The effect of Aqueous some plants Extract on *Giardia lamblia* in *vitro*

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Abstract

Six plants were purchased from herpelists shopes local. These extracts are the *Punica granetum*; *Artemisia campestris*; *Eucalyptus camaldulensis*; *Cuminum cyminum*; *Mangifera indica*; and *Achillea santolina*.

The serial double fold dilution was done to each extract in sterile labeled tubes after preparation. The stock solutions used were (2000, 1000, 500, 125, 62.5, 31.5) mg\ml. Metronidazole (flagyl) was used as standard drug at concentration of 5ml\125mg. The concentrations tested were (500, 250, 125, 62.5, 31.5, 15.75, 7.87) mg\5ml. Viability was assessed by number of viable parasite; number of dead parasite per-microscopically field. The results showed that punica *granetum; Artemisia campestris; Eucalyptus camaldulensis; and Achillea santolina* revealed significant decreases in number of *Giardia lamblia* trophozoites. While the *Cuminum cyminum and Mangifera indica* not appeared remarkable effects in number of *Giardia Lamblia* trophozoites.

تأثير المستخلص المائي لبعض النباتات على الجيارديا لامبليا في المختبر

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الخلاصة

ستة نباتات تم شرائها من محلات العشابين في السوق المحلية. وعملت منها مستخلصات نباتية وهذه المستخلصات هي قشور الرمان *Punica granetum* ونبات الشيح *Artemisia campestris* ، كالبتوز *Eucalyptus camaldulensis*،الكمون *Cuminum cyminum* ، عنبة *Mangifera indica*، القيصوم *Achillea santolina*. إستعملت طريقة التخفيف المضاعف والمتسلسل (serial double fold dilution) لكل مستخلص محضر في أنابيب مؤشرة بعد التحضير. المحاليل القياسية هي بتراكيز(2000، 1000، 500، 125، 62.5 ،31.5) مايكروغرام لكل مليلتر حجم. الدواء القياسي (المسيطر control) المستعمل هو محلول المضاد الحياتيmetronoidazole (flagyl) بتركيز 125\ml5 mg. التراكيز المختبرة للمسيطر(control) هي (500، 250، 125، 62.5، 31.5، 15.75، 7.87) ملي غرام لكل5 مليلتر حجم. الحيوية تقاس بواسطة تعداد الطفيليات الحية وكذلك تعداد الطفيليات الميتة في الحقل المجهري. واظهرت النتائج ان قشور الرمان (الدباغ) *punica granetum*، ونبات الشيح *Artemisia campestris* وقلف الكاليبتوز *Eucalyptus camaldulensis* والقيصوم*Achillea santolina* أوضحت نقص معنوي في عدد المتغذيات اللامبلية Giardia lamblia trophozoites بينما نبات الكمون *Cuminum cyminum* ونبات العنبة *Mangifera indica*. لم تظهر تأثيرات معنوية واضحة في عدد المتغذيات اللامبلية *Giardia lamblia trophozoites*.

Introduction

Giardiasis is a protozoan infection principally of the upper small intestine; it can remain asymptomatic; bring on acute self-limited diarrhea. It lead to intestinal symptoms such as chronic diarrhea, steatorrhea, abdominal cramps, bloating, frequent loose and pale greasy stools, fategne, malabsorption of fats and weight loss.

Infection agent is *Giardia lamblia* a flagellate protozoan. Its occurrence a world wide. Children are infected more frequently than adults. Prevalence is higher in area of poor sanitation and in institutions with children not toilet trained. The prevalence of stool positivity in different areas may range between 1% and 30% depending on community and age group surveyed.

The reservoir is humans; possibly beaver and other wild and domestic animals. The mode of transmission is person to person occurs by hand to mouth transfer of cysts from feces of an infected individual. Concentrations of chlorine used in routine water treatment do not kill Giardia cycts, especially when the water is cold. Unfiltered stream and lake waters open to contamination by human and animal feces are a source of infection (1).

Jokipii and Jokipii (2) indicated the need for completely new class of drug for Giardia treatment due to the suspected carcinogenicity of metronidazole. Julein, (3); Wolfe, (4), considerable variation in the sensitivity of different stocks of *Giardia lamblia* to the common drug metronidazole furazolidone (5), (6) and treatment failure (7).

Singh et,al (8) reaveled that in modern medicine also plants occupy avery significant place as a raw meterrial for some important drugs, although synthetic drugs and antibiotics brought about a revolution in controlling different disease. But these synthetic drugs are out of reach of million of people. Those who live in remote places depend on traditional healers, whom they know and trust, judicious use of medicinal herbs can even cure deadly disease that have long defied synthetic drugs.

