Original Research Article

An assessment of the epidemiology and *in–vitro* susceptibility of *Giardia lamblia* trophozoites and cysts to a variety of plant extracts

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ABSTRACT

Aims: The purpose of this study was to identify the intestinal parasite *Giardia lamblia* in patients suffering from diarrhea and primary enteritis, then determine the antiparasitic activity of virous plant extracts in comparison to the common drug metronidazole (MTZ) against *Giardia lamblia*.

Place and Duration of Study: It was performed at the laboratory of parasitology of three hospitals included Ramadi Teaching Hospital, Fallujah Teaching Hospital and Ramadi Teaching Hospital for Maternity and Children in Al-Anbar governorate of Iraq. A total of 1200 stool samples of patients were collected from both genders and ranged in age from 6 months to 65 years.

Methodology: To evaluate the anti-Giardia efficacy through the in vitro testing of five different plant extracts included (*Zumeria majda, Thymus migricus, Artemisia santolina, Sargassum*, and *Proskia*) against cysts and trophozoites of *G. lamblia* with virous concentrations of the five extracts (10, 25, 50, 75, and 100 128 µg/mL) for an exposure time of 30, 120, and 360 minutes.

Results: The mean number of the extracted cysts and trophozoites were assessed using vital 0.1% eosin staining. *G. lamblia* parasite infections accounted for 44% of all infections; males had a greater prevalence of the parasite (49.6%) than females (37.5%). In relation to other age groups. The incidences of *G. lamblia* infection were higher in the age groups of 6 months to 10 years and (11-20) years, at 55.17% and 47.6%, respectively. At the lowest concentrations of 75µg/mL and 100µg/mL following exposure times of 240 min and 360 min, all five plant extracts examined in this study included *Zumeria majda, Thymus migricus, Artemisia*

santolina, *Sargassum* and *Proskia* has a significant effect and killed 100.0% of the cysts and trophozoites of *G. lamblia* in contrast to the MTZ, which showed the highest reduction rates 68.4% against cysts and 75.4% against trophozoites.

Conclusion: To conclude, the study findings indicate that *G. lamblia* infections are more common in AL-Anbar province particularly in younger people. This can be attributed to a number of factors such as improper eating and drinking habits and an absence of respect for hygiene and public health standards. Plant-based extracts showed promising in vitro activity at low doses and short times of exposure, according to the results ~~them~~ on *G. lamblia*. These findings could ~~be~~ led to the development of a novel therapeutic alternative to treat ~~giardiasis~~ Giardiasis infections.

Keywords: *Giardia lamblia*, trophozoites, cysts, diarrhea, plant extracts, metronidazole

1. INTRODUCTION

Giardia lamblia an intestine protozoan flagellate, is responsible for the development of a condition called ~~giardiasis~~Giardiasis, which is regarded as being one of the most common protozoan infections in humans globally [1]. Giardiasis has been included in the neglected disease initiative by the World~~s~~ Health Organization (WHO), which claims that over 200 million new cases are identified with the disease globally each year [1]. Most human infections, particularly those in children occur by fecal oral route, which is caused by the direct or indirect intake of infectious cysts in both water and food. The time frame for incubating after consuming cysts ranged from 9 to 15 days [2]. Giardiasis treatment with chemotherapy and synthetic drugs conjugates has recently been recognized as the most suitable option because there is currently no effective or secure vaccine for preventing *Giardia* infections [3]. Recent research has

indicated that the use of these synthetic medications is linked to certain drawbacks, like ~~causes~~ cases which do not respond to therapy as well as adverse drugs reactions, including nausea, mild headaches, dizziness, and a metallic aftertaste, along with skin turning yellow and higher levels of liver enzymes [3-5]. Hence, in the last few decades, scientists have focused on finding novel potential anti-Giardia therapies that simultaneously are highly efficient and have low toxicity. Historically, the use of medicinal plants and their derived substances has been widespread for treating persistent illnesses rather than those that are potential fatal [5]. Several bacterial, viral, fungal, parasitic diseases have also been effectively treated with medicinal products [6].

