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Isolation and Diagnosis of the Fatty Acids from *Curcuma longa* and the Seeds of *Prunus avium L* (Cherry Plants) and Studying their Effect on the Growth of *Leishmania tropica* Promastigotes in *In Vitro*

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ABSTRACT

The aim of this study is estimated and diagnosis of fatty acids for *Curcuma longa and Prunus avium* L by GLC technique. these techniques showed that *C. longa* contains fatty acids: Pentadecanoic, Palmitic, Heptadecanaoic, Stearic, Elaidic, Oleic, Lenoli, Arachidic, Eicisenoic, Behenic, Erucic, Arachidonic, Tricasnoicm and Lignoceric, whole the Pentadecanoic, Oleic, Lenoli and Erucic was didn't found in the *P. avium* L.

The current study showed that fatty acids that isolated from C. *longa* are more beneficial than fatty acids isolated from P. *avium* L in inhibition of promastigotes growth in *in vitro* , the highest rate of inhibition at concentration of 50 µg / ml for the fatty acids of *Curcuma longa and Prunus avium* L, it was146 × 103 and 158 × 103, respectively, and the lowest inhibition rate was at concentration of 10 µg / ml it was 270 × 103 and 296 x 103, respectively, compared to the control group that was 441 x 103. The lethal concentration -50 (LC 50) of fatty acids after 96h of incubation was at concentration of 20 µg / ml, which recorded 56.0% and 51.0% of inhibition, respectively. Treating the parasite with these fatty acids has reduced the generations number of and extended the time needed to produce new generations. The current study was highlighted the use of the fatty acids of these two plants as a promising treatment against cutaneous leishmaniasis in humans being.

Keywords: *Lieshmania tropica*, fatty acids, *Curcuma longa*, *Prunus avium L.* and cherry plants.

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INTRODUCTION

Natural products are chemical compounds isolated from plants, and all of these compounds are caused by Secondary metabolites and are not directly involved in the growth or reproduction of the plants (Anulika *et al*, 2016). It is classified into three main sections, which are turbines (involving fatty acids) , phenolic compounds and alkaloids, and these compounds depend in their construction on the primary metabolism, and most of the natural products have a pharmacological activity in the treatment of many diseases, including cancer, these compounds are also starting points for discovering many manufactured drugs. Most wild and field herbaceous plants contain secondary chemical compounds of great benefit that are used for the purposes of sustaining the life of plants or protecting and defending them against other organisms known as natural products. (Lemin, 2005). Natural products, such as plant extracts, either as standardized extracts or as pure compounds, provide unlimited opportunities for new drug discoveries because of the unprecedented availability of chemical diversity (Rocha, *et al*. 2005; Cos, *et al.* 2006)

Curcuma longa, or turmeric, is a perennial Indian herb belonging to the Zingiberaceae family. Its roots are known as roots of Rhizome that grow under the soil surface and are the most useful and used part of the plant in treating its effective chemical content, as well as its use in cosmetics and cooking. (Bagchi, 2012). The native habitat of turmeric is South Asia and it needs a large amount of annual rain and temperatures ranging from 20-30 C° for growth and flowering, and Curcumin is one of the most popular plant compounds , and it is one of the compounds that reduce cholesterol in the blood in addition to that the plant contains phenol and flavonoids and others Of alkaline fatty acids and glycosides. (Okigbo, *et al*, 2009).

The *Prunus avium* L. (Sweet cherry) of the Rosaceae family, which contains more than 100 genius spread throughout most of the world (Oukabli and Mahhou, 2007). It is a deciduous and large-sized fruit tree with a height of up to 18 m, and it is a low-branch tree with a reddish-brown cylindrical stem, and its leaves are spear-edged, with dark green serrated edges on the upper surface and light on the underside. Its white and pink hermaphrodite flowers are combined in a cluster nodule and are distinguished by their dark blues (Mahmood, *et al*,2000). Sweet cherry is of economic importance because of its nutritional and health value of its fruit, as well as its medical effect because it contains effective chemical compounds that are a source of antioxidants such as phenolic compounds responsible for a number of vital activities (Ballistreri, *et al*, 2012).

