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Effect of isolated fatty acids from flaxseeds *Linumusitatissimum* against *Leishmaniatropica*In vitro.

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Abstract

The current study aimed to test the effectiveness of Fatty acid separated from the seeds of the flax plant Linum usitatissimum on the parasite *Leishmaniatropica*. Palmatic acid, Steric acid, Olic acid, Arachidic acid, Behenic acid). These fatty acids were used as a possible antilieshmanial agents in vitro, as the results showed that the concentration of 100 µg/ml gave a complete inhibition of the parasite's growth in the promastigote form in 48 hours and up by 100% compared to the control group which The growth of the parasite was witnessed at all times, while the concentration of 25 µg/ml showed a decrease in the percentage of inhibition compared to the rest of the concentrations, which was 78.8% at the time of 96 hours, and the inhibitory concentration for 90% of the parasites (LC90)) was at the concentration of 50 µg/ml. milliliters at log phase 96 hours of culture. This led to a reduction in the number of generations and an increase in the time required to produce them compared to the control group.

Key words: Fatty acid, flax, *Linumusitatissimum*, *Leishmaniatropica*.fatty acids

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Introduction

Flax (*Linum usitaissimum* L) is a dual-purpose crop that is grown for its fiber or oil, and sometimes both, The original home of the flax plant is the temperate regions of the Arab East, Europe and Asia. It is currently cultivated all over the world for its fibers, seeds and oil. Flax has been planted for at least 7000 years in the Middle East and has always received distinguished attention as a medicinal herb, as the ancient Egyptians knew and cultivated it (Yasari and Patwardhen, 2006;AL – Doori 2020). Also, the proportion of oil in flaxseeds can range between 30-45%, and flaxseed oil is a dry oil, and it is used in wood polishing and in the manufacture of dyes. It is also used in printing ink and in the manufacture of a number of soaps. saturated and the proportion reaches approximately 50% and 23% of oleic acid (Kocjan,2008).It also contains omega-3, which is the same fat that is found in fish oil, which turns into three chains of prostaglandins that are hormone-like, as they are similar to substances that are manufactured in many parts of the body, and do not come from just one organ like the rest of most hormones. Indirectly, these three chains of prostaglandins have anti-inflammatory activities, but this has not been proven and proven so far. Also, flax oil helps reduce cholesterol in the blood, and there is specific research on flax oil that indicated that it also lowers high blood pressure (Singh *et al*., 2011; Ivanova *et al*., 2011). Flax oil has many medical uses because it contains a high percentage of fatty acids and Omega-3 fatty acid,

as it is used in the treatment of cancer and heart diseases and the treatment of strokes (Charlton and Ehrensing, 2001). Plant extracts have many chemicals that have an inhibitory effect on a wide range of microorganisms, including alkaloids, glycosides, saponins, flavonoids, as well as polyphenols. The World Health Organization has shown that nearly 80% of the world's Populace, primarily from developing nations, nonetheless depend upon traditional medicine to treat common diseases (Trush, 1986).Natural products are chemicals separated from living organisms, and altogether of these mixes result from secondary metabolism and do not have a direct role in the process of reproduction and growth (Hasan *et al*, 2019).

Cutaneous leishmaniasis is one of the greatest neglected diseases in the world and affects primarily the poor, and 350 million humans are prone to contracting leishmaniasis, with one million cases appearing annually (singh, 2006; Schwarz *et al*,2017; Al Doori 2020).*Leishmaniatropica* are parasites of the family Trypanosomata. Leishmaniasis is a tropical disease that has many effects that affect human health in about 98 countries of the world, where humans are infected finished the broadcast of the pest through the bite of the shingle fly, which is the means of transmitting the disease from vertebrate animals to humans and after it turns to to whipworm cells in the intestines of sandflies(Bordbar*et al*, 2014).Cutaneous leishmaniasis is caused by two types of leishmaniasis, *Leishmaniatropica* and *Leishmaniamajora* (Sukkar,1985).

The aim of the study was to know the effect of fatty acids separated from flax plant on the parasite *Leishmania tropica* with different concentrations.

Materials and methods:

Collect flaxseeds:

The flax seeds were collected from the local markets in Mosul, and Classified within the Medicinal Plants Development Center within the Mosul Dam of the Iraqi Ministry of Agriculture. The seeds were cleaned of dust and what was suspended in them, then located in paper baggage and kept in situations faraway from moisture till use.

