

## Effect of Some Medical Plant Extracts on Metabolism of *Leishmania tropica* Promastigotes *In vitro*

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### Abstract

The study included the inhibition effect of *Equisetum arvense* and *Urtica piluifera* extracts dissolved in cold and hot water, on the growth of *Leishmania tropica* promastigotes, and the effect of these extract on metabolism activates (Total protein, carbohydrates and nucleic acids) of *Leishmania tropica* Promastigotes.

The inhibitory concentration of 50% of the promastigotes (IC<sub>50</sub>) at the log phase (96) hrs was 1.5 µg/ml of *Equisetum arvense* and 1.5 µg/ml of *Urtica piluifera* extracts dissolved in cold and hot water.

The results revealed, that these extracts were studied has inhibitory effect *L. tropica* promastigotes number, the number of *L. tropica* reduced gradually when using 0.5 to 2.5 µg/ml concentrations of extracts. Moreover, these extracts were studied has inhibitory effect on total Proteins, Carbohydrates and Nucleic acids. The results of chemical analysis of the plants has highly effect on total protein, and lees effect on total carbohydrates of cell membrane, Moreover, the extract was effect on total Nucleic acids of *Leishmania tropica* promastigotes after 96 hrs of cultivation.

**Keywords:** *Lieshmania tropica*; *Equisetum arvense*; *Urtica piluifera* Treatment; Proteins; Carbohydrates; Nucleic acid

### Introduction

Leishmaniasis is a disease caused by hemoflagellate protozoa species of the genus *Leishmania* [1]. The reservoirs of the disease are rodents, dogs, sanguine marsupials and other wild animals. The disease is transmitted by mosquitoes of the genera *Lutzomyia* and *Phlebotomus*. According to the World Health Organization, 88 countries are affected by leishmaniasis, with approximately 350 million people at risk. Comprehending 12 million infected cases, the incidence is increasing worldwide with 1-2 million new cases registered annually, despite efforts being made to fight the disease [2]. Leishmaniasis encompasses three clearly distinguishable clinical manifestations: generalized visceral infection (visceral leishmaniasis or “Kala-azar”), cutaneous leishmaniasis (oriental button), and mucocutaneous leishmaniasis (ulceration of the skin and hyper-development of the mucous membrane) [3].

Leishmaniasis can be controlled mainly by vector and reservoir control and infected case management. The first two approaches are either impractical or expensive, leaving only the case control as the most effective strategy [2].

The best drug for the treatment of leishmaniasis is the pentavalent antimonies, which have been in use for more than 50 years. High failure rates and relapses are observed, particularly in treating co-infected patients, using these drugs [4].

The chemotherapy of this disease has been undermined by resistance, variable efficacy between strains or species, toxicity, parenteral administration, and requirement for long courses of administration. There is an urgent need for new chemotherapeutic drugs for the treatment of these diseases which mainly affect people in developing countries. Extensive studies have shown that medicinal plants of several regions of the world contain compounds active against protozoa [5].

The current study was undertaken to of *Equisetum arvense* and *Urtica piluifera* extracts dissolved in cold and hot water, used for the treatment of *Leishmania tropica* promastigotes effect on growth or on change in metabolism of parasite

### Materials and Methods

#### *Leishmania tropica* promastigotes

MHOM/IQ/1992/MREC3 *Leishmania tropica* stock culture was used. The culture was obtained from the College of Medicine AL-Nahreen University, which has been characterized using isoenzyme method according to AL-Jeboori and Evans [6]. The obtained culture was cultivated in Tobie’s medium [7]. The parasites were checked under the microscope for flagella motility as an indication of viability before each experiment.

#### Collection of plants

The aerial parts of *Equisetum arvense* and *Urtica piluifera* were collected from Tikrit and Mousel city in Iraq.