"Leishmaniasis" are a complex of diseases caused by *Leishmania* spp. parasite, which transmitted by phlebotomine sand flies in tropical and sub-tropical regions (Ponte-Sucre *et al.,* 2017). and it is Globally distributed and associated with poverty. (Ibarra-Meneses, *et al.,* 2020). *Leishmania* spp. have two distinct stages in their life cycle. The moving promastigote stage lives in the digestive system of the sand fly vector, while the immovable amestigot stage resides within the phagocytes of the mammalian hosts. (Chang, *et al*., 1985). Cutaneous leishmaniasis (CL) is a neglected disease and is estimated to occur in the world around 600,000 to 1,000,000 new cases each year, mainly affecting children in poor areas. (Alvar, *et al*. 2012). CL is not life threatening, although it causes mostly distorted lesions on exposed body parts such as the face, arms and legs. Consequently, those affected are stigmatized and ostracized, and impair access to marriage, education, and well-paid jobs, which leads to economic and psychological losses. (Bennis, *et al*., 2018 and Bailey, *et al.*, 2019). CL offers

several clinical presentations based on leishmaniasis, host and transition immunity. A typical lesion of CL is a painless papule or ulcer at the site where the female sand fly is fed. In a variable proportion of cases, self-healing can take place within 3-18 months, and often develop into an ulcer covered with an adherent crust of dried secretions during this period. In the Old-World countries, CL occurs mostly by L. major, L. aethiopica and *L. tropica*. All species are capable of producing multiple lesions that tend to heal slowly and leave large, deformed scars. (Burza, *et al*., 2018).

Current treatments for CL have been poorly justified by clinical trials and have suboptimal efficacy (Gonzalez, *et al*., 2009). these treatments have been extensively reviewed in two recent publications (Burza *et al*., 2018). Treatment for a long time relied on Current medicines that are considered highly toxic for introduction into modern registration systems. Current systemic therapies, including pentavalent antimonials, amphotericin B and meltifosine used in CL, have been conceived or developed as specific therapies for CL. If the drug works with visceral visceral leishmaniasis VL, it is tested and adapted for use in CL groups, despite the fact that the pharmacokinetic properties of the drug used to treat a patient with VL are often different from those of CL patients. (Solomon, *et al*., 2013 and Guery, *et al*., 2017).

In spite of the availability of chemical compounds manufactured to treat the parasite, studies have proven that they are not free of toxic effects that appear at the length of the treatment period, as well as the high material cost of the manufactured chemical drugs, so there was a need to search for effective substances of natural origin as well as being safe and effective, Scientific research has also proven the effectiveness of plant compounds with antimicrobial properties, because of their medicinal importance in treating many diseases as well as they are often free of side effects (Oyi, *et al*, 2002). Therefore, The aim of the current study was to extract fatty acids from the Curcuma longa and Prunus avium L. plants and diagnose them with GLC technique and then compare their effect on the growth of *Leishmania tropica* promastigotes in *in vitro* , In an attempt to discover and develop alternative treatments, that are safe, available and cheap in the future, to determine the best types of fatty acids that inhibit parasite growth..

MATERIALS AND METHODS

Collect *C. longa* and *P. avium*

Turmeric Rhizome roots and sweet cherry seeds were collected from the local markets of the city of Mosul, and they were classified in the Center for Medicinal Plants Development in the Mosul Dam of the Iraqi Ministry of Agriculture, the roots and seeds were cleaned of soil and what was stuck in them, then placed in paper bags and kept in conditions away from moisture until use.

Preparing the vegetable extract using the Soxhlet

Turmeric rhizomes and sweet cherry plant seeds were ground by an electric grinder. 25 g turmeric powder and cherry seed were put into Batch of filter paper in the Soxhlet, after which 400 ml of petroleum ether was added to extract the oil. Extraction continued 7 hours per day until the solvent used in the extraction became colorless, and then concentrate the alcoholic extract with a rotary vacuum evaporator at a temperature of 40 C° (Hasan *et al*, 2019).

A. Saponification

To obtain the fatty acids we carry out the Saponification process:

Take 5 ml of the crude extract of the petroleum ether and added 100 mL of (KOH) 1N, Heating the solution for 90 minutes at 100 C°, Then, added 100 ml of distilled water and 50 ml ether solvent and put in the separating funnel, and took the aqueous layer and added the concentrated sulfuric acid H2SO4 until PH=2.In the end add 50 ml of ether and put again in the separating funnel and take the organic layer and retain well (Arthur,1972).

B. Parasite Culture

The reference *L. tropica* strain was obtained from college of medicine Al Nahrain university. These promastigotes culture of local Iraqi leishmanial strain (MHOM / IQ / 1992 / MREC3) was successfully grown in RPMI-1640 medium (Moore, *et al*., 1976) The media supplemented with 10% fetal calf serum (FCS) at 25°C.