Taxonomic position of the flax plant:

Kingdom plantae-plants
Subkingdom tracheobionta – vascular plants
Superdivision spermatophta – seed plants
Division magnoliophyta – Flowering plants
Class magnoliopsida – Dicotyledons
Subclass Rosidae
Order: Lineles
Family: Linaceae – Flax family
Genus: Linum L. – flax
Species: ***Linum usitatissimum.***

Preparation of the plant extract using a Soxhlet continuous extraction device:

The seeds of the plant were crushed by an electric mill, 25 gm of seed powder was put into a batch, and then 400 ml of fuel ether was delivered to extract the oil from the flaxseeds. The extraction continued at a rate of 7 hours a day till the solvent used within the tool became colorless, after which the extract was concentrated by way of a Rotary vacuum evaporator at a temperature of 40C° (Al-Daody, 1998).

ISSN: 1475-7192

Saponification:

5 ml of crude petroleum ether extract was taken and 100 ml of (KOH) was added to it, then the solution was warmed up for 90 minutes at 100 C°, then 100 ml of purified water and 50 ml of airflush were added and the mixture was located in a unravelingchimney, and the mixture was taken. The aqueous layer was added to it with focused sulfuric acid H2SO4 pending PH = 2. At the end, 50 km of ether was added and the mixture was placed over in the unravelingchimney, and the organic layer was taken and kept in dark bottles in the refrigerator until use and diagnosis (Arthur,1972).

Diagnosis of fatty acids using GLC technology:

The diagnosis of unglued fatty acids and unstable oils from the leaves of plants under study was carried out within the laboratories of the Ministry of Science and Technology / Department of Environment and Water, the usage of a gas chromatograph (GLC) of Japanese origin 2010 model from Shimadzu company using the FID Flame Ionization Detector with a separation column Capillary type (SE-30) with a length of (30) m and diameters (0.25 mm and 0.5 mm) and the temperature in the injection and detector area, respectively: (330 and 280) C, while the fever of the partingpillar was gradual, preliminary from (120-280). m and a rise degree of 8 degrees/min using sluggish nitrogen gas as a transporter gas at a degree of 100 KP.

Concentrations of fatty acids used under study:

The concentrations (25, 50, 75 and 100) µg/ml were ready by dissolving fatty acids with Ethylen glycol to obtain the above concentrations.

ISSN: 1475-7192

Cutaneous leishmaniasis isolation:

The original cultures of the cutaneous leishmaniasis parasite (MHOM/IQ/1992/MREC3) were obtained from Medical Research Center at College of Medicine/Al-Nahrain University.

culture medium:

1- RPMI 1640 liquid culture medium is a ready-made culture medium developed by Moore et al. in 1976. This medium contains a large amount of vitamins and amino acids.
2- Fetal calf serum according to (Sundar, et al, 2001).
3- Antibiotic: Ceftriaxone. As follows: 9.5 ml of RPMI 1640 + 0.5 ml Fetal calf serum + 5 µl of Ceftriaxone as an antibiotic to inhibit bacterial growth, filter the above mixture by passing it through a 0.22 µm Filter membrane to ensure culture purity.

The development of the parasite in glass and the study of the effect of flaxseed fatty acids on it:

The cutaneous leishmaniasis parasite was grown ex vivo and the effect of different concentrations of fatty acids in flaxseeds was studied as follows:The cutaneous leishmaniasis parasite was grown ex vivo and the effect of different concentrations of fatty acids in flaxseeds was studied as follows:

1- An amount of 4.3 ml of culture medium was placed in 25 sterile test tubes and divided into 5 organizations.
2- 0.6 ml of exceptional awareness of the extract became brought to 4 groups and the primary group was left as a manage sample.
3- 0.1 ml of growing media covering 1080 parasites was additional to the exceptional agencies.

ISSN: 1475-7192

4- The tubes have been positioned in an incubator at a temperature of 23-26C°.
5- Parasitic increase became monitored in all tubes each 24 hours by taking 0.02 and adding it to zero.4 of normal saline containing formalin at a concentration of 1% to kill parasites, and the quantity of parasites was calculated on a Neubauer hemocytometer slide

Calculating the number and time of generations:

$$n = \frac{\text{Log N} - \text{Log}}{\text{Log2}} = \frac{\text{Log N} - \text{Log}}{0.301}$$

n signifies the number of generations, n is the number of organisms at time t, and n0 is the number of organisms additional on the begin of the test, 1080 parasites.