The main aim of the present study is to evaluate the *in vitro* antigiardial activity of six crude extract of different Iraqi medicinal plant that were used previously in folk medicine in the treatment of giardiasis and other parasitic disease to examine their traditional uses and their potential to be used in the future as a source for the discovery of new drugs for the treatment of giardiasis in human and animals.

WHO (9) recommended that research shoulel continue for safe, cheap, early administered drug, bearing in mind the need also for effective treatment of patient who are unresponsive to current first line drugs. It was reported that, medicinal plant either in the form of crude drugs or their isolated medicinally active substances, will be important among the treatment choices available to physicians (10).

As conclusion, non of drugs described above can be used with completed assured safety especially to the fetus (11). There are wide varieties to medicinal plants which have pharmacological actuality against several human now (12).

Among them, many plant with antiparasitic spectrum like, *Eucalyptus comaldulensis, punica granctum, mangifera indica, caminum cynminum, Artemisia campestris* and *Achillea santolina,* which have good activity against diarrhea and dysentery (13, 14). It was reported that the plant, which contain berberine compounds, may be useful against *Giardia lamblia* (15). Because of less side effect of the plants extracts in comparison with chemical drugs. The investigatores approach to this field now days (16).

Materials and Methods

- **Parasite:**

Samples collection from individuals complaining of diarrhea were examined for *Giardia lamblia* infection. These patients attend General hospital in Ramadi; consulting laboratory at the college of Medicine and private laboratories in Ramadi city during the period of this work in 2008. Samples examination for *Giardia lamblia* trophozoites and cysts were detected by direct fecal wet preparation method according to the method mentioned by Henandez, *et al* (17).

Cysts purification from Cyst-bearing feces were obtained from infected parasites. Cysts purification was done in present work accoding to Buchel *et al.*, (18). Fecal samples were carefully diluted in distilled water (1:10). Filtered through 4 layers of gause, then through a 125 mm, 90 mm aperture metallic mesh and through a 20 mm aperture nylon mesh. Four to five ml of filtrate was plased over 3 ml of chilled 0.85 M sucrose solution in a 10ml conical centrifuge tube. After centrifugation at 4000 r.p.m for 5 min. at room temperature, the water –sucrose interface was removed. Diluted (1:10) with distilled water and centrifuged for 5 min.The pellet was resuspended in 4 ml of water and sucrose gradient was repeated. The washing step was repeated at least for three times. Purified cysts suspention were used freshly or stored at 4 c in distilled water until it was needed.

Excystation of *Giardia lamblia in vitro* was done in the present research according to the method of Bingham and Meyer (19); and the method described by Sauch (20). One volume of purified cysts suspension was added to 10 volume of HCL- saline (0.7 ml concentrated HCL 100ml of 0.85M Nacl, PH 1.5). Three of reducing solution (Hanks Balanced salt solution Ph 7.2 was supplemented with 0.098 gm glutathione and 0.1 gm of L-cysteine - Hcl) and 3 ml of solution bicarbonate prepared freshly. The suspention was mixed and incubated at 37 c for 1 hr. The suspention was centrifuged at 4000 rpm for 5 min at room temperature. The pellet was finally suspended in 5 ml of pre-warmed Excystation medium, which was prepared freshly by (0.015 gm NaHco3 and 0.01 gm of L-cysteine Hcl monohydrate were dissolved in 4 ml of 5% peptone and 1 ml of (HBSS).The final volume was adjusted to 10 ml with distilled water pH7.1 water bath with ^{0}c-The peptone was prepared by adding 5% weight \volume peptone to distilled water, boiled in stirring for 10 min. and then stored at 4c^{0}.

The sample was incubated in the Excystation medium for 30 min at 37c .Samples were removed from the Excystation tube and placed adepression slides and then covered with glass cover slip sealed with Vaseline – Position for 30 min at 37c^{0}.

Cultivation in the present research, attempts for cultiving *Giardia lamblia* trophozoites excysted *in vitro* by using three types of modified media (karapetyan; TYIS-33 and HSP-1) through a several attempts according to the study mentioned by Al-Kaissi (21).