Viability Test Assays on Promastigotes

Parasites in the promastigote stage were transferred from stock culture media to RPMI-1640 supplemented with 20% fetal calf serum (FCS), pH 7.2.

In order to get lethal concentration (LC50) of fatty acids that isolated from *C. longa* and the seeds of *P. avium* L., Five similar concentrations of fatty acids isolated from the two plants were used in the current study (50, 40, 30, 20 and 10 µg / ml) to find out which one of them was most effective against *L. tropica* promastigotes in *in vitro*, these concentrations were added to the promastigote culture medium (10 ml) were performed in test tubes. Subsequently. 23.2×10^3 promastigotes /ml were added to each tube contain 10 ml of media, was incubated at 25°C for 96h. Negative controls (culture without fatty acids) were also used, each tube mixed well in the end of each 24h, parasites were counted by the help of a hemocytometer.

STATISTICAL ANALYSIS

The present study data was statistically analyzed using the Tukey test to compare the means. The significance level of $\alpha = 0.05$ was applied to the test. (SPSS v.22) programs used to analyze current data.

RESULTS AND DISCUSSION

The identification of fatty acid compounds of *C. longa* and *P. avium* by GLC technique

The Identification of the petroleum ether extract after saponification by GLC showed the presence of the following fatty acids Table (1), Fig (1) and (2):

The fatty acids of the turmeric plant contained the Pentadecanoic fatty acid with a retention time (7.995) and with a concentration of (3.27), which is consistent with the standard sample retention time (7.034) minutes, and its absence in the cherry plant. Palmatic fatty acid appeared in

the turmeric and cherry plant with a retention time (8.504) and (8.450) with a concentration of (4.32) and (8.450), respectively, which is consistent with the standard sample retention time (9.022) minutes. heptadecanaoic fatty acid appeared in the turmeric and cherry plant with a retention time (10.372) and (10.355) with a concentration of (4.13) and (1.57), respectively, which is consistent with the standard sample retention time (10.159) minutes. Stearic fatty acid appeared in the turmeric and cherry plant with a retention time (11.761) and (11.439) with a concentration of (9.13) and (4.23), respectively, which is consistent with the standard sample retention time (11.261) minutes. Elaidic fatty acid appeared in the turmeric and cherry plant with a retention time (12.645) and (12.650) with a concentration of (6.56) and (9.72), respectively, which is consistent with the standard sample retention time (12.378) minutes. Oleic fatty acid appeared in the turmeric only with a retention time (13.563) and with a concentration of (6.56), which is consistent with the standard sample retention time (13.094) minutes. Lenoli fatty acid appeared in the turmeric only with a retention time (14.694) and with a concentration of (3.76), which is consistent with the standard sample retention time (14.361) minutes. Arachidic fatty acid appeared in the turmeric and cherry plant with a retention time (15.315) and (15.268) with a concentration of (8.57) and (4.00), respectively, which is consistent with the standard sample retention time (15.925) minutes. Eicisenoic fatty acid appeared in the turmeric and cherry plant with a retention time (16.606) and (16.558) with a concentration of (2.17) and (7.02), respectively, which is consistent with the standard sample retention time (16.700) minutes. Behenic fatty acid appeared in the turmeric and cherry plant with a retention time (18.212) and (18.137) with a concentration of (2.87) and (7.44), respectively, which is consistent with the standard sample retention time (17.618) minutes. Erucic fatty acid appeared in the turmeric only with a retention time (18.212) and with a concentration of (3.55), which is consistent with the standard sample retention time (18.592) minutes. Arachidonic fatty acid appeared in the turmeric and cherry plant with a retention time (19.496) and (19.460) with a concentration of (1.18) and (4.28), respectively, which is consistent with the standard sample retention time (19.509) minutes. Tricasnoic fatty acid appeared in the turmeric and cherry plant with a retention time (20.616) and (20.776) with a concentration of (1.41) and (9.63), respectively, which is consistent with the standard sample retention time (20.566) minutes. Lignoceric fatty acid appeared in the turmeric and cherry plant with a retention time (21.982) and (21.161) with a concentration of (3.41) and (4.72), respectively, which is consistent with the standard sample retention time (21.701) minutes.

Through the results it appears that the total concentration of fatty acids of the roots of the turmeric plant (59.71) is higher than the concentration of fatty acids of the seeds of the cherry plant (56.16) and the reason for this is the disappearance of three fatty acids from the seeds of the cherry plant. Also, the secondary metabolism activity of the turmeric plant is more effective in producing fatty acids.

