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# Antileishmanial, antioxidant, and cytotoxic activities of *Quercus infectoria* Olivier extract

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ABSTRACT

Currently, there is no effective vaccine available, and chemotherapy is the main approach for treatment of cutaneous leishmaniasis (CL). During recent decades, studies have demonstrated that a number of plant-derived compounds may act as new therapeutic tools against leishmaniasis. This study was evaluated the antileishmanial, antioxidant, and cytotoxic activities of *Quercus infectoria* Olivier (oak) extract. The total amount of phenolic and flavonoid compounds was measured in oak extract. High performance liquid chromatography (HPLC) analysis was also performed to determine the amount of quercetin and gallic acid in this plant. This extract (0–80 g/mL) was evaluated *in vitro* against promastigote and intracellular amastigote forms of *Leishmania major* (MRHO/IR/75/ER) using MTT assay and in a macro-phage model, respectively. Then oak extract was tested on CL in infected male BALB/c mice with *L. major* in order to evaluate the antileishmanial activity topically. Moreover, cytotoxicity effects of oak in murine macrophage cells were tested by MTT assay. Antioxidative activity of oak was also determined by the 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) scavenging test. The amount of phenolic and flavonoid compounds in the oak extract was 57.50 and 1.86%, respectively. The amount of quercetin and gallic acid in the oak extract was 0.0064 and 0.22%, respectively. The findings revealed that oak significantly ($P < 0.05$) inhibited the growth rate of promastigote of ($IC_{50}$ 12.65 μg/mL) and amastigotes ($IC_{50}$ 10.31 μg/mL) as a dose-dependent response. In the *in vivo* assay, after 4 weeks of treatment, 91.6, 66.66, and 50% recovery was observed in the infected mice treated with 20, 10, and 5 mg/kg of oak extract, respectively. After treatment of the infected mice with the concentration of 10 and 20 mg/kg of oak, the mean diameter of lesions, parasite load and mean number of parasites was significantly ($P < 0.05$) reduced. Selectivity index of greater than 10 for oak revealed that oak extract had no cytotoxic effects on macrophage cells. Moreover, DPPH test demonstrated that radical inhibition occurred at greater power with increasing the concentration of oak. To conclude, the present study showed potent antileishmanial and antioxidant activity of oak extract; whereas this plant had no toxic effect on mammalian cells.



## 1. Introduction

Leishmaniasis is a prevalent parasitic disease found in 98 countries in 5 continents, which causes 20,000 to 40,000 deaths per year [58]. This disease has three main types of manifestations including visceral, cutaneous, and mucocutaneous leishmaniasis. Cutaneous leishmaniasis (CL) as the most common form of leishmaniasis has the occurrence rate of 0.7–1.2 million cases per year [10]. The most affected countries include Afghanistan, Algeria, Iran, and Brazil.

There are several reports of different parasitic infections including leishmaniasis in Iran [2,23,24,30]. CL is usually characterized by chronic skin lesions and permanent scars of leaves as the deformation of the infected area [27]. Currently, there is no effective vaccine available, and chemotherapy is the main approach for the treatment of CL [31]. Several researches on new therapeutic approaches such as molecular methods and the herbal medicine effect in the treatment of leishmaniasis have been conducted

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[22,26,29,35,49]. Pentavalent antimonial compounds such as meglumine antimoniate and sodium stibogluconate are the first-line antileishmanial agents that have been used clinically since last decades [13]. Treatment of CL by antimonial drugs has some limitations in use, due to the need for intramuscular administration and long treatment periods, side effects, and emergence of resistant cases [8,47]. Furthermore, second-line drugs such as amphotericin B and pentamidine can be toxic and expensive [48]. Therefore, new alternative treatments to provide safe, cheap, and effective antileishmanial agents are urgently needed.

During recent decades, studies have demonstrated that a number of plant-derived compounds may act as new therapeutic tools against parasites such as leishmaniasis [6,25,37,44]. Oak plant with the scientific name of *Quercus*, a shrub that grows in Asia Minor, Iran, and Greece, is one of the popular medicinal plants which has been traditionally used [9,18]. Genus of *Quercus* belongs to the Fagaceae family which has more than 45 species, among which *Quercus infectoria* Olivier is highly distributed in Zagros Mountains, West of Iran [18]. Different parts of oak are known to have multiple therapeutic properties and are used widely in several folk medicine as an analgesic CNS depressant, antiparkinsonian, antidiabetic, and anti-inflammatory drug [17]. Reviews have also related various antimicrobial properties including antibacterial [56], antiviral [16], and antifungal [3] to *Q. infectoria*. Oak consists of a large number of polyphenol compounds and tannins Sakar et al., 2005. However, some factors such as plant species and parts, cultivar sex, geographical origin, harvesting time, and climatic conditions could affect the constituents and functional activity of plants [45]. To the best knowledge of the present authors, there is no study on the antileishmanial effects of oak. Therefore, this study is aimed to evaluate *in vitro* and *in vivo* antileishmanial activities of oak fruit hull (Jaft) extract and compare its adequacy with Glucantime as the reference drug. In addition, as the second objective of this study, antioxidant and cytotoxic effects of this plant are assessed.

