

# Effect of Aqueous and Alcoholic Extract of Arctium Lappa to the Growth of Leishmania Cutaneous in Samarra City

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## Abstract

The current study was conducted in the city of Samarra for the period from 1/11/2021 until 1/3/2022. Samples were collected from the injured in Samarra General Hospital, 250 samples were diagnosed for patients with cutaneous leishmaniasis, and the information about the injured was recorded according to a special information form, the ages of the injured ranged from less than one year to more than 60 years for both males and females. The results of the epidemiological study showed an increase in males and females at a lower rate, and plant extracts were used, such as burdock extract. As the burdock, plant is an effective plant in killing and inhibiting the parasite, as concentrations were used 0.5, 1, 1.5 ml / ml and at the concentration 1.5 the inhibition rate was high compared to other concentrations.

**Keywords:** Arctium lappa; alcoholic extract; leishmania cutaneous

## 1. Introduction

Leishmaniasis is a tropical and subtropical parasitic disease caused by *Leishmania* (WHO, 2015), a bi-host parasite that lives in the blood and tissues of both humans and animals and belongs to the order Kinoplasts and the family of Trypanosomatidae. The parasite passes through two stages, the first in the vector insect, the female sand fly, which is anterior to the flagellum promastegote form and is in the form of a spindle. Form Amastigotes and in humans and other animals (Wissem, 2017). It is transmitted by the bite of sand flies. It is an endemic disease in Iraq (WHO, 2015) and affects internal organs such as the liver and spleen. Cutaneous leishmaniasis, or the so-called Baghdad boil, also causes skin abnormalities or ulcers. There are two types of cutaneous leishmaniasis, depending on the source of the infection. The first is zoonotic cutaneous leishmaniasis *L. major*, known as the wet form or known locally as African type Ruval. The second is human cutaneous leishmaniasis. HCL Human Cutaneous Leishmaniasis, which causes the dry form of tropical leishmaniasis or known locally as Urban type (Elaagip & Daniel 2020).

Both diseases spread in 88 countries around the world, where the first disease affects about 500,000 people annually and its global prevalence rate is about 12 million people, and there is a third type known as mucocutaneous leishmaniasis or leishmaniasis American (WHO, 2014). The causative agent of leishmaniasis, is one of the main animals that infects mammals, including humans, through the bite of the sand fly *Phlebotomus sergenti*, a unicellular force-dwelling intracellular leishmaniasis (Reithinger et al., 2007) The infection is cutaneous, causing skin abnormalities or ulcers, and the infection is called cutaneous leishmaniasis, followed

by visceral leishmaniasis, which is caused by *Leishmania donovani* (Getty et al., 2009, Sharma and Singh, 2009). To complete deformation of the face and erosion of the membrane that separates the two nostrils, causing the so-called camel's nose (Kate et al., 1982).

Burdock is a plant that lives for two years, its stems reach a height of about one and a half meters, its leaves are oval, curved inward, and the heads of its flowers are reddish. The plant has a root covered with brown to white bark, which is spongy and becomes hard after drying. The used part of the plant: Leaves, roots and fruits that contain seeds. Burdock contains glucose yrate, the most important of which is the compound known as Arctopicrin. It also contains flavonoids, the most important of which is Arqatein. It also contains sulfur acids and volatile oil. oil, Polyacetylene, and Monophonic terpenes. Text is Inulin (Dictionary of Medicinal Herbs, Siham Khedr, 2007). Extracts prepared from different parts of the burdock plant possess different biological activities and pharmacological functions, including antioxidant, anti-inflammatory, anticancer, antidiabetic, antimicrobial, antiviral, antiallergic, anti-ulcer, anti-tuberculosis, and anti-inflammatory effects. For sterility, vasodilatation, gastrointestinal protection, antispasmodic, hepatoprotective and anti-cytotoxic, anti-acne (Petkova et al., 2017 & Jiang et al., 2020). Aqueous extract of *Arctium lappa* L. root enhanced chondrogenic differentiation induced by chondrogenic milieu (Wu & Weng et al., 2020), abdominal obesity and sex hormones in elderly women with metabolic syndrome (Ha & Yook. et al., 2020), and showed an aphrodisiac effect in experimental mice. The extracts of the roots also showed gastroprotective activity and neuroprotective effects.

## 2. Materials and Methods

### Biphasic medium

Also called Novy Mac-Neal-Nicolle NNN medium, it consists of two phases: liquid and solid for the growth and continuity of the Leishmania parasite. The promastigote phase is used to conduct the experiments under study.

#### Solid phase

The medium was prepared according to the method (Kagan & Norman., 1970), as it 37 g Brain heart Infusion

- 10 g Dextrose
- 20 g Agar
- 100 ml Defibrillated Rabbit Blood
- 1.25 ml. 80 mg/ml Gentamicin

#### Preparation of the solid phase

The medium was prepared by dissolving the above-mentioned substances except for antibiotics and blood. The solution was then sterilized at 121 °C and 1 atm pressure for 30 minutes, then cooled to 50 °C in a water bath. Then antibiotics were added, and 15% (B, O) human blood obtained from the blood bank of Samarra General Hospital was used, and then the medium was distributed into 5 mm sterile glass vials and placed on a slope until the middle hardens. The bottles were left warm at 37 degrees throughout the day to ensure that they were not contaminated, and then transferred to the refrigerator and kept at four degrees Celsius until use.