Table 1: Fatty acids identified using the GLC technique for petroleum ether extract

No.	Standard fatty acid compounds	Standard retention time (minute)	The retention time of the petroleum ether extract (minute) and concentration (mlg/gm)			
			Curcuma longa		*Prunus avium*	
			Ret. time	concentration	Ret. time	concentration
1	Pentadecanoic	7.034	7.995	3.27	--	
2	palmatic	9.022	8.504	4.32	8.450	3.54
3	heptadecanaoic	10.159	10.372	4.13	10.355	1.57
4	Stearic	11.261	11.761	9.13	11.439	4.23
5	Elaidic	12.378	12.645	6.56	12.650	9.72
6	Oleic	13.094	13.563	5.38	--	
7	Lenoli	14.361	14.694	3.76	--	
8	arachidic	15.925	15.315	8.57	15.268	4.00
9	eicisenoic	16.700	16.606	2.17	16.558	7.02
10	Behenic	17.618	18.212	2.87	18.137	7.44
11	Erucic	18.592	18.333	3.55	--	
12	arachidonic	19.509	19.496	1.18	19.460	4.28
13	tricasnoic	20.566	20.616	1.41	20.776	9.63
14	lignoceric	21.701	21.982	3.41	21.161	4.72
Total concentration				59.71		56.16

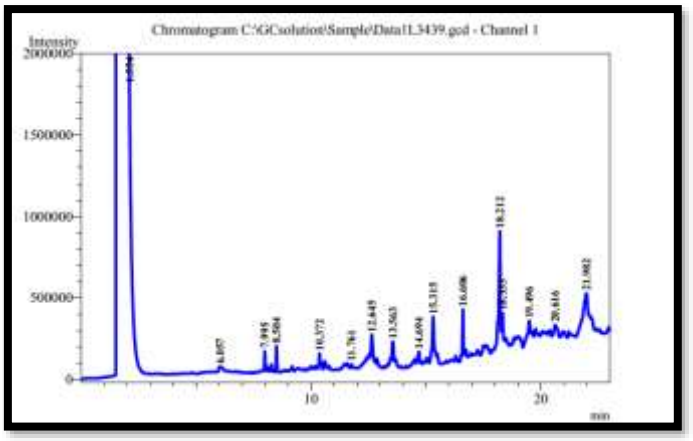

Fig. 1: Curved fatty acid compounds for *Curcuma longa.* by GLC.

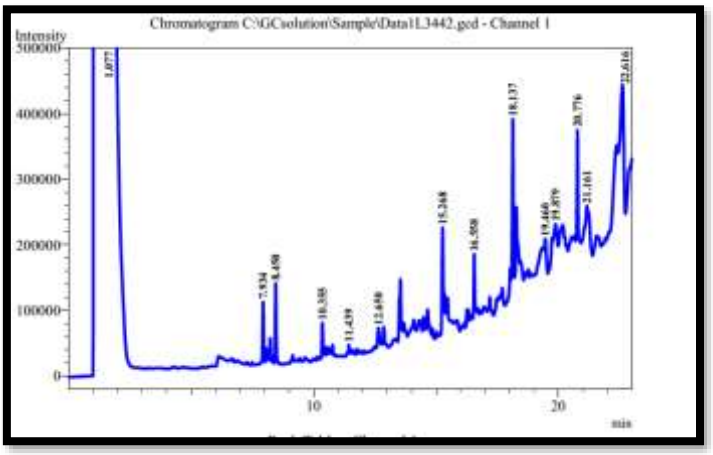

Fig. 2: Curved fatty acid compounds for *Prunus avium* by GLC.

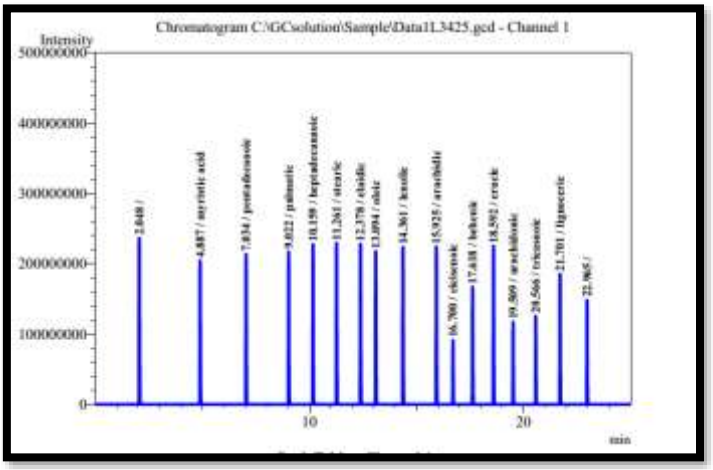

Fig. 3: standard curve of fatty acid compounds by GLC.