#### Preparation of extracts

The plant parts were cleaned with water to remove dirt, then placed in a glass with diluted chlorine (1%) for one minute, the plant

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**Received** May 24, 2014; **Accepted** September 19, 2014; **Published** September 21, 2014

**Citation:** Saeed BQ, Hassan HF, Arteen HI (2014) Effect of Some Medical Plant Extracts on Metabolism of *Leishmania tropica* Promastigotes *In vitro*. J Med Microb Diagn 3: 165. doi:[10.4172/2161-0703.1000165](http://dx.doi.org/10.4172/2161-0703.1000165)

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parts were dried at room temperature, Then kept in free of moisture conditions in paper envelopes [8].

The plant extract prepared as, A volume of (0.25 g) from each extract was dissolved in 5 ml of hot and cold water to produce a final concentration of 50 mg/ml which was used as a stock concentration in providing next dilutions (0.5, 1.0, 1.5, 2.0 and 2.5) µg/ml all extracts were sterilized by filtration through a 0.45 µm membrane filter [9].

### Cultivation and estimating numbers of parasites

1.9 ml of liquid phase was added to McCantry vials containing 5 ml solid phase slants, 0.1 ml of *Leishmania* promastigotes inoculum was taken from stock culture during logarithmic phase, so that the initial density of the organism was  $2 \times 10^5$ /ml, then the number of organisms of new culture incubated at 26°C for 4 days, was counted directly using a haemocytometer.

### Estimation of total proteins, carbohydrates, and nucleic acids (DNA and RNA)

The Total Protein quantitative determination estimated by Lowry's Method [10], while Herbert method [11] were carried out to estimate total carbohydrates, Schneider's method [12,13] was used for quantitative estimation of total Nucleic acids, DNA and RNA in treated and untreated parasites.

## Results

The results of effect of *E. arvense* on *L. tropica* promastigotes are presented in Table 1. The number of *L. tropica* decreased gradually by using 0.5 to 2.5 µg/ml concentrations of *E. Arvense* extract, Moreover, the extracts effect on number and time of generation, an inverse relationship could be established between concentration of the extract and growth mean of the parasite. Inhibitory concentration of 50% of promastigotes (IC<sub>50</sub>) was 1.5 µg/ml, whereas at logarithmic phase (96 hrs of cultivation).

Table 2 show the number of *Leishmania tropica*. promastigotes decreased gradually by using different concentrations 0.5 to 2.5 µg/ml of *Urtica piluifera* extract dissolve in cold water from 47.4 at 24 h to 15.6 after 96 hrs of cultivation, Moreover, Inhibitory concentration of 50% of promastigotes (IC<sub>50</sub>) was 1.0 µg/ml, whereas at logarithmic phase (96 hrs of cultivation).

Table 3 shows the inhibitory effects of different concentrations of the *U. Piluifera* dissolve in hot water 0.5 to 2.5 µg/ml, on *Leishmania* growth in comparison with control group, during different time intervals. Relatively all concentrations used demonstrated significant inhibitory effects against *Leishmania* promastigotes. An inverse relationship could be established between concentration of the extract and growth mean of the parasite. Inhibitory concentration of 50% of

Hours	24		48		72		96	
	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%
Control	0 ± 1.10a	100	0.005 ± 3.60a	100	0.025 ± 7.25a	100	0.011 ± 12.53a	100
0.5	0.005 ± 0.88c	80	0.028 ± 2.16c	60	0.022 ± 6.55c	90.3	0.022 ± 11.91c	95
1	0.023 ± 0.75d	68.1	0.025 ± 1.66d	46.1	0.055 ± 4.80d	66.2	0.044 ± 7.70d	61.4
1.5	0.005 ± 0.53e	68.1	0.014 ± 1.60e	44.4	0.027 ± 3.52e	48.5	0.025 ± 5.82e	46.4
2	0.022 ± 0.43f	48.1	0.021 ± 1.050f	29.1	0.014 ± 1.15f	15.8	0.011 ± 4.12f	32.8
2.5	0.006 ± 0.27g	39.0	0.01 ± 0.90g	25	0.06 ± 1.05g	14.1	0.057 ± 2.03g	16.2

\*Three replicates were used for each treatment Mean and Standard Deviation were multiplied x 105  
Different letters refers to presence of significant differences between treatments at P ≤ 0.05, according to Duncan's test [13].

