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Eggplant Calyx the Newest Alternative Cure for Leishmaniasis Disease

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Abstract

For centuries, extracts from plants have been used as folk remedies against various health problems [1]. One of the important plants and herbal medicines is eggplant plant, which has been studied extensively, but its cones wasn't studied thoroughly until now. This study is concerned with the detection and calibration of phenols in the eggplant cones. All the necessary reagents for detection of tannins, flavonoids and chlorogenic acid were positive, and then calibrated phenols with various solvent showed that the ethanolic aqueous extract has the highest percentage of phenol, followed by the aqueous then methanolic extracts. The properties of this extract in terms of its effects on the inhibition of Leishmania parasites were studied. The ethanolic aqueous extract showed a high measure in the rate of inhibition and is equivalent to IC50=39 µg/ml in vitro, the extract was formulated with cream and applied to randomly treated leishmaniasis patients in a double-blind study. The cases were studied in three groups without injection - with injection of glucantime – with liquid nitrogen treatment. Most of the cases have shown improvement or acceleration of improvement since the first week of treatment, confirming the effectiveness and importance of this plant section, which is usually wasted, that is eggplant cones as a new and undiscovered drug with a prospect to treat leishmaniasis.

Keywords: Eggplant cones; Leishmaniasis; Ethanolic aqueous extract; Methanolic extract , Total phenolic content

Introduction

Leishmaniasis is a tropical and subtropical disease caused by an intracellular parasite transmitted to humans by the bite of a sand fly, mainly Phlebotomus and Lutzomyia (Europe, Northern Africa, the Middle East, Asia, and part of South America); exceptionally, transmission has also been reported as a laboratory accident[2]. According to the World Health Organization (WHO), leishmaniasis is one of the seven most important tropical diseases and it represents a serious world health problem that presents a broad spectrum of clinical manifestations with a potentially fatal outcome [3,4]. It is found in all continents except Oceania [3,5] and is endemic in circumscribed geographic areas in North eastern Africa, Southern Europe, the Middle East, South eastern Mexico, and Central and South America.

Leishmaniasis, which is the third in vector-borne parasitic diseases, after malaria and trypanosomiasis, it is of great importance in the world due to the treatment and control difficulties [6]. According to World Health Organization (WHO) data, approximately 20 million people in 98 countries around the world are infected and 350 million people are at risk [7].The parasite is found in amastigote form in the blood and tissues of the vertebrate hosts, and in the promastigote form in the vector sand fly which are invertebrate hosts. Amastigotes are oval or round shaped, 2-4 µm in size and immobile in macrophage cells of vertebrate hosts. When the parasite multiplies in the cell it destroys the cell, and it can be seen individually or in clusters outside the cell [8]. Promastigote form can be detected in the digestive system of the vector. They are single whip shaped or shuttle shaped with a length of 10-20 µm, a width of 1.5-2.5 µm. Promastigotes proliferate in axenic cultures and in the digestive tract of the invertebrate vector [9].

Cutaneous leishmaniasis is a zoonotic disease caused by protozoa of the genus Leishmania and the genus Phlebotomus. There are two important stages in the life cycle of Leishmania: amastigotes found in humans and other non-human reservoir mammals, and promastigotes found in sand fly. In the vertebrate host the clinical outcome is depending on the parasite strain and the host immune response. The lesions are confined to the skin and to the mucous membrane. A granulomatous response occurs and a necrotic ulcer forms at the bite site. Macrophages containing amastigotes, which may be killed by sensitized lymphocytes were detected in microscopic smear. The lesion may become chronic, usually accompanied by secondary bacterial infection [10]. It has been treated by a chemical and herbal ways and those treatment give some side effects.

This study highlighted a new plant extract from eggplant calyx that has proven effective against leishmaniosis in vitro by achieving IC50 =39 µg/ml and the detection of some active substances in this wasted part of this plant, Eggplant (Solanum melongena L), which is a vegetable crop, economically important, consumed widely in Syria. It is native in South East Asian region and was domesticated over 4000 years ago [11]. Many studies have proved the effectiveness of this fruit in the treatment of many diseases such as diabetes, gonorrhoea, cholera, bronchitis, dyspnea, dysentery, debilitation and the treatment of hemorrhoids [12,13]. thus, this extract were either completely effective treatment or effective adjuvant treatment.