Prior studies have illustrated the anti-Giardia effectiveness of a variety of herbal extracts, which include those derived from *Carum copticum, Lavandula stoechas, Tanacetum parthenium,, Allium paradoxum, Allium sativum, Artemisia annua, Allium ascalonicum, Chenopodium botrys, ZizIphora clinopodioides, Zataria multiflorahad, Eucalyptus globulus, Lippia beriandievi, Punica granatum.* Additionally, the same cited study stated that the hydroalcoholic extract of *Ferula assa-foetida, Chenopodium botrys, and Tanacetum parthenium* had 100% in vitro efficacy toward G. lamblia, whereas the Allium sativum at a dose of 80 mg/mL showed the highest in vivo effectiveness regrading giardiasis [7-9]. On the other hand, inconsistent results from studies that aren't always strong enough currently hinder the widespread use of herbal treatments for ~~giardiasis~~Giardiasis. The present investigation aimed to assessed the in vitro efficacy of five plant extracts included (*Zumeria majda, Thymus migricus, Artemisia santolina, Sargassum and Proskia*) compared with available drug of metronidazole (MTZ) as an effective and first-time treatment against *Giardia lamblia*.

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2. MATERIALS AND METHODS

2.1. Preparation of plant extracts

The plant elements, which comprised aerial portions like leaves, roots and stems, had been collected from various location in Iran. Each plant material, was cleaned, then allowed to air dry in the shade at room temperature. Using maceration process and pure methanol, the powdered plant material (50g) was extracted three times in 48 hours (3 x 48 hrs) at room temperature. After being extracted using a rotary evaporator, the extracts were lyophilized using freeze dryer. Prior to testing, the concentrated extracts in transparent bottles at 4 °C in a dry, cool environment. To make the extracts into solutions, 10 mg of extract powder were weighed, and 9mL of deionized water were mixed with 1mL of methanol as a co-solvent, this resulted in a final concentration of 1mg/mL. Concentrations ranging from 10 to 100 µg/mL have been achieved by diluting 1mg/mL concentration with deionized water.

2.2. Samples Collection

From January 2023 to February 2024, a total of 1200 stool samples of patients who were referred to Ramadi Teaching Hospital, Fallujah Teaching Hospital and Ramadi Teaching Hospital for Maternity and Children in Al-Anbar governorate of Iraq. The patients were exhibiting clinical indications of diarrhea and primary enteritis. The samples were collected from both genders and ranged in age from 6 months to 65 years. Sterile plastic containers contained normal saline and labeled with particular personal information, which included name, age and gender, were used for collecting these samples and transferred to the Laboratory of Parasitology.

2.3. Isolation and collection of *Giardia lamblia* cysts

Stool textures, including mucous, serous, greasy and bloody along with a range of colors, which involves yellow, brown, semi-brown and greenish had been observed. The stools were examined directly and by formalin-ether test using a light microscope to confirm the presence of *G. lamblia* infection [10]. Using a simple sucrose of 85% gradient approach, a highly concentrated cyst solution was used to separate the cysts [10]. Normal saline was used to dilute

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stool samples containing the cyst 1:10, filtered and centrifuged for 10 minutes at 4000 rpm. After that, 5 mL of the stool solution were centrifuged at 700 g for 8 minutes at 4 °C, and 5 mL of 0.85 sucrose were placed on top of it. The *G. lamblia* cysts were gradually placed on the top of the discontinuous density gradient composed of 2- and 3-mL layers of 0.85 and 0.4 M sucrose, employing a Pasteur pipette for extracting the cysts. Lastly, until testing, the concentrated cysts were stored at 4 °C. The viability of the cysts was assessed using the conventional vital cyst staining approach, which use 0.1% eosin [11], and the appearance of the surviving and dead cysts was colorless and pink, respectively. Using a hemocytometer, the cyst count was determined and adjusted to approximately 10^5 cyst/mL in the distilled water.

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2.4. *Giardia lamblia* cysts excystation for trophozoites

G. lamblia cysts excystation was carried out employing the method described by [11]. In brief, the mixture of cyst suspension and the induction solution, aqueous hydrochloric acid (pH 2.0) was mixed at a ratio of 1:9 and stored at 37 °C for 1 hour. The combination was centrifuged at 700 rpm for 8 minutes, and the upper phase was removed. The remaining mixture was then put to the excystation medium, which included 20% heat-inactivated fetal calf serum and filter-sterilized TYI-S-33 culture medium supplemented with bovine bile. The antibiotics streptomycin (500 µg/mL) and pencilin (500 µg/mL) were added to 7 mL of the medium to improve the susceptibility and then kept warm at 37 °C in a slant.