**Table 1:** Effect of different concentrations of *E. arvense* on numbers of *Leishmania tropica*. promastigotes at different time intervals.

Hours	24		48		72		96	
	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%
Control	0.038 ± 1.18a	100	0.028 ± 4.13a	100	0.014 ± 8.74a	100	0.009 ± 20.00100 a	
0.5	0.030 ± 0.95c	80.5	0.011 ± 3.77c	91.2	0.005 ± 7.05c	80.6	0.26 ± 16.08c	80.4
1	0.018 ± 0.91d	77.1	0.009 ± 1.85d	44.7	0.022 ± 4.31d	49.3	0.43 ± 10.20d	51
1.5	0.009 ± 0.81e	68.6	0.30 ± 1.37e	33.1	0.001 ± 2.30e	26.3	0.038 ± 7.14e	35.7
2	0.002 ± 0.65f	55	0.007 ± 1.12f	27.1	0.005 ± 2.07f	23.6	0.008 ± 4.93f	24.6
2.5	0.002 ± 0.65f	47.4	0.071 ± 0.89g	21.5	0.057 ± 1.34g	15.3	0.057 ± 3.13g	15.6

\*Three replicates were used for each treatment Mean and Standard Deviation were multiplied x 105  
Different letters refers to presence of significant differences between treatments at P ≤ 0.05, according to Duncan's test [13].

**Table 2:** Effect of different concentrations of *U. Piluifera* dissolve in cold water on numbers of *Leishmania tropica*. promastigotes at different time intervals.

Hours	24		48		72		96	
	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%	Mean +/- SD*	Growth%
Control	0.076 ± 1.06a	100	0.12 ± 2.40a	100	0.43 ± 4.80a	100	0.076 ± 10.33a	100
0.5	0.003 ± 0.87c	82	0.006 ± 2.10c	87.5	0.028 ± 4.13c	86	0.008 ± 9.28b	89.8
1	0.009 ± 0.80d	75.4	0.014 ± 1.03d	42.9	0.086 ± 2.40d	50	0.016 ± 5.00c	48.4
1.5	0.011 ± 0.60e	56.6	0.002 ± 0.89e	37	0.26 ± 1.33e	27.7	0.004 ± 2.95d	28.5
2	0.028 ± 0.63e	59.4	0.004 ± 0.69f	28.7	0.025 ± 1.06f	22	0.115 ± 2.36e	22.8
2.5	0.028 ± 0.43f	40.5	0.006 ± 0.56g	23.3	0.005 ± 0.90g	18.7	0.009 ± 1.26f	12.1

\*Three replicates were used for each treatment Mean and Standard Deviation were multiplied x 105  
Different letters refers to presence of significant differences between treatments at P ≤ 0.05, according to Duncan's test [13].

**Table 3:** Effect of different concentrations of *U. Piluifera* dissolve in hot water on numbers of *Leishmania tropica*. promastigotes at different time intervals.

Treatment	IC50(µg/ml)	Total Protein µg/ml Mean +/- SD*	Total Protein %	% variation	
Control	---	2.8 ± 437.33a	100	---	---
<i>E. arvense</i>	1.5	0.175 ± 403.33b	92.2	---	7.8
<i>U. Piluifera</i> dissolve in Cold water	1	0.005 ± 372.67c	85.2	---	14.8
<i>U. Piluifera</i> dissolve in Cold and Hot water	1	0.05 ± 346.0d	79.1	---	20.9

\*Three replicates were used for each treatment Mean and Standard Deviation were multiplied×105  
Different letters refers to presence of significant differences between treatments at P ≤ 0.05, according to Duncan's test [13].