Material and Methods

Plant materials

Eggplants (Solanum melongena) were purchased from local markets in Damascus. The fruit calyx were obtained, dried, and stored away from moisture. Three types of extracts (aqueous ethanol, aqueous, and methanol) were prepared.

Preparation of the extracts

a) Methanolic extract [14]: The methanolic extract was prepared with a Soxhlet extractor. 30 g of the plant sample was extracted by 250 ml of 99% methanol for four hours, after which the extract was collected and dried using a rotary evaporator.

b) Ethanolic- Aqueous Abstract [14]: The ethanolic-Aqueous extract was prepared by a Soxhlet extractor. 30g of the plant sample was extracted with 300 ml of (ethanol-Aqueous) for four hours, after which the extracts were collected and dried using a rotary evaporator.

c) Aqueous extract [15]: 30 g of cone powder is placed in the extraction flask with 200 ml of distilled water and heated under an ascending cooler for 1 hour and the transcript is filtered and evaporated using a rotary evaporator until dryness.

Determination of total phenols (TP):

The Folin-Siocaltolin Tp method uses phenols in tungsten phosphorus molybdate acid in an alkaline medium resulting in a blue solution measured at absorption at a wavelength of 760 nm where a series of the reactions occur by electron transfer of two phenols leading to the formation of blue complexes. [16] Prepare the calibration chain: 0.5 g of gallic acid is dissolved in 10 ml ethanol → supplemented with water up to 100 ml in a calibration balloon, preferably kept in the refrigerator for up to two weeks.

To prepare the calibration curve

Add 0/1/2/3/4/5 and 10 ml of the above phenol solution to a 100 ml calibration balloon and complete the volume with distilled water. Then extend the volume with water. We will have the following concentrations of phenol: 0/50/100/150/250/500 mg / L Gallic acid. We put 20 µl in separate covets and add 1.58 ml distilled water + 100 µL foline reagent and mix well then leave 8 minutes and 30 seconds, then add 300 µL of sodium carbonate and mix well and leave the pair for two hours at 20 degrees Celsius. Absorption is measured of wavelength 760 nm versus bulk (distilled water instead of sample) [17].

Preparation of sodium carbonate solution

20% sodium carbonate solution 200 g of anhydrous sodium carbonate is dissolved in 800 ml of distilled water, boiled then cooled, and after 24 hours drain and complete volume to 1 liter [16].

Anti-promastigote assay using MTT (in vitro assay)

The experiment was designed using a cell culture plate comprising 96 wells where 500,000 parasites were taken from the homeostasis stage in each well within a final volume of 100 RPMI culture medium supported by 10% of the FBS gene. The samples were immunized in a cooled incubator at 26°C. After 48 hours, the parasite viability was tested using MTT approved viability test by treating all wells with a final concentration of 10% of the pigment for 3 hours. At the end of the incubation time, formazan crystals formed with a special solvent (MTT Solvent) The samples absorption was 540 nm wavelength using a microplate reader. The average percentage of viability of each sample is calculated using the following equation:

$$\frac{sampleabsorption}{Controlabosorption}\times 100$$

The half maximal inhibitory concentration of the parasite cell growth IC50 is calculated using the Excel program and plotted the viability curve in terms of the absorbance of all samples by the curve equation we calculate the value of IC50 given the viability values as averages ±standard deviation [18].

Clinical study of the effect of eggplant cones extracts on leishmaniasis:

This study was a randomized double-blind, included 30 patients. the treatment was applied as three cases (without injection - with glucantime injection - with liquid nitrogen) to evaluate synergistic and non-synergistic therapeutic effects. The patients were randomly assigned from the dermatology hospital in Damascus and were provided with the necessary materials from the Faculty of Pharmacy, Damascus University.

The cream containing the herbal extracts was distributed randomly and everyone was asked to use it twice a day throughout the 4-week treatment period. The patients were asked to attend at the hospital for local injection of the glucantime in the lesion, and that was only for severe cases requiring injection. Patients were diagnosed by dermatologists at the Dermatology Hospital in Damascus university by testing the swab of the lesion to confirm laboratory infection by detecting the presence of the parasite. The

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area of infection was determined by centimetre (1-2-3-4) cm and assessed its change during the treatment period. Both the degree of redness and edema, as well as the severity of sclerosis and the severity of secondary ulcers, if any, were determined using the Likert encoding scale. The clinical observation was weekly.