2.5. Efficacy of virous plant extracts on *Giardia lamblia* in vitro

Five distinct plant extracts included (*Zumeria majda, Thymus migricus, Artemisia santolina, Sargassum and Proskia*) along with MTZ were tested for their anti-Giardia impacts by subjecting *G. lamblia* cysts and trophozoites to varying concentrations (10, 25, 50, 75 and 100 µg/mL) for an exposure time of 30, 120, and 360 minutes. In brief, each test tube contained 1 mL of every concentration before 10,000 cysts or trophozoites were added. The test tubes were then incubated at 37 °C for a duration of 30, 120, and 360 minutes [12]. Following the

incubation period, the solution supernatant phase was discarded, and the remaining settled cysts or trophozoites were then treated with 50 µL of 0.1% eosin stain. Using a light microscope set to 400x magnification, smears made from residual cyst and trophozoite material were arranged individually on glass slides [12, 13]. 300 cysts and trophozoites were counted by Haemocytometer to determine the viability proportions of cysts or trophozoites, the viable and dead parasites showed up as colorless and pink respectively [11]. The cysts or trophozoites that were left untreated and those had MTZ treatment were regarded as the negative and positive controls respectively. The percentages for both cysts and trophozoites were recorded after the incubation time was completed by using the following equation:

$$\text{Trophozoites viability \%} = \frac{\text{Number of live trophozoites} \times 100}{\text{Total number of trophozoites}}$$

$$\text{Cysts viability \%} = \frac{\text{Number of live cysts} \times 100}{\text{Total number of cysts}}$$

2.6. Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software version 26 (SPSS, Inc, USA.) was used to analyze the data for the quantitative variables by calculation the mean and standard deviation. It was determined that a p-value of less than or equal ≤0.05 was statistically significant.

3. RESULTS

3.1. Distribution of *G. lamblia* infection rate according to the gender and age groups

The findings of the current study showed that a higher rate of infection caused by *G. lamblia* parasite reached 44% by examining 1200 faces samples for patients who suffered from primary

enteritis and diarrhea of some hospitals in AL-Anbar province. The recorded percentage for infection by parasite among males was 49.6% which is higher than ~~in females~~ 37.5% in females as shown in Table 1.

Table -1: The incidence of *G. lamblia* infection with regard to the gender

Gender	No. of samples	No. of infections	Percentage %
Male	640	318	49.6*
Female	560	210	37.5
Total	1200	528	44

The asterisks indicate data that are statistically significant (P≤0.001)

The current study findings revealed that *G. lamblia* infection rates were greater in the age groups 6 months to 10 years and (11-20) years, at 55.17% and 47.6% respectively compared with other age groups which were investigated, as displayed in Table 2.

Table -2: The prevalence of *G. lamblia* infection according to the years of age

Age	No. of samples	No. of infections	Percentage %
6 months –10 years	580	320	55.17**
11 – 20 years	315	150	47.6*
21 – 30 years	120	48	40
31 – 40 years	85	33	38.8
41 – 50 years	60	16	26.6
51 – 65 years	40	7	17.5
Total	1200	574	47.8

The asterisks indicate data that are statistically significant (P≤0.001)

3.2. The efficacy of virous plant extracts on cyst and trophozoite of *G. lamblia*

In the current study, five plant extracts included (*Zumeria majda, Thymus migricus, Artemisia santolina, Sargassum and Proskia*) as well as MTZ (as a positive control) were assessed against cysts and trophozoites of *G. lamblia.* The concentrations were ranged from 10 to 100 µg/mL, the time incubation 30, 120, and 360 minutes, and the findings are shown in Tables 3–7. Table 3 shows the findings for the *Zumeria majda* extract towards both trophozoites and cysts of *G. lamblia* in virous concentrations in comparison with MTZ as a positive control and TYI-S-33 medium as a negative control. It is clear that at concentration of 50µg/mL of the extract had a reduction rate of 83.2% against trophozoites after 360 min. When the concentration increased to 75µg/mL the killing percentages were improved and found to be 32.0%, 54.3% and 67.0% against cysts followed of exposure time 30, 120 and 360 min respectively. As well as the same tested concentration gave elevated reduction against trophozoites at 50.3%, 71.0% and 96.0% after 30, 120 and 360 min respectively. Interesting results were obtained from testing the concentration of 100µg/mL, as this concentration gave 61.5%, 75.4% and 100.0% killing proportions after 30, 120 and 360 min respectively against cysts. More elevated reduction rates were achieved from evaluating the same concentration of this extract against trophozoites, and showed 84.4%, 90.0% and 100.0% after incubation time 30, 120 and 360 min respectively.