In this study, we studied a comparison of the effect of fatty acids extracted from *C. longa* (CLFA) and *P. avium* L. seeds (PAFA) against *L. tropica* promastigotes in vitro. The data in Table 1 showed that *C. longa* contains fatty acids which are Pentadecanoic, Oleic, Lenoli and Erucic, while they are not present among the *P. avium* L fatty acids. And Data presented in Table (2) revealed the impact of various effective of different concentrations of the fatty acids of the two extracts on numbers of *L. tropica* promastigotes at diverse time period. the impact of various fatty acids concentrations on numbers of *L. tropica* promastigotes was significant. the highest rate of inhibition at the highest concentration was 50 µg / ml which reached 26 × 10^3 for the fatty acids of *C. longa* , while it was 28 × 10^3 for the fatty acids of *P. avium L* seeds at the same concentration after 24 hours compared to the control group, while the lowest

inhibition rate at the lowest concentration (10 µg / ml), it recorded 63 × 10^3 for fatty acids isolated from *C. longa*, and 83 × 10^3 for fatty acids isolated from *P. avium L* compared to the control group Which reached and the rate of parasites recorded 83 x 10^3

The effect increased with increasing time. After 48 hours, the highest inhibition rate at the highest concentration for the fatty acids isolated from *C. longa* and *P. avium L*, so it was recorded 44 x 10^3 and 54 x 10^3, respectively, and the lowest inhibition rate was at the lowest concentration Which 107 x 10^3 and 116 x 10^3 , respectively, compared to the control group, which recorded 152 x 10^3. and After 72hs, the rate of inhibition was increased, as it reached the highest rate of inhibition at the highest concentration (50 µg / ml) which scored 74 × 103 and 89 × 103 respectively and the lowest rate was at the lowest concentration (10 µg / ml) compared to the control group which recorded 262 × $10^{3.}$.

The inhibitory effect of *L tropica* promastigotes growth continued after 96hs and the highest rate of inhibition at concentration was 50 µg / ml for the fatty acids of both extracts as it reached 146 × 10^3 and 158 × 10^3, respectively, and the lowest inhibition rate was at the lowest concentration as it was 270 × 10^3 and 296 x 10^3, respectively, compared to the control group, which averaged was 441 x 10^3

Although the fatty acids isolated from the *C. longa* were more discouraging than those isolated from *P. avium* L., they did not record significant differences in the same concentrations, but recorded significant differences when the different concentrations also recorded significant differences in comparison with the control group at different times.

Table 2: Effect of different concentrations of fatty acids from *C. longa* and the seeds of *P. avium* L on the number of *L. tropica* promastigotes at different time intervals.

Exposure period (hrs) / Treatment (µg / ml)		24 Mean ± SE	Inh. %	48 Mean ± SE	Inh. %	72 Mean ± SE	Inh. %	96 Mean ± SE	Inh. %
Curcuma longa	Control	83±0.45 [a] D	……	152±0.63 [b] F		262±1.67 [c] D		441±1.88 [d] E	….
	10	63±0.58 [a] C	24.1	107±0.55 [b] E	29.6	140 ±0.54 [c] C	46.6	270±0.31 [d] D	38.8
	20	41±0.63 [a] B	50.6	96±1.11 [b] D	36.8	132±0.98 [c] C	49.6	194±0.88 [d] C	56.0
	30	33±0.73 [a] A	60.2	81±0.87 [b] C	46.7	126±0.82 [c] C	51.9	178±1.16 [d] B	59.6
	40	30±0.98 [a] A	63.9	70±0.83 [b] B	53.9	110±1.56 [c] B	58.0	160±1.35 [d] A	63.7
	50	26±1.14 [a] A	68.7	44±0.36 [b] A	71.1	74±1.41 [c] A	71.8	146±2.51 [d] A	66.9
Prunus cerasus	Control	83±0.45 [a] D	…..	152±0.63 [b] F		262±1.67 [c] D		441±1.88 [d] E	…..
	10	71±0.44 [a] D	14.5	116±1.14 [b] D	23.7	164±0.31 [c] C	37.4	296±0.70 [d] D	32.9
	20	48±0.89 [a] C	42,2	101±0.63 [b] C	33.6	136±1.34 [c] B	48.1	216±1.65 [d] C	51.0
	30	38±1.09 [a] B	54.2	86±0.63 [b] B	43.4	132±1.26 [c] B	49.6	193±1.04 [d] B	56.2
	40	32±0.83 [a] A	61.4	77±0.81 [b] B	49.3	129±0.72 [c] B	50.8	169±0.94 [d] A	61.7
	50	28±0.89 [a] A	66.3	54±0.69 [b] A	64.5	89±0.72 [c] A	66.0	158±1.26 [d] A	64.2