Results

Table 1: Phenolate detection in eggplant cones.

Chemical Constituents	Tests	Aqueous Extract	Ethanolic- Aqueous Extract
Tannins/Phenols	FeCl3	+ +	+ +
	Lead acetate	++	++
	Catechin	+++	+++
	Gelatin deposition	++	++
Flavonoids	Shinoda test	++	+++
	Wilson-Taubock test	++	++
	Aluminum Chlorine	++	+++
	routine Isolation	++	+++
	Interaction with zirconium salts	++	++
	Interaction with lead acetate	+	+
	Interaction with sodium hydroxide	++	++
Chlorogenic acid	Ammonia	++	++

By comparing our study with Tiwari et al. [19] study tannins were consistent with our study of iron chlorine with the difference that the reaction with lead acetate was positive in the our present study, and in the interactions of flavonoids Tiwari et al. [19] (Table 1). It was limited to reagent Shinoda only, in this present study we had five reactions that all were positive with Chlorogenic acid.

Phenolic titration results

Similar characters indicate that the statistical differences are not significant while the different letters indicate that (Table 2) the statistical differences are significant, and the value of P <0.05 was used to indicate the statistically significant difference. Statistical differences between the values of these extracts is statistically significant. The aqueous ethanolic extract shows the highest phenolic content followed by the aqueous extract then the methanolic extract (Figure 1) (Table 3). By comparing between current study and previous studies we find that total phenolic content of the aqueous extract is (1025 mg /100g) and this value within the specified range which was studied in 33 species of eggplant of different origins and its value was ranges from (740 to 1430 mg gallic acid 100/g) José et al. [20], but this study was on fruit and not on cones. In another study of total phenolic content in the cones of eggplant Diab et al. [19,21-24] it showed a significant difference in value which was greater (2869 mg / 100 g), and the probability of difference is due to the extraction method as the method of preparation was soaking with stirring for two days. The phenolic content of the ethanolic extract in our study was (1180 ± 12.83 mg / 100 g), and in the study Diab et al. [21] the result was (826 mg /100g), meaning that the ratio was higher in the extract of this present study, with the difference in the method of preparation (Table 4 & 5).

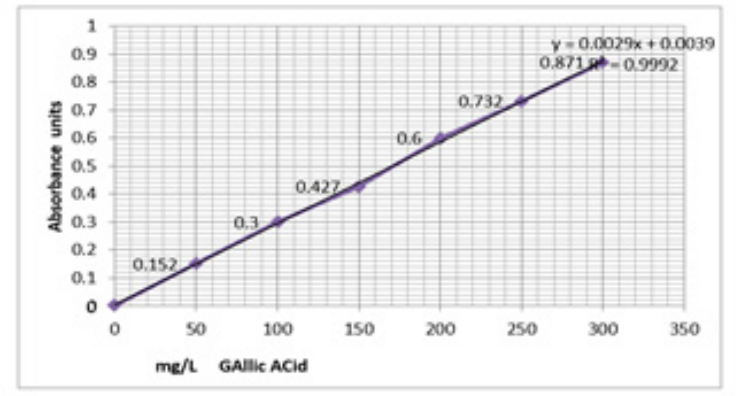

Figure 1: Graphical curve for calibration of gallic acid.

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Table 2: Phenolic titration results in the three extracts.

Extract	The Equivalent of Gallic Acid Mg / 100 G Dry
aqueous – ethanolic extract	12.83 ± b 1180
aqueous extract	21.85 ±c 1025
Ethanolic extract	16.67 a ± 895

Similar characters indicate that the statistical differences are not significant while the different letters indicate that the statistical differences are significant, and the value of P <0.05 was used to indicate the statistically significant difference.

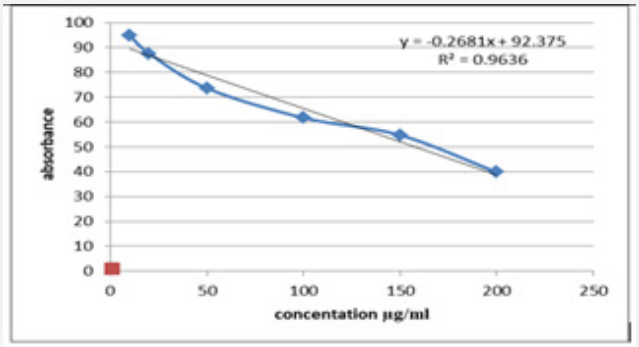
Table 3: Average readings of the standard series of gallic acid.