Table 3: The effect of *Zumeria majda* at different times and concentrations on the survival of *G. lamblia* cysts and trophozoites

Concertation	**Killing rate (%) for cysts**			**Killing rate (%) for trophozoites**		
(µg/mL)	**30 min**	**120 min**	**360 min**	**30 min**	**120 min**	**360 min**
10	4.8±32.1	8.2±25.1	9.1±50.3	14.0±35.1	18.1±30.5	42.4±50.3
25	8.0±45.9	18.2±45.9	45.0±10.1	18.9±35.1	39.7±30.1	64.8±70.4
50	10.2±50.1	30.8±37.8	49.2±23.2	19.6±35.1	51.7±68.2	83.4±20.8

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75	32.3±35.1	54.3±24.1	67.5±50.3	50.3±25.1	71.3±40.4	96.3±41.6
100	61.7±25.1	75.2±20.8	99.8±23.9	84.4v20.1	90.2±30.5	100.0±17.3
MTZ (100 µg/mL)	8.1±20.8	28.5±15.2	48.2±26.4	17.4.20.2	34.3±30.5	62.2±32.1
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The experiments were repeated three times (n=3) and the results are expressed as a mean ± the standard deviation (SD). The statistical analysis was carried out by utilizing one way analysis of variance (ANOVA). The asterisks indicate data that are statistically significant (P≤0.001) between the reduction rates of the extract at concentrations of 50, 75 and 100µg/mL compared with MTZ or TYI-S-33 medium as controls.

The results testing of *Thymus migricus* extract are shown in Table 4. Both concentrations 75 and 100µg/mL demonstrated strong activity against trophozoite and cysts. The killing rate against found to be 98.2% after 360 min whereases the MTZ tested at the same concentration and time frame which gave only 50.7%. Also, the concentration of 100µg/mL showed complete kill 100.0% against trophozoites after 360 min compared with the identical concentration and exposure time of MTZ which gave lowest reduction rate at 58.7%.

Table 4: The impact of *Thymus migricus* at virous times and concentrations on the survival of *G. lamblia* cysts and trophozoites

Concertation(µg/mL)	**Killing rate (%) for cysts**			**Killing rate (%) for trophozoites**		
	30 min	**120 min**	**360 min**	**30 min**	**120 min**	**360 min**
10	6.6±20.8	9.2±30.5	11.5±20.3	13.2±15.2	21.2±25.1	46.4±30.1
25	7.3±20.8	21.4±10.2	43.6±26.4	24.2±26.4	41.6±15.2	59.2±28.2

50	12.3±20.1	35.4±26.4	55.3±36.5	21.2±20.0	54.2±10.0	81.3±30.0
75	34.6±20.2	59.5±41.6	70.3±30.5	53.4±36.0	76.4±32.1	94.3±32.1
100	64.3±30.4	77.3±30.2	98.4±25.1	81.3±10.0	91.3±15.2	99.8±24.0
MTZ (100 µg/mL)	7.3±10.1	31.4±30.5	50.5±20.8	14.3±20.8	36.4±25.1	58.4±26.4
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The experiments were repeated three times (n=3) and the results are expressed as a mean± the standard deviation (SD). The statistical analysis was carried out by utilizing one way analysis of variance (ANOVA). The asterisks indicate data that are statistically significant (P≤0.001) between the reduction rates at concentrations of 75 and 100µg/mL associated with 100µg/mL of the extract and the MTZ or TYI-S-33 medium as controls.

The third plant extract assessed in this study was *Artemisia santolina* and the outcomes are presented in Table 5. As can be clearly seen that the concentrations 50, 75 and 100µg/mL demonstrated massive reduction rates towards both trophozoites and cysts of *G. lamblia.* At concentration of 100µg/mL, it was 98.7% killed of cysts and 99.0% of trophozoites after 360 min compared with lower reduction percentage at only 48.0% and 61.0% of MTZ respectively.

