



## Comparison Between the Effect of Metronidazole and some Medical Herbs Extracts on Viability of *Entamoeba histolytica* Trophozoites in Vitro

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### ABSTRACT

*Amoebiasis is is widely distributed in Iraq. In order to search for alternative new anti-amebic drugs, a study was planned to evaluate the efficacy of metronidazole and some medical herbs on the viability of Entamoeba histolytica trophozoites in vitro culture.*

*E. histolytica was isolated from stool examination of patients attended Pediatric Hospital Kirkuk, using wet mount technique. The active trophozoites were cultured in Lock egg medium.*

*After isolation of trophozoites tested against various concentrations of metronidazole, and medical herbs including Artemisia herba alba, Punica granatum and Tecurium polium , using viability Pandroff tube, which contain Jones broth medium. Both aqueous and alcoholic extractions of medical herbs were used, at concentrations of 50%, 25%, 12.5% and 6.25%.*

*Results: Metronidazole had highest inhibitory effect on viability of E. histolytica trophozoites than medical herbs. Medical herbs, Artemisia herba alba, recorded highest inhibitory properties against Entamoeba histolytica, followed by Punicaa granatum and Tecurium polium. The effect of alcoholic extract of all medical herbs was greater than aqueous extracts*

**Conclusions:** The efficacy of metronidazole was greater than medical herbs. The alcoholic extraction of medical herbs had greatest effect on viability of trophozoites than aqueous ones. Medical herbs can be used as an alternative of metronidazole.

**Keywords:** *Entamoeba histolytica*, metronidazole, medical herbs, in vitro.

## المقارنة بين كفاءة الميترونيدازول ومستخلصات بعض الاعشاب الطبية على

### طفيلي اميبا الزحار في الزجاج

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### الملخص

مرض الزحار الأميبي من الأمراض المنتشرة بشكل واسع في العراق، وبالتالي فإن إيجاد الطرائق الكفيلة بمعالجته والقضاء عليه من الضروريات التي يتحتم إتمامها وذلك للتخلص أو التقليل من نسب الإصابة لمنع اي مضاعفات ناتجة عن المرض , لغرض البحث عن علاج جديد ويديل ضد الاميبا ، تم تخطيط دراسة لتقييم كفاءة الميترونيدازول ومستخلصات بعض النباتات الطبية على حيوية الطور الخضري لطفيلي (اميبا الزحار) في الوسط الزرعي في الزجاج. تم استخدام وسط Locke egg medium وذلك لغرض تنمية الطور الخضري للطفيلي وعزله، وتم تنمية الأطوار الخضرية للطفيلي بنجاح باستخدام هذا الوسط. بعد عزل الأطوار الخضرية تم اجراء فحص الحيوية عليها وباستخدام قناني پاندروف الحاوية على وسط Jones medium السائل حيث تم استخدام تراكيز مختلفة من دواء مترونيدازول، إ تراكيز مختلفة من الأعشاب الطبية والتي شملت الشيح وقشر الرمان والجعدة. تم تقدير حيوية الطفيلي اعتمادا على التغيرات الحاصلة من انكماش وتحطم الغشاء الخارجي للطفيلي.

و ان تركيز المستخلصات الكحولية والمائية للنباتات الطبية كانت بتراكيز 6.25%,12.5%,25%,50% على التوالي.

سجلت النتائج فعالية كل من الميترونيدازول في القضاء على حيوية الطفيلي اكبر من لأعشاب الطبية وأما بالنسبة لمستخلصات الاعشاب الطبية فقد سجل فعالية كبيرة للشاي بمستخلصيها الكحولي والمائي في القضاء على الطفيلي ويليهما في الفعالية قشر الرمان والجعدة.

الكلمات الدالة: اميبا الزحار، ميترونيدازول، الاعشاب الطبية، في الزجاج

## 1. INTRODUCTION

Eradication of *Entamoeba histolytica* may be difficult. Available treatments using metronidazole, diloxanide furate, tetracycline and others are imperfect at best and have toxic side effect such as gastric upset, optic atrophy, bitter taste, dermatitis and possibly carcinogenic [1]. In addition, treatment failures in as many as 20% of cases raise concern about possible resistance to the used drugs and especially during pregnancy [2]. Metronidazole is the drug of choice for amoebiasis although failure following metronidazole therapy has been reported in some cases of liver abscess due to *E. histolytica* [3].