- Use 5 replicates were used for each treatment.
- The capital letters compare vertically between the transactions for each exposure period, as the different capital letters indicate that there are significant differences at the level of 0.05 between the transactions for each exposure period.
- Small letters are compared horizontally between the time periods for each treatment, as the various small letters indicate a significant difference at the level of 0.05 between the exposure periods for each transaction.

Use the Tukey test to measure the significant differences at the 0.05 level.

Table 3: Effect of different fatty acids concentrations on the generation number of *L. tropica* promastigotes at different time intervals.

Exposure period (hrs) / Treatment		24	48	72	96
Curcuma longa	(µg / ml)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
	Control	8.73±0.02 [a] A	9.93±0.008 [b] A	11.01±0.006 [c] [A]	12.04±0.004 [c] A
	10	8.19±0.01 [a] A	9.24±0.01 [b] A	9.77±0.009 [b] A	11.07±0.002 [c] A
	20	7.34±0.03 [a] A	9.02±0.04 [b] A	9.65± [0.004b] A	10.41±0.002 [b] A
	30	6.91±0.04 [a] A	8.69±0.02 [b] A	9.56±0.01 [b] A	10.24±0.01 [b] A
	40	6.72±0.05 [a] A	8.40±0.02 [b] A	9.29±0.01 [b] A	10.03±0.01 [b] A
	50	6.44±0.1 [a] A	7.48±0.01 [a] A	8.51±0.03 [a] A	9.85± 0.03 [b] A
Prunus cerasus	Control	8.73±0.02 [a] A	9.93±0.008 [b] A	11.01±0.006 [c] [A]	12.04±0.004 [c] A
	10	8.43±0.01 [a] A	9.40±0.02 [a] A	10.08±0.003 [a] A	11.25±0.005 [a] A
	20	7.65±0.02 [a] A	9.12±0.01 [b] A	9.71±0.01 [b] A	10.44±0.01 [b] A
	30	7.19±0.05 [a] A	8.80±0.01 [b] A	9.65±0.02 [b] A	10.40±0.01 [b] A
	40	6.85±0.05 [a] A	8.59±0.02 [b] A	9.61±0.009 [b] A	10.14±0.009 [b] A
	50	6.59±0.06 [a] A	7.88±0.02 [b] A	8.87±0.01 [b] A	10.01±0.01 [c] A

- Use 5 replicates were used for each treatment.
- The capital letters compare vertically between the transactions for each exposure period, as the different capital letters indicate that there are significant differences at the level of 0.05 between the transactions for each exposure period.
- Small letters are compared horizontally between the time periods for each treatment, as the various small letters indicate a significant difference at the level of 0.05 between the exposure periods for each transaction.

Use the Tukey test to measure the significant differences at the 0.05 level.

Table 4: Effect of different fatty acids concentrations on the *L. tropica* promastigotes generation time (hours) at different time intervals.

Exposure period (hrs) / Treatment		24	48	72	96
Curcuma longa	(µg / ml)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
	Control	2.75±0.006 [a] A	4.83±0.004 [b] A	6.54±0.04 [c] A	7.97±0.005 [c] A
	10	2.93±0.005 [a] A	5.19±0.004 [b] A	7.37±0.06 [c] A	8.67±0.14 [c] A
	20	3.27±0.01 [a] A	5.32±0.01 [b] A	7.46±0.02 [c] A	9.22±0.008 [d] A
	30	3.47±0.02 [a] A	5.52±0.01 [b] A	7.53±0.009 [c] A	9.34±0.009 [d] A
	40	3.57±0.03 [a] A	5.71±0.02 [b] A	7.75±0.01 [c] A	9.57±0.01 [d] A
	50	3.73±0.06 [a] A	6.42±0.01 [b] A	8.46±0.02 [c] A	9.75±0.01 [c] A