#### Liquid Phase Lock's Solution

According to the method of (Dawson et al., 1969), this medium is prepared and consists of the following substances for every one liter of water:

1. Sodium chloride 9g NaCl
2. Potassium chloride 0.42g KCl
3. hydrated calcium chloride 0.32g CaCl<sub>2</sub>.2H<sub>2</sub>O
4. Sodium bicarbonate 0.2g NaHCO<sub>3</sub>
5. D-Glucose 2 gm D-Glucose
6. Distilled water 1000 ml D.W
7. Antibiotic gentamicin 80 mg/ml 1.25 ml gentamicin

#### Preparation of the liquid phase

All substances are dissolved except for antibiotics. Then adjust the pH to 7.2±0.2. Then it is sterilized in the solid phase and distributed in bottles with a capacity of 25 ml and kept in the refrigerator until use. 2 mL of this phase was added to the vial containing the solid phase, thus forming a biphasic medium and ready to transfer the parasites to it.

#### \* Isolation of leishmaniasis

The leishmaniasis parasite was isolated from samples infected with Baghdad bean and after

confirming their infection by direct microscopic examination by injecting the largest possible amount of Luc solution at the base of the ulcer according to the method (Al-Alousi, 1979) and then withdrawing the solution slowly using a medical syringe with a capacity of 0.1 ml. Cultivated in previously prepared media, and parasite growth is confirmed through microscopic examination of the culture media 96 hours after transplantation.

#### How to make aqueous extract of Burdock

250gm of the plant was taken and soaked in pre-boiled water cooled to 50°C. It was left at room temperature on a magnetic stirrer for 5 hours, after which it was filtered, and the filtrate was washed with 100 ml of cooled boiled water at 50 °C. The filtrate was taken and dried by brushing it with plates in a light layer and the process was carried out using an Oven oven at 45 degrees Celsius for about two hours, after which the extract was collected from the dishes and kept in containers until use (Harborne, 1985).

#### How does the alcoholic extract of burdock work?

250 gm of plant powder was taken and placed in a circular flask and 500 ml of 70% ethanol was added to it. Reflex was fixed, the mixture was heated for two hours and filtered, with re-extraction over the precipitate, but adding 250 ml of 70% ethanol. Filtered and incorporated from the primary filtrate and dried in the same manner as the aqueous extract (Harborne, 1985).

## 3. Results

Table (1) shows the numbers of Leishmania parasites after activation and diagnosis, and it was adopted as a control group

Table (1) shows the control group.	
Time	Mean ± SD * 10 <sup>4</sup>
24 hr	23.700±0.264 e
48 hr	34.333±0.251 b
72 hr	45.367±0.152 a
96 hr	32.200±.200 c
120 hr	26.200±.200 d

#### The effect of burdock treatments (Arctium Lappa)

The stem and leaves of the burdock plant were used in this study, and we used plant extracts (aqueous and alcoholic at concentrations (0.5, 1, 1.5) mg/ml and then put the plant extracts on the Leishmania parasite present in the culture medium Novy Mac-Neal-Nicolle NNN medium. The following results were obtained as shown in Table No. (2) & (3).

**Table (2) Effect of aqueous extract of burdock plant on parasite growth according to concentrations and time**

Time Concentrations	Mean ± SD				
	24 hr	48 hr	72 hr	96 hr	120 hr
0.5	23.767 ± 0.205	34.167 ± 0.125	24.133 ± 0.125	27.300 ± 0.163	16.600 ± 0.163
1	24.600 ± 0.000	32.400 ± 0.327	40.600 ± 0.327	26.400 ± 0.327	19.300 ± 0.163
1.5	24.500 ± 0.245	26.400 ± 0.327	25.233 ± 0.205	16.067 ± 0.094	9.700 ± 0.082

**Table (3) Effect of the alcoholic extract of burdock on parasite growth according to concentrations and time**

Time Concentrations	Mean ± SD				
	24 hr	48 hr	72 hr	96 hr	120 hr
0.5	23.800 ± 0.163	34.233 ± 0.205	24.100 ± 0.082	27.300 ± 0.245	16.600 ± 0.327
1	25.300 ± 0.163	30.867 ± 0.125	27.600 ± 0.163	16.667 ± 0.287	11.600 ± 0.327
1.5	24.833 ± 0.125	28.867 ± 0.094	22.467 ± 0.125	13.233 ± 0.205	9.433 ± 0.205

This study showed a high inhibitory activity of the aqueous and alcoholic extract of burdock, but the inhibition activity of the alcoholic extracts was more effective than the aqueous extracts on the anterior flagella phase of cutaneous leishmaniasis as it was at a concentration of 1.5 mg / ml and at a time of 72 hours and 96 hours at a probability level of <0.05 It gave a significant difference when compared with the rest of the concentrations of the same plant in comparison with the control group, while the rest of the concentrations gave less inhibitory results and this is explained based on the nature of the plasma membrane of the parasite in terms of permeability, and the previous tables of burdock showed a slight increase in the number of Parasites 48 hours after the start of the experiment and this increase occurred due to their proliferation in the culture media and this is consistent with (Ismail et al., 2013). The results also showed the inhibitory activity of burdock plant on the frontal flagellum, which indicates that these extracts contain strong compounds such as flavonoids, and phenols and their use as anti-leishmaniasis medicines, as it agrees with (Al-Jubouri.2005) I also agreed with (Al-Fahdi. 2006), where he recommended the use of nettle and field horse tail for the treatment of leishmaniasis, which gave good results.

**Table (4) shows the percentage of parasite infection by sex**

Sex	Number of infectors	Percentage%
Male	140	56
Female	110	44
Total	250	100

#### 4. Conclusions

The current study reached some important points, which are:

1. The incidence of infection is higher in males than in females.
2. The rate of infection with Leishmania parasite was higher in January than in the rest of the months.
3. The incidence of wet cutaneous leishmaniasis is

higher than the dry type.

4. The alcoholic extract of burdock a higher inhibition effect than the aqueous extract.
5. Burdock extract was highly efficient in inhibiting parasite growth.

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