- **Plant materials:**

Six plants were purchased from herpelists shopes in local market. Selection of plants was based on ethobotanical in formation either literature or directly by personal communications with famous herpalists in Ramadi city. The identification of selective plants was kindly confirmed by Dr. Tarek. M.AL-Fahdawi (profesore of Medicinal plants) Collage of Agriculture\ University of Anbar. These studies was done in the laboratories at college of Medicine, University of Anbar. These plants were: Cuminum cyminum (Umbelliferae), on annual herb with thread like leaves, small white or rose-coloured flowers in compound umbels. Seeds are similar to caraway (22). The plant contains protein; Ca; P; Fe; carotene; Vit. A; Vit. C; fixed oil; volatile oil rich in caminic aldehyde; alpha and B-penine; Dipantene; phellondrene; Part used: Fruits

known as seeds (13). *Punica granetum*: (punicaceae), alarge tree 3-4 m high. Leaves are shiny green. Flowers are large bright orang to red. Fruit are globose to sub globose 8-12 cm in diameter with persistent tubular calyx and aleathry skin. The rinds very rich in tannin and contains the alkaloids pelletietine C8H15ON (22).The fruit rind is good for diarrhoea and dysentery. Part used fruit rinds (13). *Mangifera indica*: (Anacardiaceae). The fruit contains cellulose; yellow coloring matter; Vit. C ; sugar; CH3; albuminoids; *Mangiferin*, the bark contains tannin and the kernel. Contains tannin; gallic acid; sugar ; fat; gum; starch. Part Used are fruits (13). *Artemisia campestris* (campasitae): A wild herb 30-50cm.high with many branching stems and ovate or bicular leaves, upper leaves are smaller and in clusters. The plant contains thujone, santonin, the sterols betasitosterol and stigmasterol (14). It has an anatiheminthis activity. Part Used are whole plant.(13).

- ***Eucalyptus camaldulensis* (myrtaceae):** A high tree with grey deciduous bark and narrow lanceclate leaves. flowers 4-8 together are in stalked umbel. Fruit capsule is nearly glabours. The plant contains mostly kinotannic acid, kinain, kinored, catechin and pyrocatechin (14) .It used as astringet in diarrhea and dysentery. Part used are Leaves, flowers and bark (13).
- ***Achillea santolina* (compositae):** It is asmall perenncal herb. The ray florets are yellow and very short and the herb is hairy having afragrant odour. the plant containe volatile oil containing azulenes, terpenes, sesquiterpene lactones, choline and glycin (14). herb possesses insect repellant properties. Leaves against dysentery; intestinal colic; explusion of gases. Part used are The entire herb (13).
- **Plant Extraction:** Each of six plants were dried at room temperature garbled and powdered by rotel coffee grinder type 24 (22). 20 grams of the resulting powder from each plant were mixed with (500)ml distilled water by using electrical blender for (10) min. The suspension was kept shaking over night at room temperature, then centrifuged at 4000 rpm for 5 min and the centrifuges was dried under vacuums by rotary evaporator. The dried extracts were kept in deep freeze (-20 c) until the time of used (23).
- **Astock solution:** The stock solution of each plant was done by dissolving 200 mg of the extract in 100 ml of (HBSS) to get a concentration of 2000 m gm\ ml which was the highest concentration tested. Sterilization was done by filtration wares through a Millipore 0.45 mm and 0.22 mm. Serial double fold dilution was done to each extract in sterile labelled tubes. The concentration tested were (2000, 1000, 500, 125, 62.5, 31.25) mg\ml (24,25).
- **Standard drug:** Metronidazole (flagyl) was used as standard drug at a concentration of 5 ml 125mg the concentration tested were (500, 250, 125, 62.5, 31.25, 15.75, 7.87) mg\ml Viability was assessed as number of viable parasite, number of dead parasite per microscopic field. The percentage of viability was calculated as below:-

$$\%\text{Viability} = \frac{\text{No. living parasi\ml}}{\text{Total No.\ml}} \times 100$$

(The mean of ten trials was used)

Results and Discussion

- **Cultivation:** Using the *Invitro* Excystation procedure sample of cycts from patients with symptomatic and asymptomatic giardiasis were inoculated on manufactured media in the laboratory. These media were (karapetyan, 1962 medium; TYIS-33 medium, keister, 1983; and HSP-1 medium, Meyer, 1976) (21). Although the parasite failed to multiply for (72-hrs) in karapetyan, (1962) they live throughout the whole period of work. The parasites were maintained for (96 hrs.) as TYIS-33 media.
- **Effect of crude plants on *Giardia lamblia* trophozoites:** Some of the crude extracts of six Iraqi plants showed antigiardial activity against *Giardia lamblia* trophozoites *in vitro*. Our results showed that *punica granetum; Artemisia campestris; Eucalyptus comaldulensis; and Achillea santolina* revealed significant decreases in number of *Giardia lamblia* trophozoites, while the *Cuminum cyminum* and *Mangifera indica* not appeared remarkable effects in number of *Giardia lamblia* trophozoites. It appeared figures below:

