Effect Study of crude extracted from Coriandrum sativum on growth of Leishmania tropica promastigotes in in vitro

[*]Maksood Adil Mahmoud Al – Doori
[*]Department of Prostheses, Al-Dour Technical Institute, Northern Technical University, Saladin, Iraq
Email: maksoud.am@ntu.edu.iq

Abstract:

Analyzing of the basic oil that utilized in this examination showed that it is components to a great extent of monoterpenes, involve of β-caryophyllene (3.1%), octyl acetate (5.4%), p-cymene (3.3%), β-pinene (3.5%), sabinene (4.5%), limonene (9.4%), α-thujene (15.7%) and α-pinene (41.2%).

Coriander basic oil indicated action against Leishmania tropica promastigotes in in vitro. We used Coriandrum (Coriandrum sativum) leaves extract preparation on L. tropica. 0.1 ml of fluid phase was added to 10 ml of medium 0.1 ml of inoculums of L. tropica promastigotes are taken from stock culture after the logarithms stage, that was the underlying culture which contain for 229 parasites, these parasites was brooded at 26 C^{o} for 4 days, and afterward tallied it via haemocytometer. We take parameters on 24, 48, 72and 96 hours. In this study we found significant effect of concentration Coriandrum extract and time in the number of on numbers of L. tropica promastigotes. But don not found significant effect of concentration Coriandrum extract and time in the number of on generation numbers of L. tropica promastigotes. Between treatment do not found significant effect of concentration Coriandrum extract in the number of on generation time of L. tropica, but found significant effect of time on the number of generations of L. tropica.

Keywords: Lieshmania tropica, Coriandrum sativum, Coriandrum, RPMI 1640.

Introduction

Leishmaniasis is an infection that transmitted by the nibble of different species of sand flies, there are proximately of 20 different of Leishmania species (**Singh, et al., 2012**). The genus of Lutzomyia longipalpis of sand fly act as a vector of disease that transmitting the parasites from residential dogs which going about as a significant host have. There are 98 countries around the world endemics with Leishmaniasis and about 700,000 to 1,000,000 new instances of cutaneous leishmaniasis are happened every year. Most cases happened in six countries: Sudan, South Sudan, India, Brazil, Ethiopia and Bangladesh which form about 90 % of cases (**WHO, 2016**).

Leishmaniasis is a parasitic infections brought about by members of Leishmania species **(Torres-Guerrero, et al.2017, Alvar, et al. 2018).** this diseases are transmitted by the nibble of several type of sand flies that Belong to Phlebotomine , The Phlebotomus (Old Worlds) and Lutzomyia (New World) species are the main vectors of the Leishmaniasis. World Health Organization is thought of the cutaneous leishmaniasis is one of a neglected tropical disease. (**WHO 2019**). There are three fundamental

type of leishmaniasis: visceral, mucocutaneous, and cutaneous. Depending on the Leishmanial species. Unfortunately, there are no preventive medicines or vaccines, at the present time, available to prevent the leishmaniasis. The disease is currently being treated by several alternatives incorporate sodium stibogluconate, conventional (deoxycholate), meglumine antimoniate liposomal amphotericin B, paromomycin, miltefosine and pentamidine isethionate. Notwithstanding, these chemical drugs have suffered from extreme symptoms, toxicity , high cost, constrained availability, poor efficacy, or on the other hand creating opposition **(Solo mon et al., 2013 and Gue ryet al., 2017)** Moreover, there are often obstacles to the arrival of the drug or its arrival in limited quantities to patients in developing countries where the ailment is spreading (**Hailu, et al. 2016; Sunyoto, et al. 2018**). Fundamental vegetable oils are a perplexing mixture of unpredictable chemicals with diverse and diverse organic properties (**Firenzuoli, et al. 2014; Ba¸ser, et al. 2010**). Easily obtainable vegetable essential oils give minimal effort treatment choices against leishmaniasis. These anti-parasitic exercises of fundamental oils and their segments have been checked on. (**Monzote, et al. 2012; Bero, et al. 2014)**. Commercially obtainable essential oils have been examined to work against a variety of human pathogens **(Mikus, et al. 2000; Powers, et al. 2018; Orchard, et al. 2018; Serra, et al. 2018; Cannas, et al. 2016; Andrade, et al. 2116**) and for cytotoxic activity (**Pow ers et al. 2018**; **Cannas et al. 2016**). antimonial sodium stibogluconate (Pentostam®) is the basic drugs containing of the administration of and Amphotericin B and pentavalent

