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Diagnosis of phenolic compounds of Curcuma longa and the seeds of Prunus avium L (cherry plants) by HPLC technique and studying their effect on the growth of leishmania tropica promastigotes in in vitro

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Abstract: *Leishmaniasis is a sickness brought about by Leishmaniasis protozoa parasites. The sickness stays a worldwide general wellbeing danger that requires powerful chemotherapy for control and treatment. In this examination, the impact of some chosen phenolic mixes on promastigote leishmaniasis was researched. The mixes were inspected for their exercises against leishmaniasis against prostagogues and intracellular. Phenolic mixes, which are optional plant metabolites present in the eating routine, have been accounted for among other common aggravates that have inhibitory impacts against protozoan parasites. In this investigation, we considered the impact of phenolic mixes on intracellular promastigotes of tropical leishmaniasis and propose a component for their activity against the parasite.*

Kew words: *leishmania tropica, phenolic com pounds, Curcuma longa, Prunus avium l. and cherry plants.*

I. INTRODUCTION

Phenolic compounds are among the most important natural products that are widely found in vascular plants. Phenolic acids are found in soluble forms that are associated with sugars or organic acids and are included in the composition of complex compounds such as lignin, anthocyanins, tannins, flavonoids, etc (Lattanzio, 2013).

Turmeric (*Curcuma longa*) is an eternal grass plant that belongs to the Zingiberaceae family and is widely plowed in Asia, especially in India and China, it leaves are large, narrow oblong to the base (Goel et al., 2008). The rootstocks are the most important parts of the plant and are used in medicine and give a yellow powder. Turmeric is used to treat infections, eczema,wounds, treating acne, parasitic infections, boils, jaundice, bruises, stomach cramps, skin diseases bleeding, and insect bites, and it is also used as a flavor in cooking (Labban, 2014). The most important active compounds in turmeric are flavonoid curcuminoids as well as other components that include resins, sugars and proteins. Curcumin is one of the most studied compounds in research studies, which includes 0.3-5.4% of raw turmeric (Zaman and Akhtar, 2013).

The sweet cherry plant, *Prunus avium* L., of the Rosaceae family, includes 100 genera scattered around the world (Oukabli and Mahhou, 2007). It is one of the most known organic products, and it is characterized by charming colors and scrumptious taste. Cherries are devoured new just as in candy ,wine, jams and other manufactured products, and the cherry has antioxidant properties because it contains phenolic compounds (Budak,

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2016). It has been used as a medicinal substance in the treatment of many as a diuretic or as a medicine for bladder disorders (Karlidag *et al*, 2009). The original sweet cherry fruits vary in size, shape, color and taste and can have unique nutritional and organic qualities (Ercisli, 2004).

Cutaneous leishmaniasis is a medical issue in most of the countries of the world, especially the Mediterranean basin regions (Alvar *et al*, 2012). In these areas, two types of cutaneous leishmaniasis occur: *L. tropica* or *L. major*, the clinical symptoms are similar for both types both types result in skin ulcers and nodular lesions (Shehata *et al*, 2009). Cutaneous leishmaniasis can cause instinctive leishmaniasis, which are not kidding maladies that require an altered treatment routine (Porrozzi *et al*, 2004).

Leishmania in its life cycle goes through two phases, the first amastigote in the host and the second in the vectors, the natural transmission may be animal or human in nature, usually by sand fly (order: Diptera, family: Psychodidae; subfamily Phlebotominae) (Ready, 2008).

A long time ago, naturalistic items secluded from different naturalistic sources, especially those extracted from plants, were used in the treatment of many human diseases, and throughout this period, scientific research through natural products proved to be the best in discovering new drugs and with excellent qualities, the natural products are the result of three pathways to secondary metabolism: chickmic, amino acids, and acetic acid, these resulting compounds have an important effect in treating many diseases through the use of active compounds and plant extracts (Gurnani *et al*, 2014).