Concentration µg/ml	0	50	100	150	200	250	300
Absorbance	0	0.152	0.3	0.427	0.6	0.732	0.871

The results of detecting the effect on leishmania parasite

Table 4: The results of detecting the effect of leishmaniasis with aqueous extract.

Concentration µg/ml	10	20	50	100	150	200
Absorbance	94.76	87.6	73.58	61.68	54.73	39.8

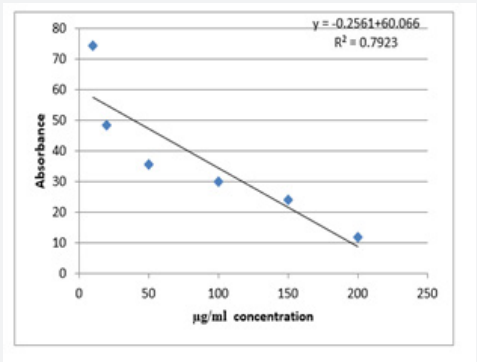


IC_{50}= 159.09µg/ml

Figure 2: The results of detecting the effect of leishmaniasis with aqueous extract.

Table 5: The results of detecting the effect of leishmaniasis with ethanolic-aqueous extract.

Concentration µg/ml	10	20	50	100	150	200
Absorbance	74.4	48.4	35.8	30.13	24.04	11.89



IC_{50}= 39.2µg/ml

Figure 3: The results of detecting the effect of leishmaniasis with ethanolic-aqueous extract.

Table 6: Results of detection of leishmaniasis with methanolic extract.

Concentration µg/ml	10	20	50	100	150	200
Absorbance	73.63	63.22	51.7	22.74	9.48	5.35

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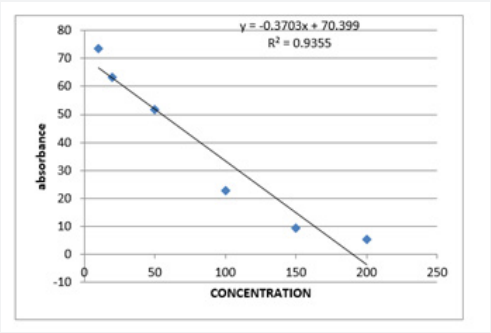


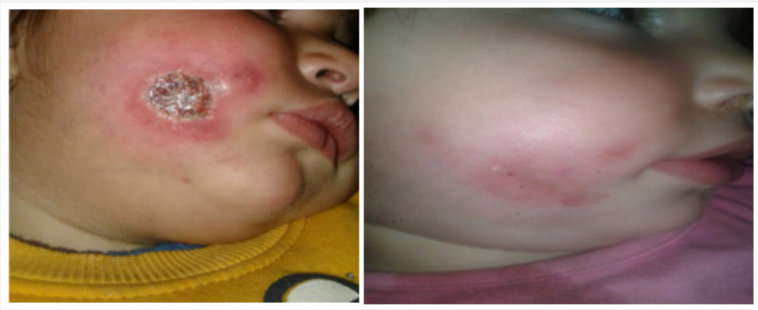
Figure 4: The results of detecting the effect of leishmaniasis with methanolic extract IC50=55.1µg/ml.

Discussion and Clinical Conclusions

In this present study, the eggplant cones contain phenolic substances, useful in the treatment of many diseases, where the results of the calibration of phenolic substances showed that the ethanolic-aqueous extract has the highest phenolic content followed by the aqueous extract. Each of these components has its own therapeutic effect as Flavonoids so it play a good role in treating damaged and inflamed tissues [25] strengthening the walls of capillary veins, anti-inflammatory, anti-fungal, antimicrobial, antibacterial [26,27], antiviral, anti-cancer, anti-vascular, anti-allergic, anti-oxidant [28] preventing injuries caused by free radicals by Inhibiting the free radical enzyme, activation and regeneration of antioxidant systems and has role in reducing the permeability and fragility of the veins [29] And The rutin witch founds in the extract is one of the most important types of flavonoids because it reduces the capillary permeability of blood vessels [30]. Tannins have the strongest effect among phenols for their role in strengthening soft tissues, reducing excess secretions [31-33]and repairing damaged tissues [34].

