Effect of Aqueous Extracts of *Nerium oleander* and *Melia azedarach* on Growth *Leishmania tropica* promastigotes In Vitro

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Abstract
The present study tacked the effect of the aqueous plant extracts of *Melia azedarach* fruits and *Nerium oleander* leaves and fruits on *Leishmania tropica* promastigotes growing on Tobie's medium to which 15 % of human blood is added.
The results indicated that these extracts have poisonous effect on the promastigotes of *L. tropica*. The used concentrations were 0.75, 1, 1.5, 2.5, 5 mg/ml for fruits extract of *M. azedarach*, 0.05, 0.25, 0.5, 1, 2.5 mg/ml for leaves extract of *N. oleander* and 0.5, 1, 1.75, 2.5, 5 mg/ml for fruits extract of *N. oleander*, and caused gradual inhibition of promastigotes number with the increase of concentration through different growth periods 24, 48, 72, 96 hours.
The high concentrations 5 mg/ml for fruits of *M. azedarach* 2.5 mg/ml for leaves of *N. oleander* and 5 mg/ml for fruits of *N. oleander* caused inhibition of the promastigotes growth of *L. tropica* at rates 97 % , 71.9 % and 81.5 respectively during 96 hours of growth.
The IC50 concentration for extracts were determined which were 0.75, 1, 1.75 mg/ml for *M. azedarach* fruits, *N. oleander* leaves and fruits extracts respectively during 96 hour of growth.
Key wards : Leishmania , Plant extracts.

Introduction
IRAQ is considered one of the countries in which a lot of plants, of medical use, grow widely like: *Citrullus colocynthis, Thymus vulgaris* and *Glycerrhiza globra* etc. [1] . The chemical and pharmalogical studies in the last fifteen years proved the therapeutic activities of these plants [2]. Extracts of different plants were used in the treatment of diseases caused by bacteria, viruses, parasites, fungi, worms and insects. A study testing the effects of a number of plants growing in Guatimala on the growth of *Trypanosoma cruzi, Plasmodium falciparum* and *Leishmania spp.* and the most effective plants were *Wigandia urens* and *Neurolaenal lobata* [3].
In (2002) [4] , found that compounds extracted from *Diospyros montana* showed an inhibitory effect on the growth of *L. donovani* promastigotes, and in another study Maes et al., [5] showed that alcohol extract of the leaves of *Maesa balansae* had an inhibiting effect on the vesciral species of Leishmania, while the alcohol extracts of the leaves and roots of *Moringa stenopetala* had an inhibitory effect on *Trypanosoma cruzi* and *L. donovani* [6]. *Citrullus colocynthis* and *Capparis spinosa* were tested for their effect on growth and metabolism of *L. major* as they proved to have toxic effect, on these parasites [7]. This study aims to test more plants for their effect on *L. tropica.*

Materials and methods
Leishmania tropica used in this work were cultured promastigote forms (MHOM/IQ/1992/MREC$_3$) which were obtained from the laboratory of leishmainia, College of Medicin, University of Nahrain, which was identified enzymaticaly using isoenzymes method [8]. Promastigotes were grown in modified Tobie's medium [9]. Which consists of two phases blood agar and Locke's solution, 5 ml of solid phase were dispensed in to 25 ml flasks, and just before use, 2 ml of Locke's solution were added to the flasks, then inoculated with promastigotes 2×10^5 parasite/ml.
The effect of aqueous extracts of *Nerium oleander* and *Melia azedarach* on premostigote growth was investigated by adding different concentrations to the culture at zero time. Culture without extracts were used as control.
All cultures were incubated at 26 c˚ for 5 days, and the number of parasites were counted daily under a microscope 40 × using a haemocytometer.

Preparation of extracts
Nerium oleander and *Melia azedarach* plants collected from areas around Mosul city/IRAQ, and the aqueous extracts of fruits of both plants and leaves of *Nerium oleander* were prepared according to the method used by [10]. Different concentrations prepared 0.75, 1, 1.5, 2.5, 5 mg/ml of fruits extract of *M. azedarach*, 0.05, 0.25, 0.5, 1, 2.5 mg/ml of leaves extract of *N. oleander* and 0.5, 1, 1.75, 2.5, 5 mg/ml of fruits extract of *N. oleander.*

Results
The gradual decrease in the number of promastigotes indicated that there is a clear toxic effect of the water extracts of *N.oleander* and *M. azedarach* on the growth of *L. tropica*, and that there is aproportional relation between the concentration of the extract and it's inhibitory effect. The highest concentrations of the extracts the fruits of *M. azedarach* 5 mg/ml, the leaves of *N. oleander* 2.5 mg/ml and the fruits of *N. oleander* 5 mg/ml caused inhibitions at a ratios of 97 % , 71 % and 81.5 % respectively during the logarithmic phase of growth (Tables, 1,2,3).The results indicated that the IC50 of the fruits of *M. azedarach*, leaves of *N. oleander* and fruits of *N. oleander* were 0.75 mg/ml, 1.0 mg/ml and 1.75 mg/ml respectively (Tables , 1,2,3).