II. MATERIALS AND METHODS

A. Collect *Curcuma longa* rhizomes and *Prunus avium* seeds:

Turmeric rhizomes and sweet cherry seeds were obtained from the local markets of the city of Mosul, and were classified at the Medicinal Plants Research Center in the Mosul Dam of the Iraqi Ministry of Agriculture.

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 for defatting. And then, 400 ml of ethanol 70% was added to crude extract. Extraction continued 7 hours per day until the solvent used in the extraction became colorless, and then concentrate the ethanol extract with a rotary vacuum evaporate at a temperature of 40 C° (Hasan *et al*, 2019).

The separation of phenolic compounds from the ethanol extract by the acid hydrolysis process:

5 ml of raw ethanol extract were taken for both plants separately and 25 ml of HCl (1N) were added. Then the thermal escalation was carried out at a temperature of 100 C°. for one hour, then the solution was cooled and placed in the separation funnel and 50 ml of ethyl acetate was added to it the organic layer was taken, 3 g of $MgSO_4$ anhydrous magnesium sulfate was added to it, the samples were preserved inside sealed and dark vials and placed in the refrigerator until identification by the HPLC (Harborne, 1998).

B. Parasite Culture: The reference *Leishmania 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 moved 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 phenolic compounds that isolated from *Curcuma longa* and the seeds of *Prunus avium* L., serial dilutions of both (1, 0.9, 0.8, 0.7, 0.6 and 0.5 mg / dl) were added to the promastigote culture medium (10 ml) were performed in test tubes. Subsequently.

20.3×10^3 promastigotes /ml (initial culture) were added to each tube contain 10 ml of media, was incubated at 25°C for 96h. Negative controls (culture without phenolic compounds) were also used, each tube mixed well in the end of each 24h, parasites were checked by the assistance of a hemocytometer.

III. STATISTICAL ANALYSIS

Information of current investigation were examined by utilizing Tukey test to analyzed between implies . A degree of essentialness of α=0.05 was applied to test. (SPSS v.22) programs used to dissect current information. **(this** paragraph **putted in end of material and method section).**

IV. RESULTS AND DISCUSSION

The identification of phenolic compounds of *Curcuma longa* and *Prunus avium* by HPLC technique.

The chromatographic identification showed that the sweet cherry plant seeds contained the phenolic Quercetin compound with a retention time of (6.143) minutes which is in line with the time of retention the standard sample (6.04) minutes, and the Identification demonstrated that the compound did not appear in turmeric plant table (1).

The identification also demonstrated that turmeric and cherry plant contained phenolic compound Catchine with a retention time of (7.460) minutes and (7.487) minutes, respectively, and was in agreement with the time of standard sample (7.247) minutes table (1).

Also, from the same table it appears that the turmeric plant contains the phenolic compound Keampferol with a retention time of (8.957) minute capture and is consistent with the time of standard sample retention (8.07) minute, and the absence of the compound in sweet cherry plant table (1).

Finally, the diagnosis showed that the cherry plant contained the phenolic compound Rutin with a retention time of (11.313) minutes, which was in line with the time of retention the standard sample (11.90) minutes, and that the compound did not appear in the turmeric plant table (1).

Table 1: Phenolic compounds identified using the HPLC technique for ethanol extract

No.	**Standard phenolic compounds**	**Standard retention time (minute)**	***Curcuma longa* (Ret. time)**	***Prunus avium* (Ret. time)**
1	Quercetin	6.04	--	6.143
2	Catchine	7.247	7.460	7.487
3	Keampferol	8.07	8.957	--
4	Rutin	11.90	--	11.313