Table 5: The efficacy of *Artemisia santolina* at variety of times and concentrations on the survival of *G. lamblia* cysts and trophozoites

Concertation (µg/mL)	**Killing rate (%) for cysts**			**Killing rate (%) for trophozoites**		
	30 min	**120 min**	**360 min**	**30 min**	**120 min**	**360 min**

10	8.3±10.0	11.3±10.0	17.3±30.0	14.3±20.8	25.2±10.0	49.1±15.2
25	9.4±20.8	24.4±30.5	48.1±21.2	27.2±15.2	44.2±10.0	65.3±20.8
50	13.1±15.2	38.3±10.0	58.2±20.8	25.4±25.1	56.1±15.2	78.4±25.1
75	39.1±15.2	61.3±15.2	73.2±25.1	57.1±15.2	79.4±51.3	96.3±30.5
100	69.5±43.5	79.6±83.2	98.5±20.8	84.5±30.5	95.3±40.4	99.3±32.1
MTZ (100 µg/mL)	5.1±15.2	29.3±10.0	48.2±25.1	13.2±20.0	39.4±20.0	61.4±36.0
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The experiments were repeated three times (n=3) and the results are expressed as a mean± the standard deviation (SD). The statistical analysis was carried out by utilizing one way analysis of variance (ANOVA). The asterisks indicate data that are statistically significant ($P \leq 0.001$) between the reduction rates of the extract and the MTZ or TYI-S-33 medium as controls.

Both concentrations 75 and 100µg/mL of *Sargassum* extract were demonstrated superior activity against cysts and trophozoites in all tested times 30-360 min as shown in Table 6. Fascinating results obtained from tested this extract, as it showed full reduction at 100.0% following exposure time of 360 min, compared to 100µg/mL of MTZ which achieved about half killing rates at only 54.2% against cysts and 58.0% against trophozoites.

Table 6: The influence of *Sargassum* at different times and concentrations on the survival of *G. lamblia* cysts and trophozoites

Concertation	**Killing rate (%) for cysts**			**Killing rate (%) for trophozoites**		
(µg/mL)	**30 min**	**120 min**	**360 min**	**30 min**	**120 min**	**360 min**

10	6.3±10.0	9.1±20.8	14.5±10.0	13.2±20.0	22.3±30.0	45.1±15.2
25	10.1±15.2	21.4±11.5	44.5±26.4	23.4±20.0	42.1±15.2	58.6±26.4
50	17.4±20.8	40.2±10.0	55.1±15.2	29.3±10.0	51.3±10.0	74.3±20.8
75	44.3±15.2	67.3±10.0	77.4±10.0	60.4±20.8	80.3±15.2	94.3±20.8
100	70.1±15.2	80.3±15.2	100.0±17.3	79.2±20.8	93.4±20.0	100.0±17.3
MTZ (100 µg/mL)	7.2±20.8	36.2±20.0	54.4±26.4	18.5±15.2	44.3±25.1	58.2±20.0
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The experiments were repeated three times (n=3) and the results are expressed as a mean± the standard deviation (SD). The statistical analysis was carried out by utilizing one way analysis of variance (ANOVA). The asterisks indicate data that are statistically significant ($P \leq 0.001$) between the reduction rates of the extract and the MTZ or TYI-S-33 medium as controls.

The last plant extract tested in this investigation was *Proskia* and the findings are presented in Table 7. The results are very interesting as this extract enhanced the reduction rates compared to the controls and the other tested plant extracts. The concentration of 50µg/mL provide great reduction percentages after 360 min, and gave 90.3% for cysts and 94.0% against trophozoites. The killing rates were improved when the concentration increased up to 75µg/mL and gave 97.3% for cysts after 360 min, while for trophozoites the reduction rate was found at 98.3% after 120 min and entire kill 100.0% followed 360 min of time exposure. A 100µg/mL concentration caused 100.0% reduction for cysts and trophozoites after 120 and 360 min of incubation time compared to MTZ which gave lower kill of rate following the same exposure period.