Amphotericin B and pentavalent antimonial sodium stibogluconate (Pentostam® and Glucantime®) is the basic drugs consists of the administration for leishmaniasis. All these medications are limited to some extent by their lack of efficacy and toxicity, high cost and the requirement for hospitalization. **(Kaur, et al. 2013)** Leishmaniasis has become a very important disease in the past few decades because parasites have developed resistance to chemical drugs. During the search for new drugs affecting on leishmaniasis, in developing countries the disease is representing significant burden, the discovery of new chemotherapeutic agents that are less toxic through the changing activities of existing treatments, the related symptoms and the absence of any vaccine (**Celso and Tânia, 2010; Ahmed, et al. 2017; Grover, et al.2012).**

Anti-leishmania Natural Products.

From Rolliniae marginata (Annonaceae) reported the four acetogenins were identified with antiprotozoal activity by bioassay-guided screening method against different Leishmania species. (**Kayser, et al. 2003)**.

Coriandrum sativum (coriander):

For deadly activities against nematodes, tested 28 types of commercial basic oils against nematodes from pine wood, Bursaphelenchus xylophilus, and the best pesticides for these worms were from coriander

(**Kim, et al. 2008**). Anthelmintic activities have been observed when using crude hydro-alcoholic extracts of coriander seeds in in vitro. It had effects on adult and egg nematode Haemonchus contortus.

in sheep infected with Haemonchus contortus the coriander aqueous extract had been activity as anthelmintic in vivo. the concentration of 0.5 mg/ml of Both concentrates of coriander inhibited hatching of eggs totally. ED50 (effective dose) of coriander hydroalcoholic extract was 0.18 mg / dl. While that of aqueous concentrate was 0.12 mg / ml. There was no measurably critical contrast among hydroalcoholic and aqueous extracts ($p>0.05$). The aqueous concentrate appeared lowest efficacy in vitro against grown-up worms than the hydroalcoholic extract. Crude watery extract of coriander was given to sheep that was experimentally infected with Haemonchus contortus at 0.45 and 0.9 g/kg portion levels. Efficiency tried via the total worm tally decrease (TWCR) and fecal egg check reduction (FECR) after two days of treatment. Huge FECR was distinguished in the gatherings that treated with higher portion of Coriandrum sativum ($p<0.05$) and albendazole ($p<0.001$). The importance of TWCR ($p<0.05$) was recognized uniquely for the higher portion of extraction contrasted with the benchmark group (untreated gathering) Reduction in female worms was lower than male worms. Treatment with both of coriander dosages didn't assist the creatures with maintaining or improve their PCV, while the treated with albendazole demonstrated expanding in PCV ($p<0.05$) **(Eguale, et al. 2007)**.

The antiparasitic effected of essential oils of coriander was studied by two extracted in vitro examines on Haemonchus contortus utilizing larval development test (LDT) and egg hatch test (EHT) essential oils of coriander were displayed a dose-dependent impact in the egg hatch test, is that inhibiting 81.2% the larvae hatching of Haemonchus contortus at a convergence of 2.5 mg/ml. The viable of focus to hinder of 50% (EC50) of egg bring forth was 0.63 mg/ml. in larval development test, coriander at concentration of 10 mg/ml was inhibited 97.8% of H. contortus of the advancement larva **(Macedo, et al. 2013).** in vitro effecting of coriander fractions on amastigotes and promastigotes of L. infantum was studied in additional to its poisonousness against the murine monocytic cells RAW 264.7. All coriander portions were successful against the L. infantum promastigotes and didn't vary from the pentamidine (positive control) ($p>0.05$). The coriander methanol fraction, was the best against amastigotes as well as amphotericin B (positive control) ($p>0.05$) (**Rondon, et al. 2011**). The biological action of basic oil of coriander seeds was tried against grown-up Tribolium confusum and Callosobruchus maculatus in a progression of lab tests. The mortality of of insect pests' grown-ups was expanded with focus from 43 to 357 µl/l air of seed essential oil of coriander and with presentation time from 3 to 24 h. In the probit investigation, LC50 values indicated that C. maculatus (LC50 = 1.34 µl/l air) was more susceptible than T. confusum (LC50 = 318.02 µl/l air) (**Khani and Rahdari, 2012**). The fundamental oil of coriander fruits was evaluated as repellent activities and larvicidal against Aedes albopictus Skuse (Diptera: Culicidae). Highlighted that coriander fundamental oil was a decent anti-agent against A. albopictus, it is going about as harmful action against A. albopictus hatchlings: LC90 was 531.7 ppm while LC50 was 421 ppm. (**Benelli, et al. 2013**). The leaf oil of coriander had critical poisonous impacts against the Aedes aegypti hatchlings with a LC_{50} estimation of 26.93 ppm and a LC_{90} estimation of 37.69 ppm, and itis stem oil has harmful impacts against the A. aegypti hatchlings with a LC_{50} estimation of 29.39 ppm and a LC_{90} estimation of 39.95 ppm **(Chung, et al. 2012a)**. And seed oil also had a critical harmful effect against the Aedes aegypti larvae with an LC50: 21.55 ppm and LC90: 38.79 ppm. The significant compounds in the coriander basic oil assume