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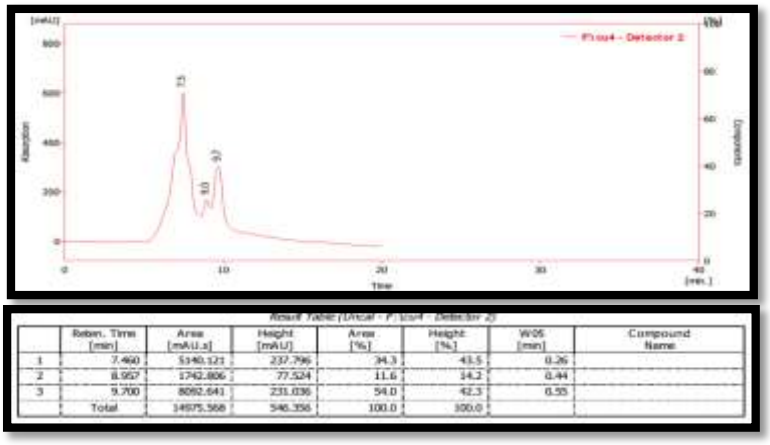
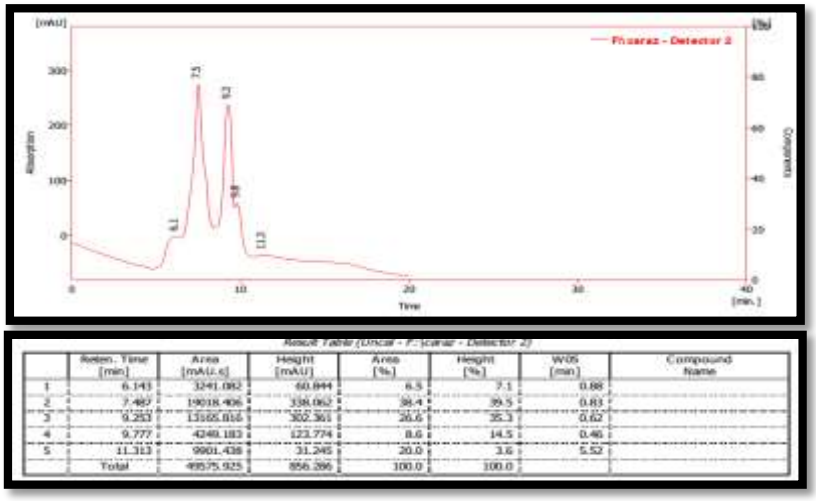


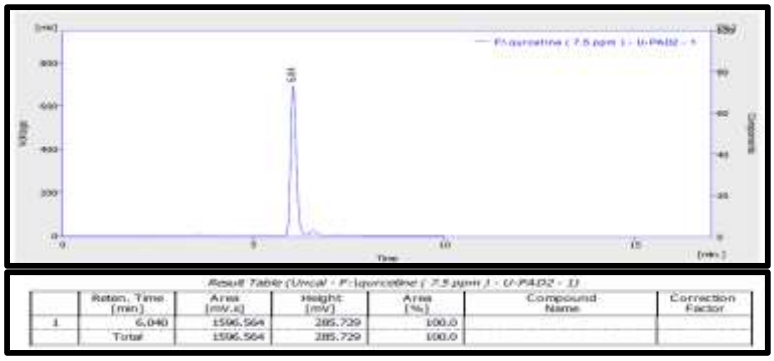
	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.460	5340.121	237.796	34.3	43.5	0.26	
2	8.957	1742.806	77.524	11.6	14.2	0.44	
3	9.700	8092.641	231.036	54.0	42.3	0.55	
	Total	14975.568	546.356	100.0	100.0		

Figure 1: Identification of the phenolic compounds of the turmeric plant with HPLC technology.



	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.143	3241.092	60.844	6.5	7.1	0.88	
2	7.487	19018.406	338.062	38.4	39.5	0.83	
3	9.253	13165.816	302.361	26.6	35.3	0.62	
4	9.777	4248.183	123.774	8.6	14.5	0.46	
5	11.313	9901.438	31.245	20.0	3.6	5.52	
	Total	49575.925	856.286	100.0	100.0		

Fi**gure 2:** Identification of the phenolic compounds of the sweet cherry plant with HPLC technology.



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Compound Name	Correction Factor
1	6.040	1596.564	285.729	100.0		
	Total	1596.564	285.729	100.0		

Figure 3: Qurcetin phenolic compound curve with HPLC.