Prunus cerasus	Control	2.75±0.006 [a] A	4.83±0.004 [b] A	6.54±0.04 [c] A	7.97±0.005 [c] A
	10	2.85±0.004 [a] A	5.11±0.02 [b] A	7.14±0.002 [c] A	8.53±0.004 [c] A
	20	3.14±0.01 [a] A	5.26±0.008 [b] A	7.42±0.01 [c] A	9.03±0.01 [c] A
	30	3.34±0.02 [a] A	5.45±0.009 [b] A	7.46±0.01 [c] A	9.23±0.01 [d] A
	40	3.50±0.02 [a] A	5.59±0.01 [b] A	7.49±0.002 [c] A	9.45±0.009 [d] A
	50	3.64±0.03 [a] A	6.09±0.01 [b] A	8.12±0.01 [c] A	9.59±0.01 [c] A

- Use 5 replicates were used for each treatment.
- The capital letters compare vertically between the transactions for each exposure period, as the different capital letters indicate that there are significant differences at the level of 0.05 between the transactions for each exposure period.
- Small letters are compared horizontally between the time periods for each treatment, as the various small letters indicate a significant difference at the level of 0.05 between the exposure periods for each transaction.
- Use the Tukey test to measure the significant differences at the 0.05 level.

The lethal concentration -50 (LC 50) of fatty acid that extraction from *C. longa*, and *P. avium L* after 96 h of incubation was at concentration of 20 µg / ml, which recorded 56.0% and 51.0% of inhibition, respectively.

As for the effect of different concentrations of the fatty acids that extraction from *C. longa*, and *P. avium* L on generation number (Table 3), there are inverse correlation between generation number and concentration were observed, generation number at log phase (after 96hrs) ranged from 11.07 generations at 10 µg / ml culture to 9.85 generations at 50 µg / ml for fatty acid of *C. longa* and from 11.25 generations at 10 µg / ml culture to 10.01generations at 50 µg / ml for fatty acid of *P. avium* L when compared with control group (12.04 generations).

However, generation time appeared to depend upon the concentration of the fatty acid (Table 4). Generation time increased (direct correlation) when concentration increased. At log phase, generation time values ranged from 8.67h. at 10 µg / ml to 9.75h at 50 µg / ml for fatty acid of *C. longa* and from 8.53hrs. at 10 µg / ml to 9.59h at 70 µg / ml for fatty acid of *P. avium* L when compared with control group (7.97h). This means that the parasite has taken longer to produce new generations compared to the control group.

The current study showed that fatty acids that isolated from *C. longa* are more beneficial than fatty acids isolated from *P. avium* L in inhibition of promastigotes growth in in vitro , that is due to containing on a another fatty acids that will did not found in the *P. avium* L seed which are Pentadecanoic, Oleic, Lenoli and Erucic. which It makes it a promising drug in the treatment of cutaneous leishmaniasis in humans being.

REFERENCES

1. Alvar, J.; Ve´lez, I.D.; Bern, C.; Herrero, M.; Desjeux, P.; Cano, J.; Jannin, J. and den, Boer M. (2012). WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence., PloS One, 7 (5): e35671
2. Anulika , N. P.; Ignatius, E.O. ; Ezenweani, Raymond, S. ; Osasere, O.I. and Abiola, A.H. (2016). The chemistry of natural product: plant secondary metabolites. International journal of technology enhancements and emerging engineering research, vol 4, pp: 1-8.
3. Arthur, I. Vogel (1972). "Practical organic chemistry including qualitative organic analysis, 3rd edition, 445.
4. Bagchi, Anamika (2012). Extraction of Curcumin. Journal of Environmental Science, Toxicology and Food Technology, Volume 1, Issue 3 PP 01-16.
5. Bailey, F.; Mondragon-Shem, K.; Haines, L.R.; Olabi, A. ; Alorfi, A.; Ruiz-Postigo, J.A.; Alvar, J.; Hotez, P.; Adams, E.R.; Velez, I.D.; Al-Salem, W.; Eaton J.; Acosta-Serrano A. and Molyneux, D.H. (2019). Cutaneous leishmaniasis and co-morbid major depressive disorder: a systematic review with burden estimates PLoS Neglected Trop. Dis., 13.
6. Bennis, I.; DE Brouwere, V.; Belrhiti, Z.; H. Sahibi, H. and Boelaert, M. (2018). Psychosocial burden of localised cutaneous Leishmaniasis: a scoping review BMC Public Health, 18, p. 358
7. Burza, S.; Croft, S.L. and Boelaert, M. (2018). Leishmaniasis Lancet, 392 (2018), pp. 951-970.
8. Chang, K.-P.; Fong, D. and Bray, R. S. (1985). "Leishmaniasis," ed. by Chang K.-P., Bray R. S., Elsevier, London, pp. 1—30.
9. Cos, P.; Vlietinck, A. J.; Berghe; D. V. and Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept' Journal of Ethnopharmacology.;106(3):290–302. doi: 10.1016/j.jep.2006.04.003. [PubMed] [CrossRef] [Google Scholar]
10. Gonzalez, U.; Pinart, M.; Rengifo-Pardo, M.; Macaya, A. and Alvar, J.A. (2009). Twee Interventions for American Cutaneous and

Mucocutaneous Leishmaniasis Cochrane Database Syst Rev (2009), p. CD004834.