Medical herbs have recently been widely used around the world due to their natural constituent and absence of any side effect. Medical herbs used were selected for their efficiency and prosperities in treatment of abdominal disturbance, amoebic dysentery, and other intestinal parasitic and bacterial infection [4].

This study was planned to evaluate the amoebic potency of metronidazole and some medical plant extracts (*Artemisia herba- alba*, *Punica granatum* and *Tecurium polium*).

## 2. Materials and Methods

### Collection of stool samples

Stool samples were collected from Kirkuk Pediatric hospital, Kirkuk General Hospital, Primary Health Care Center (PHC) from beginning of February 2014 to end of January 2015. The samples were examined for the presence of *Entamoeba histolytica* using wet mount technique. When the samples showed active trophozoite motility they were cultured in Boeck and Dboholve's Locke egg medium [5].

### Viability testing

After cultivation of *Entamoeba histolytica* for 48 hours in culture medium and successful production of large number of trophozoite, the trophozoites were prepared for use in the viability tests. *Entamoeba histolytica* trophozoites from cultures of different samples were used. The medium solution was centrifuged for 2 minutes at 2000 rpm then transferred into sterile serum tube, 0.2 ml was taken from this solution and trophozoite number was counted using haemocytometer chamber, then mixed with 0.3 ml of Jones medium and added into pandroff tube, incubated at 37°C for 12 days, being examined everyday by using a haemocytometer chamber. The viability was assessed as number of viable parasite, number of dead parasite per microscope field. The percentage of viability was calculated as a following formula [6].

$$\% \text{ viability} = \frac{\text{No. living parasite/ml}}{\text{Total No./ ml}} \times 100$$

### Preparation of Medical herbs

Plants were obtained from a local grocery which patronizes traditional plants in Kirkuk province, and the species were confirmed in the traditional plant center/ College of Science / Tikrit University. Medical herbs were firstly cleaned from dust, unwanted parts excluded using sieve. They were cut thoroughly into small parts to increase surface area; the 500 mg of plant was weighted. Added to Pyrex beaker, 500 ml of Distilled Water was added into beaker. The mixture was boiled for one hour by a hot plate at 60°C. Heat source was removed and the beaker left to cool at room temperature. Solution filtered by sterile gauze then by filter paper. (Schleichers and Schuell GmbH filter paper, Germany). The filtrate was transferred into a suitable plastic container, and kept in refrigerator until used.

Two methods were used in medical plants preparation; Maceration method, which is also called alcoholic extraction, the extraction solvent was ethanol 95% [7] and Infusion method. in which, the lighter parts of plants (leaves, flower, light stems) were steeped in boiled water for 15-30 minute [8].

### 3. Results

Table (1) show the effect of three concentration of metronidazole on *Entamoeba histolytica* during days of incubation, totally reduction of viability at (100 mg /ml) was occur on day 3; on day 6 at 50 mg/ml and 25 mg/ml.

**Table (1):** The effect of different concentration of metronidazole on viability of *Entamoeba histolytica* in comparison to control

Metronidazole Concentration	Days (%)						T test	P value
	2	4	6	8	10	12		
100mg/ml	1.6	0	0	0	0	0	-3.54	<0.05
50mg/ml	3.35	1.65	0	0	0	0	-3.52	<0.05
25mg/ml	5	3.35	0	0	0	0	-3.48	<0.05
Control	259.2	153.8	63.3	50	11.6	0		

Note: (%) represent viable parasite.

*Artemisia herba Alba* aqueous extraction shows good lethal activity, especially at concentration 50% when totally reductions occur on day 8; and on day 10 at concentration 25%, 12.5% and 6.25%, with significant difference (P<0.05) in comparison to the control. Alcoholic extraction of *Artemisia herba- alba* shows high efficacy in killing parasite in comparison to control (P<0.05), totally reduction of viability occurred on day 6 at concentration 50% and 25%, and on day 8 at 12.5% and 6.25% concentration Table (2).

**Table (2):** The effect of different concentration of *Artemisia herba alba* extraction (aqueous & alcoholic) on viability of *Entamoeba histolytica* in comparison to control.