Table (1) shows viability of *Giardia lamblia* parasites in *cuminum cyminum* plant at percentage 25% viability with concentration 2000 mg\ml. This plant showed less effect on the density of parasite trophozoites. Table (2) shows viability of *Giardia lamblia* parasites in *Punica granetum* plant. This plant showed a high effect on the density of parasite trophozoites. The result showed the best activity of this plant. Table (3) shown that the aqueous extract of *mangifera indica* had lowered statistically ($P<0.05$) effect even at 2000mg\ml. Table (4) shows viability of *Giardia Lamblia* parasites in *Artemisia compestris* plant. The aqueous extract of this plant had good effect on trophozoites parasite. Table (5) revealed that viability of *Giardia lamblia* trophozoites were remained unchanged at concentration (31.25), (62.5) mg\ml. although the effect of *Eucalyptus camaldulensis* plant an *Giardia lamblia* trophozoites were finally good effection. Table (6) shows viability of *Giardia lamblia* parasites in *Achillea santolina* plants. That plant showed good effect on *Giardia lamblia* trophozoites, and the effection began from 62.5con.mg \ml (viability 87%). The viability was 0% at concentration 2000 mg\ml. The results of mine studies had been showed the effect of aqueous plants extract on *Giardia lamblia* trophozoites *in vitro*. Up to mine knowledge, there were no studies in Iraq concerning antigiardial activity of medicinal plants. So that, this study in from for the first time that Iraqi medicinal plants have an effect against *Giardia lamblia* parasites although these parasites are fastidious .The lowest concentration (31.25 mg\ml) showed no activity anal forms in a comparison with controls. The on way analysis of variance of the results on six plants indicated that two well separated groups of plants were found. The first group represents *Caminum cyminum and Mangifera indica*. These plants demonstrated no significant activity ($P> 0.05$) against the parasite. This result agrees with Al-Wahach (26). The highest concentrations (2000 mg\ml) were eliminated all the parasites and showed good activity in all forms represented the second group consist of *Punica granetum; Artemisia campestris; Eucalyptus comaldulensis; and Achillea santolina*. These plants revealed significant activity ($P< 0.05$) against *Giardia lamblia* trophozoites. These viability percentage at concentration (1000mg\ml) showed (25%); (28.5%); (42.8%); and (57%). The highest concentrations eliminated all the parasites, where no considerable activity was observed at the lowest concentrations. *Punica granetum* had good activity for diarrhea and dysentery (13). It contains the Alkaloids pelletierine (14). *Artemisia campestris* contains santonin, resin, abinthin and thujone and also it was reported to yield camphor (27). Hajawi , *et al* (28) reported that plant *Eucalyptus comaldulensis* contains ecalyptol; phellanderene and pinene. Al-sawah (29)

was reported that the plant *Achillea santolina* contains sesquiterpene lactones which has an insecticidal activity.

The usage of plants may be with less toxicity as reported by Al-Jeboory (30) (drugs isolated from plants are generally less toxic than synthetic drugs), while all available literature agrees that all drugs currently used in the treatment of *Giardia lamblia* are carcinogenic and expensive (31), on the other hand, the procedure of extraction that were adopted in the present study is not too costly since we needed distilled water only as solvents in addition to the non-costly types of plants and their availability in local market. Plants had played an important role in the history of humanity for providing crude drugs along the past centuries. In addition the natural plants drugs served as useful prototypes for even better medicine (32). According to the above results which recorded an *in vitro* antigiardial activity in some extracts of Iraqi medicinal plants, and the *in vitro* activity may be used as a rough predictor *in vivo* activity (33). Thus, it is concluded that these plants are promising and important for the future treatment of giardiasis and research should continue to prove their *in vivo* activity and their safety for humane use without side effects in order to achieve the aim of using the plants extracts safely and effectively in the treatment of Giardiasis and research should continue to prove their *in vivo* activity and their safety for human use without side effects. In order to achieve the aim of using the plants extracts safely and effectively in the treatment of human and animals giardiasis I am like to recommend that the use of laboratory animals, infected with *Giardia lamblia*.

And studying the effect of the crude extracts in the treatment of these infected animals. Further studies, including sub-fractionation of the extracts using appropriate pharmacognostic method such as chromatography to isolate, identify, and study the active compounds as aphytotherapy, to facilitate their used in human and animals (34).

