml was 86.95% on the tenth day of treatment, and the lowest killing rate by concentration was 58.13%, 66.66%, and 72.72%, respectively, compared with the positive control group and the Metronidazole drug group.

The ethanolic extract of pomegranate peel (Punica granatum) and pumpkin seeds (Cucurbita pepo) was found to have anti-helminthic properties against Ascaridia galli in both in vitro and in vivo tests of anthelmintic effectiveness. Fenbendazole was added to extracts of the two herbs at 25, 50, and 75 mg/ml for in vitro comparison. fenbendazole at a 5 mg/ml concentration. Pomegranate peel extract demonstrated a lethal effect in vitro, while all concentrations of pumpkin seed extract displayed an almost similar effect to fenbendazole for 48 hours in vivo. As an anthelmintic, pumpkin seed extract outperformed pomegranate peel extract [19].

The impact of the alcoholic pumpkin seed extract (C. pepo) on the larvae of two different worm species (Trichinella spiralis and Trichinella britovi) in the ex vivo culture medium was verified by the Boros et al., 2021[20] study. The extract completely inhibited the movement of worm larvae after 48 hours of treatment, showing effects comparable to the alcoholic solutions used in the control groups.

Table 2. Percentage killing of E.histolytica using different concentrations of ethanolic extract of seeds Cucurbita pepo.

Time period / hour						
Dose concentration	**24 hours**	**27 hours**	**120 hours**	**168 hours**	**240 hours**	**Average concentration**
Control +	**0**	**0**	**0**	**0**	**0**	**0**
200 µg/ml	**2.32**	**13.95**	**30.23**	**48.83**	**58.13**	**30.692 E**
300 µg/ml	**9.52**	**21.42**	**42.85**	**59.52**	**66.66**	**39.994 D**
400 µg/ml	**13.63**	**27.27**	**47.72**	**61.36**	**72.72**	**44.540 C**
500 µg/ml	**15.21**	**30.43**	**58.69**	**73.91**	**86.95**	**53.038 B**
Meteronidazole 250 \ kg	**23.91**	**52.17**	**84.78**	**100**	**100**	**72.172 A**
Average period	**10.765**	**24.207 d**	**44.045 c**	**57.27 b**	**64.077 a**	

The objective of the Baies et al. (2023) [21] study was to assess the gastrointestinal tract of Cucurbita pepo seeds' antiparasitic potential against nematodes and protozoa present in pigs. Following the collection and analysis of samples, a number of parasite species were identified, including Ascaris suum, Trichuris suis, Oesophagostomum spp., Balantidium coli, Eimeria spp., and Cryptosporidium spp. The plant seed extract was given to the animal for a certain amount of time at a dose concentration of 500 mg/kg/day. When compared to the control group, the C. pepo extract demonstrated a strong anthelmintic effect and proved to be an efficient countermeasure against the aforementioned parasitic protozoa over a 28-day period. He made the point that considerable losses in productivity and reproduction are caused by parasitic infections.Over the past ten years, the use of plant remedies has grown dramatically because of their high bioavailability, low toxicity, non-polluting nature, and, to some extent, anti-parasitic properties.

4. Conclusion

The ethanolic extract derived from Cucurbita pepo seeds has demonstrated notable efficacy in the elimination of Entamoeba histolytica in vivo. This significant finding not only corroborates the traditional medicinal use of this plant for treating amoebic dysentery but also underscores its potential as a valuable therapeutic agent against this pathogen. The demonstrated effectiveness of the extract suggests promising avenues for the development of novel treatments for amoebic infections, potentially offering a safer and more accessible alternative to existing pharmaceutical options. However, further research is warranted to elucidate the precise mechanisms underlying the observed anti-amoebic activity, optimize dosage regimens, evaluate long-term efficacy and safety profiles, and explore potential synergistic effects with other therapeutic agents. Such investigations hold the potential to enhance our understanding of the therapeutic properties of C. pepo and facilitate its integration into mainstream medical practices for combating amoebic dysentery and related illnesses.

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