Medical Herb Concentration	Days (%)						T-test	P-value
	2	4	6	8	10	12		
Ar.h.a. Aqueous 50%	8.35	6.65	1.65	0	0	0	-3.42	<0.05
25%	13.35	10	3.35	1.65	0	0	-3.32	<0.05
12.5%	13.75	7.5	5.0	1.65	0	0	-3.28	<0.05
6.25%	28.5	12.5	3.35	1.65	0	0	-3.10	<0.05
Ar.h.a.Alcoholic 50%	5.0	1.65	0	0	0	0	-3.51	<0.05
25%	5.0	1.65	0	0	0	0	-3.49	<0.05
12.5%	5.85	5.0	1.65	0	0	0	-3.44	<0.05
6.25%	11.25	6.7	3.35	0	0	0	-3.39	<0.05
Control	259.2	153.8	63.3	50	11.6	0		

Key's: Ar.h.a= *Artemisia herba Alba*,

Note: (%) represent viable parasite.

In comparison to control, aqueous extraction of *Punica granatum* shows significant effect ( $P < 0.05$ ) especially at 50% and 25% concentration, on day 8 at 12.5% concentrations; and on day 10 at 6.25%. Alcoholic extraction of *Punica granatum* shows a similar effect to the aqueous ones in reduction the viability of *Entamoeba histolytica*. Total reduction occurred on day 8 at 50%, 25% and 12.5% concentrations, but at 6.25% on day 10 Table (3).

**Table (3):** The effect of different concentrations of *Punica granatum* extraction (aqueous & alcoholic) on viability of *Entamoeba histolytica* in comparison to control.

Medical Herb Concentration	Days (%)						T-test	P-value
	2	4	6	8	10	12		
Pu. G Aqueous 50%	7.9	3.35	3.35	0	0	0	-3.43	<0.05
25%	11.85	5.0	1.65	0	0	0	-3.40	<0.05
12.5%	20.4	6.7	5.0	0	0	0	-3.22	<0.05
6.25%	30.55	16	8.35	3.35	0	0	-3.11	<0.05
Pu. G Alcoholic 50%	5.0	3.35	1.65	0	0	0	-3.48	<0.05
25%	5.85	5.0	1.65	0	0	0	-3.44	<0.05
12.5%	20.15	6.7	1.65	0	0	0	-3.31	<0.05
6.25%	21.85	8.35	5.0	1.65	0	0	-3.21	<0.05
Control	259.2	153.8	63.3	50	11.6	0		

Key's: *Pu. g.* = *Punica granatum*

Note: (%) represent viable parasite.

Aqueous extraction of *Tecurium polium* shows low effect on viability of *Entamoeba histolytica* reduction of viability occur on day 10 in concentrations 50% , 25% and 12.5%, on day 12 at concentration 6.25%, *Tecurium polium* showed non significant effect at concentration 12.5% and 6.25%. The viability of *Entamoeba histolytica* treated with alcoholic extraction of *Tecurium polium* was affected very slowly. Total reduction of viability occur on day 10 at 50%, 25%, and 12.5%, and on day 12 at concentration 6.25% but the results were considered significant statistically (P<0.05) as seen in Table (4).

**Table (4):** The effect of different concentrations of *Tecurium polium* extraction (aqueous & alcoholic) on viability of *Entamoeba histolytica* in comparison to control.

Medical Herb Concentration	Days (%)						T-test	P-value
	2	4	6	8	10	12		
Te. P.Aqueous 50%	24.35	21.6	15.0	3.35	0	0	-3.00	<0.05
25%	49.2	17.5	13.3	5.0	0	0	-2.71	<0.05
12.5%	117.5	89.5	50	5.0	0	0	-1.53	>0.05
6.25%	165.9	104.5	45	10	1.65	0	-0.97	>0.05
Te. P.Alcoholic 50%	19.95	12.5	10	5.0	0	0	-3.71	<0.05
25%	45.3	17.5	10	5.0	0	0	-2.85	<0.05
12.5%	70.7	22.0	13.3	3.35	0	0	-2.51	<0.05
6.25%	69	25.6	32.2	13.3	1.65	0	-2.14	<0.05
Control	259.2	153.8	63.3	50	11.6	0		

Key's: Te.p. = *Tecurium polium*

Note: (%) represent viable parasite.