Table 7: The impact of *Proskia* at several times and concentrations on the survival of *G. lamblia* cysts and trophozoites

Concertation (µg/mL)	**Killing rate (%) for cysts**			**Killing rate (%) for trophozoites**		
	30 min	**120 min**	**360 min**	**30 min**	**120 min**	**360 min**
10	8.3±10.0	11.4±20.8	27.2±30.5	17.4±20.0	29.4±20.8	48.4±26.4
25	15.5±25.1	23.4±26.4	58.2±15.2	28.2±15.2	44.3±20.8	73.3±25.1
50	25.3±25.1	72.2±23.0	90.3±20.8	34.3±20.0	67.6±23.0	94.3±26.4
75	54.2±10.0	88.3±20.8	97.3±15.2	72.4±20.8	98.3±20.8	100.0±17.3
100	86.4±20.8	100.0±17.3	100.0±17.3	91.4±20.0	100.0±17.3	100.0±17.3
MTZ (100 µg/mL)	11.2±20.8	44.3±20.8	68.4±10.0	21.3±20.8	54.4±20.8	75.4±20.0
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

The experiments were repeated three times (n=3) and the results are expressed as a mean± the standard deviation (SD). The statistical analysis was carried out by utilizing one way analysis of variance (ANOVA). The asterisks indicate data that are statistically significant ($P \leq 0.001$) between the reduction rates of the extract and the MTZ or TYI-S-33 medium as controls.

4. DISCUSSION

There was a very high rate of infection by *G. lamblia* in patients suffering from diarrhea who visited three different hospitals including the Ramadi Teaching Hospital, the Fallujah Teaching Hospital and the Ramadi Teaching Hospital for Maternity and Children in the Cities of Ramadi

and Fallujah in Iraq. Out of 1200 stool samples only 528 samples (44%) tested positive, whereas the remaining 672 (56%) were negative. ~~An increased~~ Increased incidence of infections was observed in male patients accounting for 49.6%, while the proportion of infections in female patients stood at merely 37.5%. These findings are comparable with those of Shahatha [14] who observed that the same three hospitals had a 41.7% *G. lamblia* prevalence and that male infection rates were 47.8%, greater than female infections at 35.4%. The results of this research differ from those of a pervious study [15] which examined 1804 stool samples and found that only 356 residents of Al-Ramadi City tested positive for intestinal parasites (19.7%), with *G. lamblia* having the lowest incidence overall of 9.8%. In comparison with prior published research conducted in serval Iraqi cities [16-21], which demonstrated that the prevalence of *G. lamblia* was 14.30%, 10.80%, 17.9%, 38.5%, 10.31%, 5.94% respectively, the rate of *G. lamblia* isolated in the present study appeared to be higher. Additional studies [22, 23] undertaken in Kurdistan region of Iraq identified lowest percentages 0.2% and 4.2% respectively, these outcomes contradict those of the current study.

The variation in results may explained by differences in the size of samples examined, testing techniques, laboratory conditions, different ~~areas~~ regions of study, alterations in environment situations, methods for handling waste, sanitary systems and individual hygiene. Furthermore, for the reason that men are likely then woman to be exposed to the parasites simply because of their natural tendency to spend time playing outside and eating from unhealthy places. It is peddlers who disregard public health and hygienic regulations. Following this, the age groups of 6 months to 10 years manifested an infection rate of 55.17%, indicating significant statistical relevance ($P \leq 0.001$) in relation to other age groups. Consequently, the age brackets of 11-20 years revealed an infection rate of 46.6%, with significant statistical ($P \leq 0.001$) when contrasted with other age groups. These results ~~in~~ are congruent with previously published research in Al-Ramadi City [14, 15].

As reported in Table 3, the *Zumeria majda* extract showed great activity at lower concentrations 75µg/mL and 100µg/mL with short duration of exposure at 360 min achieved complete reduction 100.0% against cysts and trophozoites of *G. lamblia* compared with MTZ which gave only 48.0% reduction against cysts and 62.0% kill against trophozoites. A recent study [24] found that the *Astragalus maximus* extract significantly ($p<0.0001$) reduced the survival of *G. lamblia* cysts; after 240 and 360 min of incubation process the extract killed 100% of *G. lamblia* cysts in both concentrations of 22.5 mg/mL and 45 mg/mL. The findings further shown that following 120 and 240 min of exposure 100% of *G. lamblia* trophozoites were eradicated by the extract at the two concentrations of 22.5 mg/mL and 45 mg/mL, due to the authors of the cited study tested their extracts at very high~~est~~ concentrations ~~of~~ ranges in mg/mL, these results are inconsistent with ~~mine.~~ the current study.