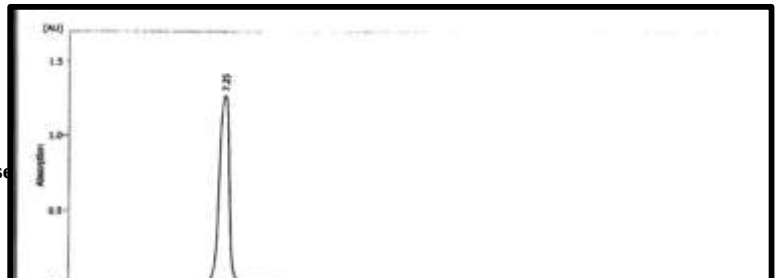
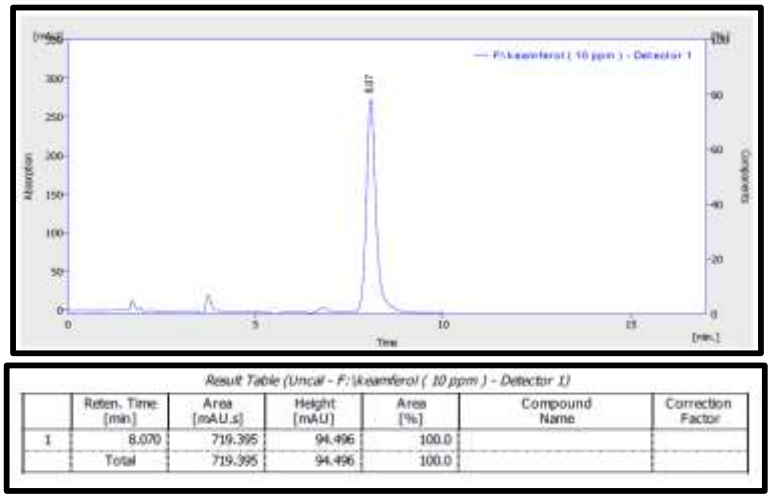


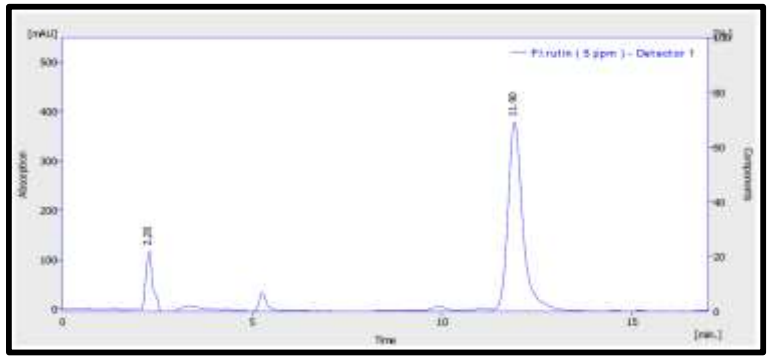
Figure 4: The Standard Curve for Phenolic Compound Catchine



Result Table (Uncal - F:\keamferol (10 ppm) - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	8.070	719.395	94.496	100.0		
	Total	719.395	94.496	100.0		

Figure 5: Keampferol Phenolic Standard Curve with HPLC



Result Table (Uncal - F:\rutin (5 ppm) - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	2.283	708.603	84.547	37.8		
2	11.903	1166.119	99.556	62.2		
	Total	1874.721	184.103	100.0		

Figure 6: Rutin Phenolic Standard Curve with HPLC

In this investigation, the counter conceptive impact of curcumin (Curcuma longa), the dynamic element of ground dried roots against neighborhood reference leishmania strains, Leishmania major, Leishmania tropica, was contemplated.

Curcumin has shown an average microM Against prostigiates of various strains of leishmaniasis it is a lot of lower contrasted with pentamidine which is one of the principle medicines against leishmaniasis.

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Curcumin has shown an average Against prostigiates of various strains of leishmaniasis it is a lot of lower contrasted with pentamidine which is one of the fundamental medicines against leishmaniasis. To these tests performed on promastigotes is heterogeneity of results contrasted with those acquired with intracellular amastigotes or with in vivo impact.

Table 2: The different concentrations of phenolic compounds that extracted from *Curcuma longa* on numbers of *L. tropica* promastigotes at various timespans.