The law for determining generation time is:

where g is the cohort time in hours, t is the contact time, and n is the number of peers.

$$g = \frac{t}{n}$$

Results and discussion:

The diagnosis of fuel ether excerptafterward soaping process by GLC presented the attendance of the next fatty acids as in Figure (1) and (2), which included: Lauric acid at a holding time of (10,810) minutes and corresponds to the standard compound at a holding time of (10.582) minutes

ISSN: 1475-7192

, Myristic acid at a holding time of (13.849) minutes, which agrees to the normalmultiple at a holding time of (13.233) minutes, and Palmatic acid at a holding time of (18.063) minutes, which agrees to the normalmultiple at a time of detention (18.126) minutes, and acid Stearic acid at a holding time (20.389) minutes, which agrees to the normalmultiple at a holding time (20.519) minutes, and oleic acid at a holding time (22.498) minutes, which agrees to the normalmultiple in a holding time (22.498) minutes, and arachidic acid acid at a holding time of (25,581) minutes, which agrees to the normalmultiple, at a holding time of (25,221) minutes, and behenic acid at a holding time of (30.324) minutes, which is reliable with the normalmultiple and at a holding time of (30.333) minutes.

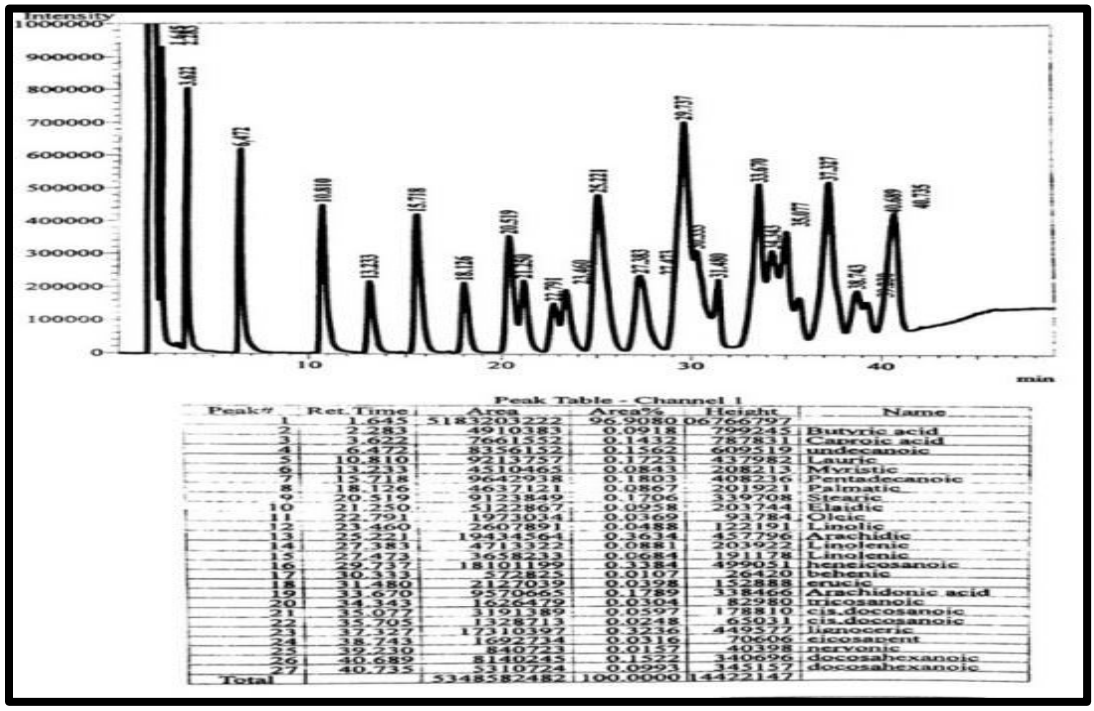
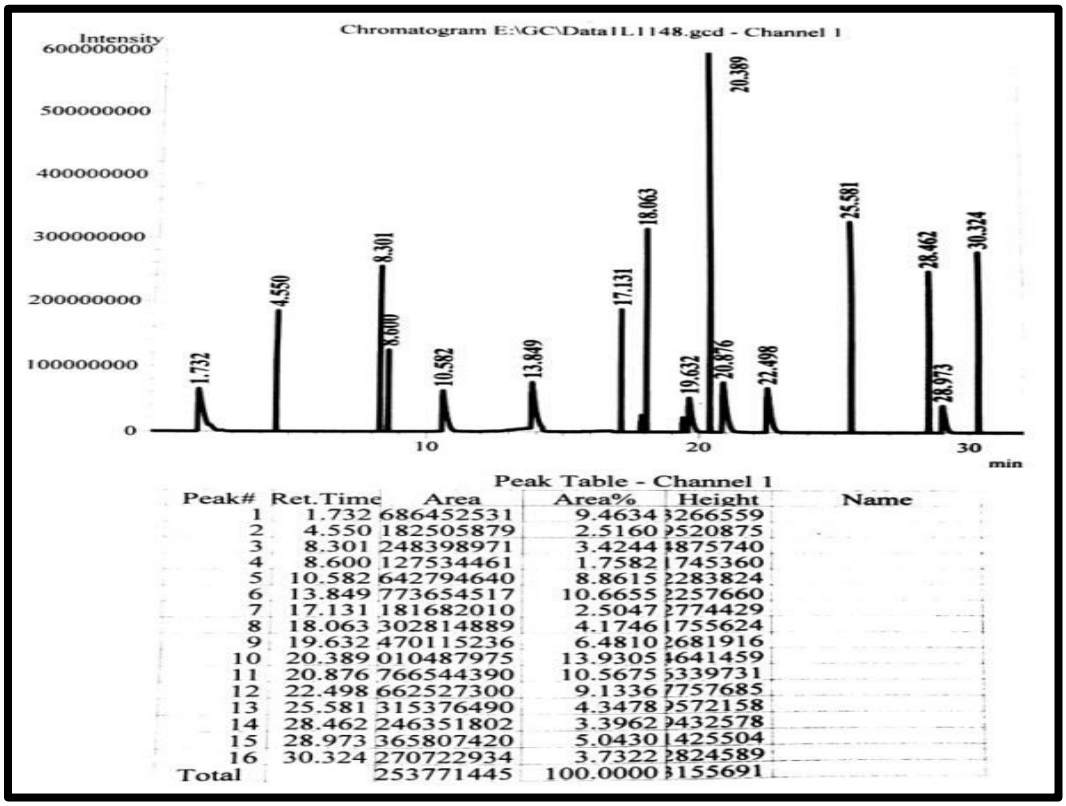