Table 1 : Effect of different concentrations of fruits extract of *M. azedarach* promastigotes ($\times 10^6$) during different periods of growth (No. of cultured promastigotes 2×10^5 cells/ml)

Exposure period (hrs) / Treatment (mg/ml)	24		48		72		96	
	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition
Control	^{ef}1.35 ± 0.12	--	^{ki}3.06 ± 0.005	--	^{o}7 ± 0.2	--	^{q}12.93 ± 0.005	--
0.75	^{de}1.03 ± 0.003	23.8	^{gh}2.06 ± 0.20	32.7	^{m}4.16 ± 0.85	40.6	^{n}6.19 ± 0.26	52.2
1	^{de}0.97 ± 0.006	28.2	^{fg}1.7 ± 0.43	44.5	^{hi}2.3 ± 0.26	67.2	^{i}3.5 ± 0.50	73
1.5	^{cde}0.9 ± 0.005	33.4	^{ef}1.35 ± 0.13	55.9	^{gh}2.0 ± 0.18	71.5	^{ij}2.55 ± 2.25	80.3
2.5	^{bcde}0.86 ± 0.006	36.3	^{de}1.0 ± 0.1	67.4	^{ef}1.3 ± 0.2	81.5	^{abcd}0.7 ± 2.002	94.6
5	^{abcd}0.74 ± 0.004	45.2	^{abcd}0.6 ± 0.008	80.4	^{de}1.0 ± 0.2	85.8	^{abe}0.4 ± 0.008	97

* The reading represent average of three repeating ± SD.

** Different letters at the end of some readings indicate significant differences, while similar letters indicate no significant differences existed, using Dunken's test. ($P \geq 0.05$) .

Table 2 : Effect of different concentrations of leafs extract of *N. oleander* promastigotes ($\times 10^6$) during different periods of growth (No. of cultured promastigotes 2×10^5 cells/ml)

Exposure period (hrs) / Treatment (mg/ml)	24		48		72		96	
	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition
Control	^{ab}1.325 ± 0.12	--	^{de}6.25 ± 0.72	--	^{k}16.14 ± 0.009	--	^{q}27.35 ± 1.3	--
0.05	^{a}1.08 ± 0.002	18.5	^{d}5.4 ± 0.4	13.6	^{ij}14.09 ± 0.5	12.8	^{o}23.31 ± 0.9	14.8
0.25	^{a}0.98 ± 0.1	26.1	^{c}4.1 ± 0.26	34.4	^{h}12.85 ± 1.1	20.4	^{n}21.87 ± 0.006	20.1
0.5	^{a}0.95 ± 0.12	28.4	^{c}3.89 ± 0.18	37.8	^{g}10.06 ± 0.37	37.7	^{i}17.68 ± 0.87	35.4
1	^{a}0.7 ± 0.008	47.2	^{b}2.06 ± 0.3	67.1	^{ef}6.96 ± 0.25	56.9	^{j}14.6 ± 0.4	46.7
2.5	^{a}0.6 ± 0.008	54.8	0.15 ± ^{a}1.1 ±	82.4	^{c}4.3 ± 0.3	73.4	^{f}7.7 ± 0.3	71.9

* The reading represent average of three repeating ± SD.

** Different letters at the end of some readings indicate significant differences, while similar letters indicate no significant differences existed, using Dunken's test. ($P \geq 0.05$) .

Table 3 : Effect of different concentrations of fruits extract of *N. oleander* on number of L. tropica promastigotes ($\times 10^6$) during different periods of growth (No. of cultured promastigotes 2×10^5 cells/ml)