Exposure Time (hrs)	24	Inh. %	48	Inh. %	72	Inh. %	96	Inh. %
Treatment mg/ml	Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
control	[a] 66.33±0.88 D	…..	[b] 180.67±1.45 D	…..	[c] 363.67±2.03 G	….	[d] 678.00±0.58 F	
1	[a] 33.00±0.58 A	50.2	[b] 88.00±1.15 A	51.9	[c] 116.67±1.20 A	67.9	[d] 186.00±1.73 A	72.6
0.9	[a] 36.00±0.58 A	45.7	[b] 92.00±0.58 A	49.1	[c] 126.00±1.53 B	65.4	[d] 197.67±1.45 A	70.8
0.8	[a] 43.33±2.03 B	34.7	[b] 95.67±1.45 A	47,0	[c] 135.67±1.45 C	62.7	[d] 210.33±2.03 B	69.0
0.7	[a] 48.67±0.33 B	26.6	[b] 103.33±0.88 B	42.8	[c] 145.00±1.15 D	60.1	[d] 234.67±2.91 C	65.4
0.6	[a] 53.33±0.88 C	19.6	[b] 116.67±0.88 C	35.4	[c] 158.00±0.58 E	56.6	[d] 252.67±1.76 D	62.7
0. 5	[a] 60.00±0.58 D	9.5	[b] 134.00±1.53	25.8	[c] 176.00±0.58 F	52.0	[d] 287.00±1.15 E	57.7

			D					

For each treatment, use 3 repeats.

The capital letters compare vertically between the transactions for each exposure period, whereas the different capital letters indicate a significant difference at the level of 0.05 between the transactions for each exposure period.

Small letters compare 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: The different concentrations of phenolic compounds that extracted from *Prunus avium* on numbers of *L. tropica* promastigotes at various timespans.

Exposure Time (hrs)	**24**	**Inh. %**	**48**	**Inh. %**	**72**	**Inh. %**	**96**	**Inh. %**
Treatment mg/ml	**Mean ± SE**		**Mean ± SE**		**Mean ± SE**		**Mean ± SE**	
control	[a] 66.33±0.88 C	.....	[b] 180.67±1.45 E	.....	[c] 363.67±2.03 G	....	[d] 678.00±0.58 D	.....
1	[a] 44.33±1.45 A	**33.2**	[b] 97.67±1.33 A	**45.9**	[c] 134.67±1.45 A	**63.0**	[d] 320.33±1.45 A	**52.8**
0.9	[a] 53.33±1.20 B	**19.6**	[b] 105.67±1.76 A	**41.5**	[c] 149.67±2.03 B	**58.8**	[d] 340.67±1.45 A	**49.8**
0.8	[a] 55.33±0.88 B	**16.6**	[b] 121.33±2.19 B	**32.8**	[c] 162.33±2.91 C	**55.4**	[d] 394.00±4.58 B	**41.9**
0.7	[a] 57.33±2.19 B	**13.6**	[b] 125.33±1.76	**30.6**	[c] 192.00±1.53 D	**47.2**	[d] 407.67±3.53 B	**39.9**

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			B					
0.6	[a] 58.00±2.08 B	**12.6**	[b] 139.00±2.08 C	**23.1**	[c] 217.33±1.76 E	**40.2**	[d] 420.00±5.77 B	**38.1**
0. 5	[a] 60.00±1.73 B	**9.5**	[b] 154.33±3.48 D	**14.6**	[c] 243.33±2.60 F	**33,1**	[d] 496.67±4.98 C	**26.7**

For each treatment, use 3 repeats.

The capital letters compare vertically between the transactions for each exposure period, whereas the different capital letters indicate that there are significant differences at the level of 0.05 between the transactions for each exposure period.