The next extract ~~was~~ evaluated was *Thymus migricus*, greater activity was observed against cysts and trophozoites of *G. lamblia* after 360 min of exposure provided a complete kill at 100% compared to MTZ which exhibited around half of reduction 50.7% against cysts and 58.7% against trophozoites. At 50, 75 and 100µg/mL treatments, *Artemisia santolina* extract demonstrated remarkable reductions towards cysts and trophozoites of *G. lamblia*, whereas at 100µg/mL MTZ, minimum level of mortality occurred. *Astragalus maximus* extract was evaluated in a study by [24] and the results showed that 100% of *G. lamblia* were killed by the extract at concentrations of 22.5 mg/mL and 45mg/mL following 240 min and 360 min of treatment. Furthermore, within 120 min and 240 min the same tested extract at concentrations of 22.5 mg/mL and 45 mg/mL exhibited 100% reduces of trophozoites. Considering the concentration used in the cited study differed, the results had reached in contrast with the findings from the current investigation. As opposed to MTZ, which demonstrated lowest reduction at 54.2% for cysts and 58.0% for trophozoites, *Sargassum* accomplished a 100% death rate occurred for cysts and trophozoites at concentration of 100µg/mL after 360 min of exposure time~~.~~, ~~Compared~~

compared to previous research ~~by~~ [25] ~~who~~ that found that the chloroformic extract of *Artemisia annua* was effective in eradicating cysts and trophozoites of *G. lamblia* after 180 min at an elevated dose of 100 mg/mL.

Incredibly surprising outcomes from evaluating *Proskia* extract on *G. lamblia* cysts and trophozoites at lowest concentrations 75 and 100µg/mL gave a full kill 100.0% for cysts and trophozoites after 360 min, in relation to MTZ which was showed only 68.4% against cysts and 75.4% against trophozoites. These findings are consistent with my previously published research papers that I have undertaken for *Streptococcus* species and *Acanthamoeba* spp. [26, 27]. The same tested extracts were found to have superior activity in lowest concentrations on cysts and trophozoites of *Acanthamoeba* spp. as well as against isolates of *Streptococcus* species which obtained from dental decay infection. The MTZ is especially destructive to *Giardia*, however, this drug killing mechanisms with this parasite has not been fully understood. By drawing a comparison between MTZ antibacterial activity against bacterial species and trichomonads, it has been suggested that the drug metabolic reduction produces free nitro radicals, which oxidize DNA and cause strand breakage and eventually the death o cells [28]. When MTZ interacts with DNA it causes strand breaks and loss of helical DNA shape in addition to diffusing throughout the organism and inhibiting protein production. As a result, it led to cell death in species that are unvenerable [29]. The mechanisms of action for the tested plant extracts are unclear, however, it has been noted that several of the extracts attracted attention in the literature due to interesting biological activities. These extracts commonly disrupt important target in parasitic organisms, like microtubules, DNA (intercalation, alkylation) the integrity of membranes and neural transmission of signals [30]. Thus, the existence of flavonoid molecules may be linked to the anti-Giardia activity of certain plants. This is the first in vitro study to evaluate the parasitic activity of these plant extract because, despite my best efforts to search the literature, I was unable to find studies that tested

these five plant extracts *Zumeria majda, Thymus migricus, Artemisia santolina, Sargassum and Proskia* on *G. lamblia* infections.

5. CONCLUSION

The findings of this study showed high-level of infection caused by G. lamblia at 44% and it was sharpest in males at 49.6% than females 37.5%, as well as the infection rates effect the age groups ranged from 6 months to 10 years at 55.17% followed by age groups 11-21 years at 47.6%. The results of the current study demonstrated that exhibited potential in vitro anti-*Giardia* impacts of new tested plant extracts included (*Zumeria majda, Thymus migricus, Artemisia santolina, Sargassum and Proskia*) against both cysts and trophozoites of *G. lamblia.* The greatest influence at the concentrations 75µg/mL and 100µg/mL and the exposure time was short at 240 and 360 min. According to my best of knowledge, this study for first time assessed these types of plant extracts towards cysts and trophozoites of *G. lamblia* and the outcomes displayed promising activity at lowest concentrations and short exposure time. However, this study suggested that further in vivo investigations particularly in animal models and clinical setting are needed in order to ensure from the precise effectiveness and the mode of action of the tested extracts.

CONSENT

Informed written consent was obtained from all the study participants.

ETHICAL APPROVAL

Based on the Ethical Guidelines of Research from the College of Veterinary Medicine at the University of Fallujah in Iraq and ethical committee of the hospitals, the ethical and research committee granted this study ethical approval.

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