11. Guery, R.; Henry, B.; Martin-Blondel, G.; Rouzaud, C.; Cordoliani, F.; Harms G.; , Gangneux, J.P.; Foulet, F.; Bourrat, E.; Baccard, M.; Morizot, G.; Consigny, P.H.; Berry, A.; JBlum, J.; Lortholary, O.; Buffet, P. and French , G. (2017) . CUTANEOUS LEISHMANIASIS Study, N. THE Leishman Liposomal amphotericin B in travelers with cutaneous and muco-cutaneous leishmaniasis: not a panacea PLoS Neglected Trop. Dis., 11.
12. Hasan, M.; Khorsheed, A. Ch. And Saleh, Sh, M. (2019). Chromatographic Investigation of Alkaloids (atropine and hyoscine) and fatty acid compounds of *Datura stramonium* and study of its antioxidant effect. Plant Archives Vol. 19 No. 2, pp. 4115-4120.
13. Ibarra-Meneses, A.V.; Moreno, J. and Carrillo, E. (2020). New strategies and biomarkers for the control of visceral leishmaniasis Trends Parasitol., 36 (1), pp. 29-38
14. Lemin, D. (2005). Introduction to Natural Products and Medicinal Chemistry. Japan Society for the Promotion of Sciences, Post-Doctoral Fellowship, Chiba University.
15. Llistreri, G, Continella, A.; Gentile, A.; Amenta, M.; Fabroni, S. and Rapisarda, P. (2013) Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. Food Chem.; 140:630–638.
16. Mahmood, K.; Carew, J.G.; Hadley, P. and Battey, N.H. (2000). The effect of chilling and post-chilling temperatures on growth and fl owering of sweet cherry (*Prunus avium* L.). J. Hortic. Sci. Biotechnol. Vol. 75. 598–601
17. Moore, G.E. and Woods, L.K. (1976). Culture Media for Human Cells- RPMI 1603, RPMI 1634, RPMI 1640 and GEM 1717. Tissue Culture Association Manual. 3, 503-508.
18. Okigbo, R.N.; Anuagasi, C.L.; Amadi, J.E. and Ukapabi UJ. (2009). Potential inhibitory effect of some African tuberous plant extracts on Escherichia coli Staphylococcus aureus and Candida albicans. International journal of interactive biology.;6(2):91-98.
19. Oukabli, A and Mahhou A. (2007). Dormancy in sweet cherry (*Prunus avium* L.) under Mediterranean climatic conditions, Biotechnol. Agron. Soc. Environ, Vol. 11. 133-139.
20. Oyi, A.R.; Onaolapo, J.A. and Adigun, J.O. (2002): Phytochemical and antimicrobial screening of the latex of *Jatropha curcas* Linn (Euphorbiaceae). *Journal of Phytomedicine and Therapeutics* Vol. 1(1-2): Pp. 63-74.
21. Ponte-Sucre, A.; Gamarro, F.; Dujardin, J.C.; Barrett, M.P.; López-Vélez, R.; García-Hernández, R.; Pountain, A.W.; Mwenechanya ,R. and , Papadopoulou, B. (2017) . Drug resistance and treatment failure in leishmaniasis: a 21st century challenge PLoS Neglected Trop. Dis., 11.
22. Rocha, L. G.; Almeida, J. R. G. S.; Macêdo, R. O. and Barbosa-Filho J. M. (2005). A review of natural products with antileishmanial activity. Phytomedicine.;12(6-7):514–535. doi:10.1016/j.phymed.2003.10.006. [PubMed] [CrossRef] [Google Scholar]
23. Solomon, M.; Pavlotzky, F.; Barzilai, A. and Schwartz, E. (2013) Liposomal amphotericin B in comparison to sodium stibogluconate for Leishmania braziliensis cutaneous leishmaniasis in travelers J. Am. Acad. Dermatol., 68, pp. 284-289.