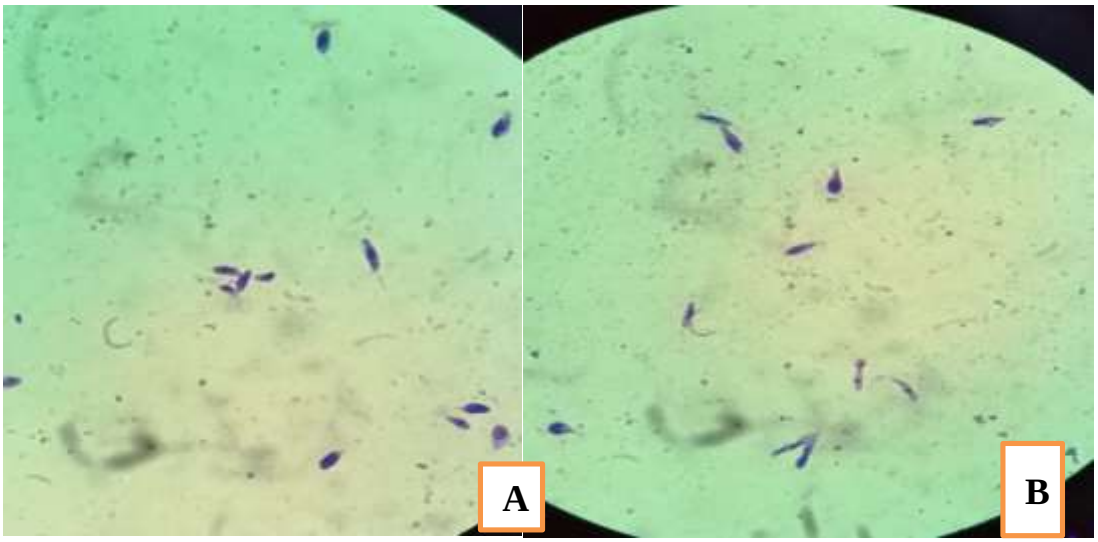
Small letters compare horizontally between the time periods for each treatment, as the various small letters indicate a significant difference at the level of 0.05 between the exposure periods for each transaction.

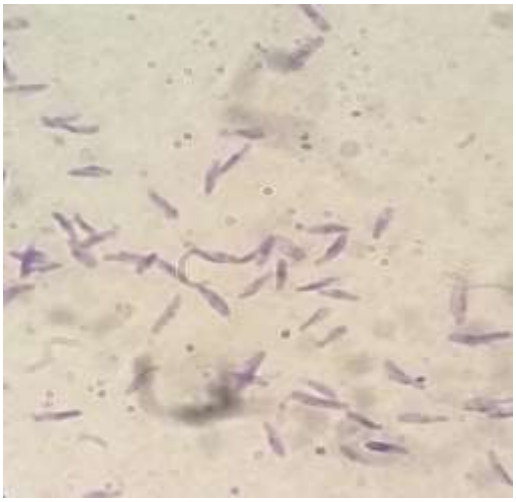
Use the Tukey test to measure the significant differences at the 0.05 level.

By and large, these examinations showed a more noteworthy level of shared collaboration between counter acting agent reactions to the essential leishmaniasis stages than the investigations we found. The openness of explicit leishmania tropica promastigote (extracellular) and (intracellular) films might be connected to the overall abilities of the two phases to animate host insusceptible reactions. To inspect presentation to membranous antigens on inhabitant macrophages, delicate to macrophages

Impervious to inhabitant macrophages, the two stages were broke down by electrophoresis for polyacrylamide gel and immunomodulation after explicit marking and extraction strategies. Auxiliary investigations of protein, utilizing the metabolic markers of prostigots, have shown that the two structures have numerous inside incorporated proteins.

These investigations think about in detail the outside availability and articulation of L. tropica promastigote layer atoms. Different reports for the most part centered around the promastigote phase of different leishmanias, and basically stressed the generally perplexing nature of the promastigot layers. However, little information is available concerning the membrane constituents.





A. *L. tropic*a promastigotes after 96h that treated with *Curcuma longa* phenolic compounds (X 100)

B. L. tropica promastigotes after 96h that treated with *Prunus avium* phenolic compounds (X 100)

C. Control group; We note: *L. tropic*a promastigotes after 96h of incubation (X 100)

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