**Table 4:** Effect of IC50 of *E. arvense* and *U. Piluifera* dissolve in Cold and Hot water on the amount of total protein (µg/ml) of *L. tropica*. promastigotes at log-phase (96 hrs).

Treatment	IC50(µg/ml)	Total Protein µg/ml Mean +/- SD*	Total Protein %	% variation	
Control	---	5.61 ± 165.0a	100	---	---
<i>E. arvense</i>	1.5	2.38 ± 152.5b	92.2	---	7.6
<i>U. Piluifera</i> dissolve in Cold water	1	2.88 ± 137.3c	83.2	---	16.8
<i>U. Piluifera</i> dissolve in Cold and Hot water	1	0.46 ± 135.0d	81.8	---	18.2

\* Three replicates were used for each treatment Mean and Standard Deviation were multiplied×105  
Different letters refers to presence of significant differences between treatments at P ≤ 0.05, according to Duncan's test [13].

**Table 5:** Effect of IC50 of *E. arvense* and *U. Piluifera* dissolve in Cold and Hot water on the amount of total Carbohydrates (µg/ml) of *L. tropica*. promastigotes at log-phase (96 hrs).

Treatment	IC50(µg/ml)	DNA µg/ml Mean +/- SD*	% Concentration	% Decrease	RNA µg/ml Mean +/- SD*	% Concentration	% Decrease	Total Nucleic acid (DNA & RNA) µg/ml Mean +/- SD*	% Concentration	% Decrease
Control	---	1.73 ± 33.0a	100	---	0.1 ± 74.0a	100	---	0.57 ± 107.0a	100	---
<i>E. arvense</i>	1.5	2.8 ± 30.0b	90.9	9.1	2.7 ± 50.5b	67.5	32.5	2.8 ± 80.0b	74.7	25.3
<i>U. Piluifera</i> dissolve in Cold water	1	2.8 ± 23.0c	69.6	30.4	2.7 ± 50.0c	67.5	32.5	3.2 ± 73.0 c	68.2	31.8
<i>U. Piluifera</i> dissolve in Cold and Hot water	1	0.02 ± 22.3d	67.5	32.5	2.8 ± 46.6 d	62.9	37.1	4.1 ± 68.9 d	64.3	35.7

Three replicates were used for each treatment Mean and Standard Deviation were multiplied×105  
Different letters refers to presence of significant differences between treatments at P ≤ 0.05, according to Duncan's test [13].

**Table 6:** Effect of IC50 of *E. arvense* and *U. Piluifera* dissolve in Cold & Hot water on the amount of nucleic acid (µg/ml) of *L. tropica* promastigotes at log-phase (96 hrs).

promastigotes (IC50) was 1.0 µg/ml, whereas at logarithmic phase (96 hrs of cultivation).

The number of *L. tropica*. promastigotes decreased gradually for 2.5 from 40.5 after 24 hour to 18.7 after 72 hour and to 12.1 after 96 hrs of cultivation.

The *Equisetum arvense* and *U. Piluifera* dissolve in Cold and Hot water found to cause reduction in protein content in *Leishmania tropica*. promastigotes that were treated with IC50 of the tested extracts (Table 4). The extracts found to reduce to (7.8%), (14.8%) and (20.9%) respectively at log-phase (96 hrs), Moreover, this study show the effect of *E. arvense* and *U. Piluifera* dissolve in Cold and Hot water on total Carbohydrates of *L. tropica*. promastigotes (Table 5), The table shows that the carbohydrates rate *L. tropica*. decreased to (7.6%), (16.8%) and (18.2%) respectively by IC50 concentrations of the extract at log-phase (96 hrs).