Figure 1: Standard curve of GLC-mediated fatty acid complexes.



Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	1.732	686452531	9.4634	3266559	
2	4.550	182505879	2.5160	9520875	
3	8.301	248398971	3.4244	8875740	
4	8.600	127534461	1.7582	1745360	
5	10.582	642794640	8.8615	2283824	
6	13.849	773654517	10.6655	2257660	
7	17.131	181682010	2.5047	2774429	
8	18.063	302814889	4.1746	1755624	
9	19.632	470115236	6.4810	2681916	
10	20.389	010487975	13.9305	3641459	
11	20.876	766544390	10.5675	5339731	
12	22.498	662527300	9.1336	7757685	
13	25.581	315376490	4.3478	9572158	
14	28.462	246351802	3.3962	2432578	
15	28.973	365807420	5.0430	1425504	
16	30.324	270722934	3.7322	2824589	
Total		253771445	100.0000	3155691	

Figure 2: Curved fatty acid complexes of *Linumusitatissimum* mediated by GLC.

Effect of fatty acids on the growth of Leishmania parasite In vitro:

Table (1) indicates that the different concentrations of fatty acids have an inhibitory effect on the growth of the leishmaniasis parasite ex vivo, as it is clear from the table that there is a relationship between the concentration and growth rate with the reduction of the number of frontal flagella during the exposure period in an inverse relationship with the increase in fatty acid concentrations, which led to An increase in the inhibition of parasite growth during different growth periods, as the concentration of 100 µg/ml showed a total inhibition of the growth of the parasite at 48, 72 and 96 hours at a percentage of 100% compared to the control group, which showed an increase in the growth of the parasite at all times, while the concentration

showed 25 µg /ml showed a decrease in the percentage of inhibition compared to the rest of the concentrations, which was 78.8% at the time of 96 hours, while the inhibitory concentration of LC90 (lethal concentration 90) from parasites was at a attentiveness of 50 µg/ml at the logarithmic phase of 96 hours of culture.

When highlighting the growth index in Table No. 2 and 5, we note the result of the dissimilarattentions of fatty acids on the number and time of the frontal flagella generations, respectively. In Table No. 4, it was noted that there is an inverse relationship between the number of generations and fatty acid concentrations, as the concentration resulted in 100 micrograms/ml. It killed all parasites at logarithmic time 48, 72 and 96, and the number of generations was zero for the concentration of 100 µg/ml, 3.34 generations at the concentration of 75 µg/ml, and 4.79 generations at the concentration of 50 µg/ml, and 6.62 generations at the concentration of 25 µg /ml compared with the control group, which produced 8.86 generations after a period of 96 hours.