Exposure period (hrs) / Treatment (mg/ml)	24		48		72		96	
	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition	Mean ± SD	% inhibition
السيطرة	$^{ab}1.39 \pm 0.006$	--	$^{g}4.66 \pm 0.30$	--	$^{k}10.23 \pm 0.34$	--	$^{p}19.9 \pm 0.83$	--
0.5	$^{ab}1.12 \pm 0.009$	19.5	$^{ef}3.75 \pm 0.254$	19.6	$^{j}8.85 \pm 0.37$	13.5	$^{o}17.48 \pm 0.68$	12.2
1.0	$^{ab}0.96 \pm 0.002$	31	$^{de}3.15 \pm 0.13$	32.5	$^{i}7.5 \pm 0.43$	26.7	$^{m}15.78 \pm 0.22$	20.8
1.75	$^{ab}0.9 \pm 0.24$	35.3	$^{e}2.45 \pm 0.48$	47.5	$^{h}6.25 \pm 0.60$	39	$^{ki}10.83 \pm 0.33$	45.6
2.5	$^{ab}0.9 \pm 0.13$	35.3	$^{b}1.5 \pm 0.13$	67.9	$^{fg}4.2 \pm 0.2$	59	$^{i}7.5 \pm 0.66$	62.4
5.0	$^{a}0.73 \pm 0.005$	47.5	$^{ab}0.98 \pm 0.10$	79	$^{cd}2.9 \pm 0.17$	71.7	$^{ef}3.7 \pm 0.3$	81.5

* The reading represent average of three repeating ± SD.

** Different letters at the end of some readings indicate significant differences, while similar letters indicate no significant differences existed, using Dunken's test. ($P \geq 0.05$) .

Discussion

The obtained results concurred with the results of [7] on *Citrullus colocynthis* and *Capparis spinosa* indicating the inhibitory effect on *L. major* IC50 , 1.75 and 3.25 mg/ml respectively. Also the results of [5] indicated the inhibitory effect of the alcoholic extract of the Viatnamese plant *M. balansae* has an inhibitory effect on the vesciral species of Leishmania *In vitro* IC50 , 0.04 Mg/ml. The inhibitory effect is due to presence of Triterpene saponins compounds. In (2002) [11] showed that the water extract of *C. colocynthis* and *C. spinosa* have an inhibitory effect on the growth of *Trichomonas vaginalis in vitro* with an IC50 of 1.5 mg/ml and 4.25 mg/ml respectively , and the effect is due to the presence of alkaloids , saponins and flavonoids.

Alhamdani in [12] showed the inhibitory effect of the alcohol extrat of *M. azedarach* on *Spodoptera exigua* as 10 p.p.m. caused the abnormal growth.

The inhibitory effect of these extracts could be due to the presence of active compounds effecting the growth of the promastigotes *In vitro* as the fruit of *M. azedarach* contains tetranitroterpenoid and saponin , while leaves and fruits of *N. oleander* contains srteroidalglycosides like oleandrin and nirin, [1][13][14].

The results showed that the effect of *M. azedarach* is more than *N. oleander* although that there is avariation between the effects of leaves and fruits of *N. oleander*, and this could be due to difference in content and concentrations of the terpenes, saponins, alkaloids and stroidalglycosides.

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تأثير المستخلصات المائية لنباتي الدفلة *Nerium oleander* والسبحبح *Melia azedarach* على نمو طفيليات اللشمانيا الاستوائية أمامية السوط promastigotes *Leishmania tropica* خارج الجسم الحي

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الملخص

تناولت هذه الدراسة بيان تاثير المستخلصات المائية لثمار السبحبح *Melia azedarach* واوراق وثمار الدفلة *Nerium oleander* على طفيليات اللشمانيا الاستوائية *Leishmania tropica* امامية السوط Promastigotes النامية في وسط توبي Tobie's medium الذي اضيف اليه دم انسان بنسبة ١٥%.

وبينت النتائج ان لمستخلص كل من ثمار السبحبح بتراكيز ٠,٧٥، ١، ١,٥، ٢,٥، ٥ ملغم/ مل واوراق الدفلة بتراكيز ٠,٠٥، ٠,٢٥، ٠,٥، ١، ٢,٥ ملغم / مل، وثمار الدفلة بتراكيز ٠,٥، ١، ١,٧٥، ٢,٥، ٥ ملغم/مل تاثيراً سمياً واضحاً على هذه الطفيليات من خلال ملاحظة الانخفاض التدريجي في عددها مع ازدياد التركيز لكل مستخلص بعلاقة عكسية خلال فترات النمو ٢٤، ٤٨، ٧٢، ٩٦ ساعة، واظهرت ايضاً ان التراكيز العالية للمستخلصات ٥ ملغم / مل من ثمار السبحبح و٢,٥ ملغم / مل من اوراق الدفلة و٥ ملغم / مل من ثمار الدفلة، ادت الى حدوث تثبيط في نمو امامية السوط بنسب ٩٧% و ٧١,٩% و٨١,٥% على التوالي خلال فترة ٩٦ ساعة من النمو.

وقد تم تحديد قيمة Inhibitory Concentration (IC50) لكل مستخلص حيث بلغ ٠,٧٥ و١ و١,٧٥ ملغم/مل لمستخلصات ثمار السبحبح واوراق وثمار الدفلة على التوالي عند فترة ٩٦ ساعة من النمو.