It works at the same time to stop bleeding due to its astringent effect in addition to its antiseptic effect [35] and it has an important role in the protection of inflamed surfaces of human body [36]. It is also considered anti-tumour because it inhibits the proliferation of cancer cells and prevents DNA from vandalism [31,37] .In addition to these properties, tannins have great antioxidant capabilities due to their Phenol nuclei. Most of the antioxidant effects of phenolic compounds are due to its own Oxidation Reduction properties, which make them Reduction agents. Flavonoids and phenolic acids are among the most potent antioxidant compounds. Phenolic acids also have interesting biological properties such as Anti-inflammatory and antipyretic [38], One of these phenolic compounds is chlorogenic acid it has a role as antioxidant [31,33], protect the inflamed surfaces of the mucous membranes, an anti-tumour that inhibits the proliferation of cancer cells and protects the DNA from destruction [39]. With these auxiliary effects of phenols in skin restoration and strengthening of blood vessels, they support the inhibitory effect of leishmaniasis parasites, especially in the ethanolic-aqueous extract. where the correlation in the results was corresponds in terms of the highest rate of phenols and the highest inhibition of leishmaniasis parasites compared with other extracts (Figure 3 -4).

The efficacy in inhibiting the growth of leishmaniasis parasites in the synthetic medium of ethanol-aqueous extract is very close to that of glucantime, the drug traditionally used for the treatment of leishmaniasis. IC50 for the extract =39µg/ml-IC50 for glucantime=35µg/ml [40]. This is consistent with the clinical results of the cream use containing the extract as the healing of cases that require liquid nitrogen and glucantime injection was faster by using the cream and in the case of using it alone the amount of edema gradually reduced and the severity of infection and sepsis significantly lessened from the first week and pictures show that.



Before case(1) After

Figure 5: case 1: This case was injected with glucantime twice monthly and apply the cream twice daily for 4 weeks (this case arrived to the Dermatology hospital after it was injected by glucantime for 6 time in one year in Raqqa city and the case was without any improvements)

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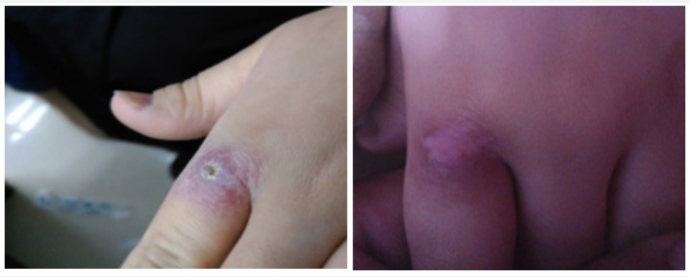
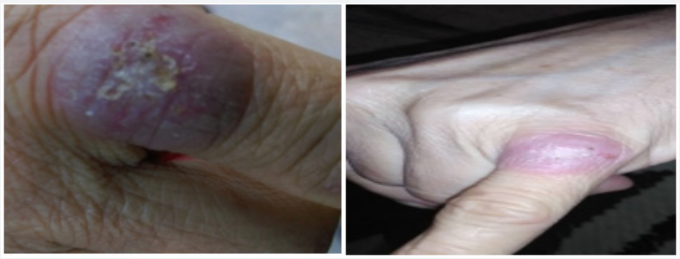


Before case(2) After

Figure 6: Case 2: This case was one time-treated with liquid nitrogen for 2 weeks with applying the cream twice daily



Before case(3) After

Figure 7: Case 3: This case was treated with cream twice daily for only one week without liquid nitrogen and without glucantime injection.

Conclusion

We can say that ethanolic- aqueous extract of eggplant cones(calyx), which is neglected in medical and nutritional use showed a high inhibitory effect of Leishmania parasites close in its efficiency to the glucatiem effect. In addition to the presence of active substances in it, including high percentage of phenols, as it helps to repair the affected area and Accelerates its recovery. therefore, the use an effective cream to treat leishmaniasis disease will benefit the patient all over the world which is more economical, easier to apply, without side effect and less painful in the treatment, so this is the goal that we seek to achieve.

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