a significant job as immunotoxicity on the A. aegypti (**Chung b, et al., 2012b**). In this paper we study the effect of crud water extraction of Coriandrum sativum leaves on development of L. tropica promastigotes in vitro at different times.

Materials and Methods

A. Plant sample: Coriandrum (Coriandrum sativum) leaves were collected from local garden, washed, dried in the shade at room temperature, ground using a coffee grinder and were used for extract preparation.

B. Leishmania used:

The L. tropica strain (MHOM / IQ / 1992 / MREC3) was gotten from the Research Center / College of Medicine / Al-Nahrain University / Iraq, which had been taken from patients suffered from cutaneous leishmaniasis on Iraqi country, and that strain was diagnosed by molecular biology methods. the parasites in promastigotes form was cultured in RPMI 1640 medium according to Moore and Woods (1976), and 5 % fetal calf serum was added for enrichment media.

C. Cultivation and estimating numbers of parasites:

100µl of stock culture has been added for 10 ml of medium, taken 100µl of Leishmania promastigotes from stock culture during the phase of logarithm it was the initial culture was contained about 229 parasites and then incubated four days at 26 C^{o}, the parasites were directly counted by used of haemocytometer.

Statistical analysis

Information of current examination was dissected by utilizing of Tukey test to look at between midpoints (implies). A degree of hugeness of α=0.05 was applied to test. Furthermore, utilized the SPSS v.22 programs to investigate current information.

Results and discussion:

Data presented in Table (1) revealed the different concentrations effects of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes at various time periods. The different concentrations effects of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes was significant. The lowest numbers of L. tropica promastigotes at 24 hours was recorded in treatment 14 mg/ml followed by 0.7, 0.35, 0.175 and control, respectively. The lowest numbers of L. tropica promastigotes at 48 hours was recorded in treatment 14 mg/ml followed by0.7, 0.35, 0.175 and control, respectively. The lowest numbers of L. tropica promastigotes at 72 hours was recorded in treatment 14 mg/ml followed by0.7, 0.35, 0.175 and control, respectively. The lowest numbers of L. tropica promastigotes at 96 hours was recorded in treatment 14 mg/ml followed by 0.7, 0.35, 0.175 and control, respectively.

The effect of Coriandrum sativum leaves on numbers of L. tropica promastigotes at various time periods. the impact of various concentrations of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes was significant. The lowest numbers of L. tropica promastigotes in control was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 14 mg/dl was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 0.7 mg/ml was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 0.35 was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 0.175 was recorded in 24h followed by 48, 72 and 96, separately.

Data presented in **Tabie (2)** revealed the various concentrations effects of crud water extraction of Coriandrum sativum leaves on age number of L. tropica promastigotes at various timespans. the effect of various concentrations of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes was not significant.

The Effect of various concentrations of crud water extraction of Coriandrum sativum leaves on age number of L. tropica promastigotes at various timespans. the effect of various concentrations of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes was not significant.

Table 1: The different concentrations Effects of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes at various timespans.