## 2. Materials and methods

### 2.1. Ethical statement

This study was carried out in strict accordance with the recommendations in Guide for the Care and Use of Laboratory Animals of National Institute of Health (publication no. 85-23, revised in 1985). The protocol was approved by Committee on the Ethics of Animal Experiments, Lorestan University of Medical Sciences (permit number: 89/6). Moreover, all the efforts were made to minimize suffering.

### 2.2. Chemicals

Meglumine antimoniate (Glucantime®, MA) as a control drug was purchased from Aventis, France. Penicillin and streptomycin were obtained from Alborz Pharmacy, Karaj, Iran, and stored at room temperature (25 °C) until testing. MTT powder [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide], and Schneider's medium were prepared from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) and RPMI-1640 medium with L-glutamine were also purchased from Gibco- BRL, Gaithersburg, MD. All other chemicals and solvents had the highest commercially available purity.

### 2.3. Collection of plant materials

Oak fruit hulls were collected from the rural regions of Khorramabad district, Lorestan Province, west of Iran, in September 2013. They were identified by a botanist from Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences (Khorramabad, Iran). A voucher specimen (RH 1165) of the plant materials was deposited at the herbarium of Razi Herbal Medicine Research Center, Khorramabad, Iran.

#### 2.3.1. Preparation of hydroalcoholic extract

The dried materials of the plants were milled and extracted using methanol solvent and soxhlet extractor at 50 °C [32]. The obtained extract was condensed by vacuum rotary, completely dried under shadow, and stored at 4 °C for later use. The extraction efficiency was reported as 45%.

#### 2.3.2. Determination of total phenolic compounds

The total amount of phenolic compounds was measured using Folin-Ciocalteu [51]. In this method, 500 μL of the extract with 2.5 mL Folin (Folin 0.2 N in 50% methanol) was mixed and incubated at room temperature for 5 min. Then, 2 mL of sodium carbonate (75 g/L) in water was added. After 1 h incubation, the absorption of the samples against the blank (water) was measured at 765 nm.

#### 2.3.3. Determination of total flavonoid compounds

The total amount of flavonoid compounds was measured by Dowd method [1]. In this method, 4 mL of oak extract was mixed with 4 mL of AlCl3 2% in methanol and incubated for 15 min at room temperature. Then, the absorption of the samples was measured at 415 nm. The blank was 4 mL of methanol in 4 mL of oak extract without AlCl3.

#### 2.3.4. High performance liquid chromatography (HPLC) analysis

HPLC device, model Shimadzu (SCL-10AVP), with C-18 column, model Wakosil II 5C18R, with the length of 24 cm, diameter of 6.4 mm, filler particle size of 5 μm, and a protective column with the length of 1 cm was used to analyze the actual sample. This device was equipped with a reciprocating pump, an oven, a continuous degassing device, a sample loop with the size of 20 μm, and a UV/Visible detector, model SPD-10 AVP. Class-VP V.R 6.1 was used to control the HPLC device and process the data. A 100 μL micro-syringe, made by Hamilton Company, was used to collect the sample from the container and inject it into the device. Quercetin was detected using HPLC device by isocratic elution program with methanol, isopropanol solvent, and 20 mM phosphate buffer with pH equal to 2 with the ration of (v:v:v) 70:10:20. The UV detector was set at 380 nm. The chromatograms were run at the flow rate of 0.7 mL/min. Also, gallic acid was detected using HPLC device by isocratic elution program with methanol solvent, water, and phosphoric acid with the ration of (v:v:v) 20:79.9:0.1. The UV detector was set at 210 nm. The chromatograms were run at the flow rate of 1 mL/min [41].

### 2.4. Antioxidant activity

In order to perform the experiment, 0.3 mL with different concentrations of solutions containing the extract and butyl hydroxy tuloene standard (BHT) anti-oxidant was separately poured into the tubes and 2.7 mL of 2,2-diphenyl-1,1-picrylhydrazyl (DPPH) methanol solution ($6 \times 10^{-5}$ M) was added to each tube. The resulting solution was stirred in a continuous shaker device for 60 min under darkness. Then, using a spectrophotometer, the absorptivity of the solution was measured at 517 nm. The free radical scavenging activity of DPPH was calculated according to the following equation [52]:

$$\mathrm{RSA}(\%) = 100 \times \left(\frac{A_{\mathrm{blank}} - A_{\mathrm{sample}}}{A_{\mathrm{blank}}}\right)$$