The data obtained from the present study demstrated *in vitro* that the a queaus exract of some plants on viability percrntage of *G-lamblia* trophozaites in differentes concentrations

Plants	31.25 mg/ml	62.5 mg/ml	125 mg/ml	250 mg/ml	500mg/ml	1000mg/ml	2000mg/ml	Conc.
	%viable	%viable	%viable	%viable	%viable	%viable	%viable	%viable
Cominum Cyminum	100%	100%	100%	85.7%	85.7%	62.5%	25%	
Punica Granetum	100%	87.5%	75%	62.5%	50%	25%	0	
Magifera Indica	100%	100%	100%	100%	100%	87.5%	75%	
Artemisia Campestris	100%	87.5%	75%	57%	50%	28.5%	0	
Eucalyptus Camaldulensis	100%	100%	85.7%	71.4%	57%	42.8%	0	
Achillea Santolina	100%	87.5%	75%	71.4%	66.6%	57%	0	

Table (1) The mean viability of *Giardia Lamblia in Caminum Cyminum*

31-25 mg/ml				62 mg/ml				125mg/ml				250mg/ml			
V	d	T	%	V	d	T	%	V	d	T	%	V	d	T	%
8	0	8	100	8	0	8	100	8	0	8	100	6	1	7	85.7
500mg/ml				**1000mg/ml**				**2000mg/ml**							
V	d	T	%	V	d	T	%	V	d	T	%				
6	2	8	85.7	5	3	8	62.5	2	6	8	25				

v=viable
d=dead
T=total
%=percentage

Table (2) The mean viability of *Giardia Lamblia in Punica Grantium*

31-25 mg/ml				**62 mg/ml**				**125mg/ml**				**250mg/ml**			
V	d	T	%	V	d	T	%	V	d	T	%	V	d	T	%
7	0	7	100	7	1	8	87.5	6	2	8	75	5	3	8	62.5
500mg/ml				**1000mg/ml**				**2000mg/ml**							
V	d	T	%	V	d	T	%	V	d	T	%				
4	4	8	50	2	6	8	25	0	8	8	0				

v=viable
d=dead
T=total
%=percentage

Table (3) The mean viability of *Giardia Lamblia in Mangifera indica*

31-25 mg/ml				**62 mg/ml**				**125mg/ml**				**250mg/ml**			
V	d	T	%	V	d	T	%	V	d	T	%	V	d	T	%
6	0	6	100	6	0	6	100	7	0	7	100	8	0	8	100
500mg/ml				**1000mg/m**				**2000mg/ml**							
V	d	T	%	V	d	T	%	V	d	T	%				
8	0	8	100	7	1	8	87.5	6	2	8	75				

v=viable
d=dead
T=total
%=percentage

Table (4) The mean viability of *Giardia Lamblia in Artemisia campestris*

31-25 mg/ml				62 mg/ml				125mg/ml				250mg/ml			
V	d	T	%	V	d	T	%	V	d	T	%	V	d	T	%
8	0	8	100	7	1	8	87.5	6	2	8	75	4	3	7	57
500mg/ml				**1000mg/m**				**2000mg/ml**							
V	d	T	%	V	d	T	%	V	d	T	%				
3	3	6	50	2	5	7	28.5	0	8	8	0				

v=viable
d=dead
T=total
%=percentage

Table (5) The mean viability of *Giardia Lamblia in Eucalyptus camaldulensis*

31-25 mg/ml				62 mg/ml				125mg/ml				250mg/ml			
V	d	T	%	V	d	T	%	V	d	T	%	V	d	T	%
7	0	7	100	8	0	8	100	6	1	7	85.7	5	2	7	71.4
500mg/ml				**1000mg/m**				**2000mg/ml**							
V	d	T	%	V	d	T	%	V	d	T	%				
4	3	7	57	3	4	7	42.8	0	7	7	0				

v=viable
d=dead
T=total
%=percentage

Table (6) The mean viability of *Giardia Lamblia in Achillea santolina*

31-25 mg/ml				62 mg/ml				125mg/ml				250mg/ml			
V	d	T	%	V	d		%	V	d		%	V	d		%
8	0	8	100	7	1	8	87.5	6	2	8	75	5	2	7	71.4
500mg/ml				**1000mg/m**				**2000mg/ml**							
V	d	T	%	V	d	T	%	V	d	T	%				
4	2	6	66.6	4	3	7	57	0	7	7	0				

v=viable
d=dead
T=total
%=percentage

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