Exposure Time (hrs)	**24**	**%**	**48**	**%**	**72**	**%**	**96**	**%**
mg/ml	**Mean± SE**		**Mean± SE**		**Mean± SE**		**Mean± SE**	
Controls	1009.95[a]± 17200.00 D		21650.00 1090.48[b] ± D		29750.00 629.15[c]± C		45750.0 1376.89 [d]±0 D	
14	457.34[a]±6 250.00 A	63.7	8550.00 206.15[a]± A	60.5	14050.00 457.34[b]± A	52.8	741.05[c]±2 0150.00 A	**56.0**

0.7	250.00[a]±8450.00 B	50.9	427.20[b]±11750.00 B	45.7	478.71[c]±15650.00 A	47.4	506.62[d]±25700.00 B	**43.8**
0.35	369.68[a]±9500.00 B	44.8	298.60[b]±13450.00 B	37.9	492.44[c]±17950.00 A	39.7	637.70[d]±28200.00 B	**38.4**
0.175	500.00[a]±13300.00 C	22.7	499.16[b]±18150.00 C	16.2	412.31[c]±23900.00 B	19.7	860.23[d]±36000.00 C	21.3

Use 4 replicates to every treatment. The capital letter shows different between the treatments at (P≤ 0.05). The small letter shows different between the times at (P≤ 0.05). Use Tukey test to show the significant differences at (P ≤ 0.05)

Table 2: various concentrations Effects of crud water extraction of Coriandrum sativum leaves on age number of L. tropica promastigotes at various timespans.

Exposure Time (hrs) Treatment	24	48	72	96
mg/ml	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Control	43.19±.26[a] A	44.21±.22[a] A	45.63±.09[a] A	47.54±.13[a] A
14	38.69±.32[a] A	40.11±.10[a] A	42.30±.14[a] A	43.68±.30[a] A
7	40.05±.13[a] A	41.51±.160[a] A	42.79±.13[a] A	44.99±.08[a] A
3.5	40.57±.17[a] A	42.11±.09[a] A	43.40±.12[a] A	45.40±.09[a] A

1.75	42.06±.16[a] A	43.40±.11[a] A	44.67±.07[a] A	46.48±.10[a] A

Use 4 replicates to every treatment. The capital letter shows different between the treatment at (P≤ 0.05). The small letter shows different between the times at (P≤ 0.05). Use Tukey test to show the significant differences at (P≤ 0.05)

Data presented in **Table (3)** revealed the different concentrations Effects of crud water extraction of Coriandrum sativum leaves on age time (hours) of L. tropica promastigotes at various timespans. The different concentrations effects of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes was not significant.

The Effect of various concentrations of crud water extraction of Coriandrum sativum leaves on age time (hours) of L. tropica promastigotes at various timespans. The different concentrations effects of crud water extraction of Coriandrum sativum leaves on numbers of L. tropica promastigotes was significant.

The lowest numbers of L. tropica promastigotes in control was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 1.6 mg/dl was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 0.8 mg/dl was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 0.4 was recorded in 24h followed by 48, 72 and 96, respectively. The lowest numbers of L. tropica promastigotes in 0.2 was recorded in 24h followed by 48, 72 and 96, individually.

Table 3: Different concentrations effects of crud water extraction of Coriandrum sativum leaves on age time (hours) of L. tropica promastigotes at various timespans.

Exposure Time (hrs) Treatment	24	48	72	96
mg/ml	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Control	.55±.002[a] A	1.08±.006[b] A	1.58±.004[c] A	2.02±.005[c] A
14	.62±.004[a] A	1.20±.004[b] A	1.70±.006[c] A	2.19±.015[c] A

7	.59±.002[a] A	1.15±.004[b] A	1.68±.004[c] A	2.13±.004[c] A
3.5	.59±.002[a] A	1.14±.004[b] A	1.66±.004[c] A	2.11±.005[c] A
1.75	.57±.004[a] A	1.10±.002[b] A	1.61±.002[c] A	2.06±.006[c] A

Use 4 replicates to every treatment. The capital letter shows different between the treatment at (P≤ 0.05). The small letter shows different between the time at (P≤ 0.05). Use Tukey test to show the significant differences at (P≤ 0.05)

Conclusion

In this study we found significant effect of concentration Coriandrum sativum extract and time in the number of on numbers of L. tropica promastigotes.

But don not found significant effect of concentration Coriandrum sativum extract and time in the number of on generation numbers of L. tropica promastigotes.

Between treatment don't found significant effect of concentration Coriandrum sativum extract in the number of on age time of L. tropica promastigotes, but found significant effect of time on in the number of on age time of Leishmania tropica.

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