Also, the generation time appeared depending on the different concentrations of the fatty acids isolated from flaxseed, as it increased with the increase in concentration in a direct relationship, so the parasite needed 28.83 hours at a concentration of 75 mcg / ml, and 20.25 hours at a concentration of 50 mcg / ml and 14.51 hours at a concentration 25 micrograms/ml compared to the control group, in which the parasite needed 10.83 hours after a period of 96 hours, and the concentration of 100 micrograms/ml did not produce any generations because of the absence of parasite growth after the time 48 hours and upwards, Table No. (3).

Table (1): The effect of different concentrations of fatty acids of flaxseed on the number of leishmaniasis parasites In vivo:

Exposure period (hrs) / Treatment	24	Inh. %	48	Inh. %	72	Inh. %	96	Inh. %
control	**11600±0.68a** E***	------	**19800±0.80b** E	-----	**31200±1.56c** E	-----	**50000±1.41d** E	----
100	**270.00±25.50b** A	**97.67**	**0.00±0.00a** A	**100**	**0.00±0.00a** A	**100**	**0.00±0.00a** A	**100**
75	**430.00±25.50a** B	**96.29**	**530.00±25.50a** B	**97.32**	**820.00±56.12b** B	**97.37**	**1100.00±83.67c** B	**97.8**
50	**710.00±43.01a** C	**93.87**	**920.00±40.62b** C	**95.35**	**1700.00±148.32c** C	**94.55**	**3160.00±556.42d** C	**93.68**
25	**1320.00±33.91a** D	**88.62**	**2400.00±70.71b** D	**87.87**	**6600.00±551.36c** D	**78.84**	**10600.00±533.85d** D	**78.8**

* Use 5 replicates for each treatment.

The dissimilar English literaturesdesignate the presence of importantchanges, while the similar letters designate that there are no importantchanges at the 0.05 level, rendering to Tukey's test. ** Small letters are compared horizontally amid the time periods for each treatment *** and the upper letters are compared vertically between the transactions.

Table 2: Effect of different concentrations of fatty acids of flax seeds on the number of generations produced by the parasite during different periods outside the vivo.

Exposure period (hrs)	24	48	72	96

Treatment µg/cm^3				
	* Average ± standard deviation	Average ± standard deviation	Average ± standard deviation	Average ± standard deviation
control	6.75±0.08a** C***	7.52±0.06a D	8.17±0.07a D	8.86±0.04a D
100	1.30±0.14b A	0.00±0.00a A	0.00±0.00a A	0.00±0.00a A
75	1.99±0.09a A	2.30±0.07a B	2.92±0.10a B	3.34±0.10a B
50	2.71±0.09a A	3.09±0.06a B	3.96±0.13a B	4.79±0.25a B
25	3.62±0.03a B	4.48±0.04a C	5.92±0.11b C	6.62±0.07b C

* Use 5 replicates for each treatment.

The dissimilar English letters designate the presence of importantchanges, while the alike letters designate that there are no importantchanges at the 0.05 level, rendering to Tukey's test. ** Small letters are compared horizontally amid the time periods for each treatment *** and the upper letters are compared vertically between the transactions.

Table 3: Effect of different concentrations of fatty acids of flax seeds on the generation time needed by the parasite during different periods outside the vivo.

Exposure period (hrs)	24	48	72	96

Treatment µg/cm^3	* Average ± standard deviation	Average ± standard deviation	Average ± standard deviation	Average ± standard deviation
control	3.55±0.04a** A***	6.38±0.05b B	181.27±172.43d E	10.83±0.05c B
100	19.26±2.13b D	0.00±0.00a A	0.00±0.00a A	0.00±0.00a A
75	12.15±0.57a C	21.09±0.77b E	24.76±0.87b D	28.83±0.88c E
50	8.88±0.28a B	15.55±0.33b D	18.25±0.62b C	20.25±1.04b D
25	6.63±0.06a B	10.71±0.10b C	12.29±0.26b B	14.51±0.16b C

* Use 5 replicates for each treatment.

The dissimilar English letters designate the presence of importantchanges, while the alike letters designate that there are no importantchanges at the 0.05 level, rendering to Tukey's test. ** Small letters are compared horizontally amid the time periods for each treatment *** and the upper letters are compared vertically between the transactions.

Conclusion

The results of the research showed that the fatty acids of flaxseed have an inhibitory effect on the growth of cutaneous leishmaniasis ex vivo by up to 100% at a concentration of 100 µg/ml.

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