#### 4. Discussion

Metronidazole treated tubes showed high declining in trophozoite count, total reducing of viability reached at 3<sup>rd</sup> day in 100 mg/ ml concentration, and on 5<sup>th</sup> day and 6<sup>th</sup> day in 50mg /ml and 25 mg/ ml respectively. In a relative study metronidazole showed total reducing of viability after 48 hours at 100 µg/ ml [9]. Metronidazole activity was due to trapping electrons by virtue of its very low redox potential. The generation of H<sub>2</sub> from pyruvate is halted and the organism soon becomes depleted of NADH and NADPH. The nitro ring of metronidazole becomes cleaved in the process producing toxic substances that hasten cell death [10]. Metronidazole requires at least 10 days at high dosage to eradicate luminal amoebae [11].

Aqueous extraction of *Artemisia herba-Alba* shows significant effect on reduction of the viability of *Entamoeba histolytica*, complete reduction of viability occurs on day eight of incubation using 50% concentration and on day nine in 25%, 12.5%, 6.25% concentrations. The efficacy of alcoholic extraction was greater than the aqueous one, total reduction of



viability reported after four days of incubation in 50% and 25% concentration, and after seven days in 12.5% and 6.25% concentration. From these results, it appears that alcoholic extraction of *Artemisia herba alba* was more effective than the aqueous ones in the inhibition of the *Entamoeba histolytica* growth and considers very close to effect of metronidazole. AntiParasitic effect of *Artemisia herba alba* may be due to the presence of sesquiterpene lactones [12], and flavonoids [13], thujone, santonin, the sterols beta- sitosterol and stigmasterol [14].

The same results for *Punica granatum* aqueous and alcoholic extraction effect were reported against *Entamoeba histolytica*, except variance in the trophozoite count which is lower in alcoholic extraction. Total reduction of viability occur on day 7 from incubation at 50% and 25% concentration and occur in day 8 and 9 at 12.5% and 6.25% respectively. In relative studies (15) showed that alcoholic extraction for *Punica granatum* was more effective than aqueous ones in inhibitory properties. *Punica granatum* efficiency on amoebic dysentery may be attributed to the presence of tannins and alkaloids. In a study to evaluate biological activity of alkaloids extracted from roots and rinds (aqueous extraction) of *Punica granatum* on axenic cultures from *Entamoeba histolytica* and *Entamoeba invadens*. It has been shown [16] that 2 milliliters of aqueous extraction have complete inhibitory effect on *Entamoeba histolytica* and *Entamoeba invadens* in about 100% and 40% respectively. It was found that extraction made from the rind of *Punica granatum* have great competency against parasitic infection in which its action was attributed to alkaloids [17]. Although *Punica granatum* contain pelleltierine alkaloids which are considered as antihelminthic (especially cestodes) and large scale of protozoa [18], several studies showed that extraction of *Punica granatum* roots, rinds, and bark can be treated as amoebic dysentery because these components change the protinous nature of the intestine and reduces fluid flow, in addition to their antibacterial and relieving of bacterial poisons [19].

Aqueous extraction of *Tecurium polium* shows low effect on the viability of *Entamoeba histolytica*, which persists until day 10 at 50% and 25% concentrations, while no significant differences were recorded in comparison to control at 12.5% and 6.25%, alcoholic extraction shows results near to that recorded from aqueous extraction except that it shows significant differences at 12.5% and 6.25% concentration in comparison to control. The herbs possess hypoglycemic, insulintropic, and anti- inflammatory activities [20]. *Tecurium polium* have antispasmodic and anti bacterial activity [21]. These properties promote the use of *Tecurium*



*polium* against *Entamoeba histolytica* in the present study. Lower activity of *Tecurium polium* against *Entamoeba histolytica* may be due to antioxidant prosperities which promote the anaerobic condition [22], and the anaerobic conditions are preferred by *Entamoeba histolytica* to grow at good scale in the culture medium [23].

It is concluded that the effect of metronidazole is the best against *E. histolytica* in vitro studies in comparison with medical herbs. Medical herbs (*Artemesia herba alba* and *Punica granatum*) have competent effect on *E. histolytica* viability, while *Tecurium polium* had lowest effect on viability of trophozoite. The effect of alcoholic extracts was greater than aqueous ones. TLC could be used for isolation and identification of chemical compounds. The best solvent system was the solvent consisting of butanole, acetic acid and distilled water.

It is recommended to carry on in vivo studies in order to determine the best concentration that can be used against amebic dysentery. Specific studies on medical herbs should be done with more advanced techniques like HPLC in order to identify the active chemical compounds present in medical herbs and to use it as alternative for chemical drugs with undesired side effects.

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