Table 6 includes the result of effect of *E. arvense* and *U. Piluifera* dissolve in Cold and Hot water on the total of Nucleic acid (DNA and RNA) of *L. tropica*. promastigotes at log-phase (96 hrs), the total (DNA and RNA) reduced to (9.1%) for DNA, (23.5%) for RNA and ( 25.3%) for total nucleic acid when treated with IC50 of the *E. arvense* extract, and reduced to (30.4%) DNA, (32.5%) RNA and ( 31.8%) total nucleic acid when treated with IC50 of the *U. Piluifera* dissolve in cold water, moreover, the IC50 of *U. Piluifera* dissolve in Hot water reduced of

the DNA (32.5%), RNA (37.1%) and (35.7%) of total nucleic acid with IC50 extract at log-phase (96 hrs).

## Conclusion

Therapeutic evaluations for medicinal plants are essential because of the growing interests in alternative therapies and the use of natural products. Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans [14].

*Equisetum arvense* is a plant with wide prospectus. In folk medicine, *Equisetum arvense* is used for, pulmonary and gastric haemorrhages, for brittle fingernails and loss of hair, for rheumatic diseases, gout, poorly healing wounds and ulcers, swelling and fractures and for frostbit. The plant is reported to contain a number flavonoids, alkaloids, minerals, phenolic petrosins, triterpenoids, saponins, phytosterols [15], moreover, *Urtica piluifera* have a long history of use in the home as a herbal remedy. The whole plant is antiasthmatic, diuretic, it is also used to treat anaemia, haemorrhoids, rheumatism and skin complaints, especially eczema. It is used in the treatment of ailments such as bites and stings, burn and hives [16].

The *Equisetum arvense* and *Urtica piluifera* extracts dissolved in cold and hot water were tested for their antileishmanial properties *in vitro*. The significant inhibitory effect of extracts on the growth of *Leishmania tropica*. promastigotes may be elucidated by different

reasons. May be these plants have poisoning effect on the promastigotes because the *Equisetum arvense* contain a number of flavonoids and like nicotine and many active materials, as well as, the *Urtica pilulifera* contain a number of amines, minerals like Calcium, Potassium and may be contain lignans [17,18].

On the other hand, The *Equisetum arvense* and *Urtica pilulifera* extracts effected on total Protein and nucleic acids of treated promastigotes.

Proteins of protozoan cells have similar characters of those in higher organism [19], many antiprotozoan agents affect metabolism of proteins and nucleic acids of the target organism like ketokonazole, allopurinol, chlorpromazine, ethidium, dihydroemite and soramine [20,21], these results of this study the result of effect of effects of crude alkaloids isolated from *Peganum harmala* seeds on the growth and metabolism of *Leishmania tropica*. promastigotes [22], Furthermore, some plant extracts that contain alkaloids have been found to have inhibitory effects on *lieshmania* metabolism, for example, [23] and result of [24,25] revealed that treating of ehrlich ascites tumor cells with Daphnoretin compound extract effect on total protein of treated cells, as well as, the Ketoconazole drag reduce the total protein of treated *Leishmania tropica* and *Lieshmania donovani* promastigotes [26].

The nucleic acids (DNA, RNA) are very important structure in the living cell, in this study may be the effect of *Equisetum arvense* and *Urtica pilulifera* extracts on *Leishmania tropica*, promastigotes because this extract includes many active compounds can effect on total nucleic acids of *Leishmania tropica*. promastigotes or effect on the enzymes of parasite [27].

This study includes the effect of IC50 of *Equisetum arvense* and *Urtica pilulifera* extracts on energy metabolism (carbohydrate content) of *Leishmania* promastigotes in vitro.

All the parasites has carbohydrates on the serves of the cell membrane [28], the result show no more effect of *Equisetum arvense* and *Urtica pilulifera* extracts on total carbohydrates of *Leishmania tropica*. Promastigotes, this results show the cell membrane become mechanical barrier between the extract and parasite [23,24].

Present study concludes that *Equisetum arvense* and *Urtica pilulifera* extracts show a promising in vitro antileishmanial activity and can be considered as new lead structures in the search for novel antileishmanial drug. Therefore, there is a need for investigation about the efficacy of *Equisetum arvense* and *Urtica pilulifera* extracts as antileishmanial agents in vivo (in animals) to determine therapeutic indices of such compounds.

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