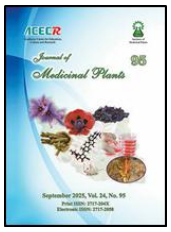
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Research Article

In vitro anti-leishmanial activity of *Boswellia sacra* gum resin extract on *Leishmania major* promastigotes

Sahar Hamoonnavard[1,*], Hossein Rezvan[1], Fatemeh Behdarvand[1], Behnaz Bazargani-Gilani[2], Mahshid Mousavi[1]

[1] *Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran*
[2] *Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran*

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ABSTRACT

Background: The adverse effects of traditional chemical treatments have driven interest in herbal compounds for leishmaniasis therapy. **Objective:** This study aimed to investigate the anti-leishmanial effect of *Boswellia sacr*a gum resin extract on the survival of *Leishmania major* promastigotes. **Methods:** *Leishmania major* strain (MHOM/IR/75/ER) was adjusted to 2 × 10^5 parasites per well. Promastigotes were exposed to a range of *B. sacra* gum resin extract concentrations (15, 25, 50, 70, 100, 200, 400, 800, 1600, and 3200 µg/ml) for 24 hours. Viability and cytotoxic effects were assessed using the MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) with absorbance measured at 450 nm. **Result:** The *B. sacra* gum resin extract reduced promastigotes viability at various concentrations, with statistically significant effects observed at 800, 1600, and 3200 µg/mL ($P \leq 0.05$). Cytotoxic effects at 1600 and 3200 µg/mL were significantly greater than those at 15, 25, and 50 µg/mL ($P \leq 0.01$). **Conclusion:** Based on the *in vitro* anti-leishmanial activity observed, *Boswellia sacra* resin extract warrants further investigation as a potential agent against cutaneous leishmaniasis. Future *in vivo* studies are recommended to evaluate efficacy and safety.

1. Introduction

Cutaneous leishmaniasis (CL) is a parasitic disease caused by the protozoan *Leishmania*. The parasite is transmitted by the bite of infected sandflies and is widespread in tropical and subtropical regions, including Iran, with many people affected each year [1]. Although chemical treatments exist, concerns about drug resistance and adverse effects have spurred interest in herbal preparations [2]. Glucantime (Meglumine antimoniate) is commonly used as a first-line therapy for leishmaniasis in Iran; however, it is contraindicated in patients with hepatic or renal impairment due to potential liver toxicity and other systemic effects. Resistance to Glucantime, along with its cost, side effects, and treatment-related pain, has further motivated exploration of alternative

Abbreviations: APC, Antigen-presenting cells; Bas, Boswellic Acids; CL, Cutaneous leishmaniasis; IC_{50}, Inhibitory concentration; MIC, Minimum inhibitory concentrations; NK, Natural killer cells; Th, T helper.

*Corresponding author: s.hamoonnavard@basu.ac.ir

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approaches [3]. Consequently, researchers are increasingly investigating medicinal plants as safer options with fewer adverse effects. Various studies have reported anti-leishmanial activity of medicinal plants against *Leishmania major*; for example, *Aloe vera* has shown activity against both promastigotes and amastigotes, potentially by modulating macrophage activity; *Thymus vulgaris* exhibits strong anti-leishmanial effects; and *Mentha pulegium* essential oil has been reported to inhibit parasite growth [4, 5].

Boswellia sacra (Indian frankincense), commonly known as frankincense or olibanum, is a deciduous tree native to India, Africa, and the Arabian Peninsula also known as *Boswellia carteri* belongs to the Burseraceae family. The sap from *B. serrata* is sometimes used to make frankincense or olibanum, frankincense is an aromatic oil gum-resin extracted from *B. sacra* and used as a home remedy [6, 7]. The oil of *B. sacra* contains monoterpenes (97.3 %), octyl acetate (39.9 %) followed by 1-octanol (11.9 %), limonene (33.5 %) and, α-pinene (15.1 %) as the main component in olibanum from *B. sacra* [7-9]. The therapeutic uses of essential oil of different *Boswellia* spp resins have been investigated and used as a traditional composition for cardioprotective, antimicrobial and, anti-inflammatory activities [10]. The antimicrobial properties of *B. sacra* (frankincense) resin have been studied; frankincense oil has shown mild antibacterial activity against methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Pseudomonas aeruginosa; in vitro* studies using minimum inhibitory concentrations (MIC) indicated that frankincense oil has mild antibacterial properties [11, 12]. While the total essential oil concentration was different among the three varieties of *B. sacra*, a comparative analysis of the essential oils showed that all samples had strong antibacterial activity, especially, against fungal infections such as *Candida albicans* [12]. Frankincense oils have been shown to prevent the development of Gram-positive and Gram-negative bacteria. Essential oils extracted from various *B. sacra* resin have shown varying degrees of efficacy against dermatological bacteria such as *Propionibacterium acnes* [12, 13]. Boswellic Acids (BAs) are a group of oleanane or ursane pentacyclic triterpenoids derived from the gum resin of *B. sacra*, BAs characterized by a carboxylic acid group at the C-4 position and are classified into *β*-BAs and *α*-BAs. These compounds have demonstrated pharmacological effects including anti-inflammatory, antimicrobial and anti-arthritis properties by suppression of leukotriene biosynthesis in neutrophil granulocytes [14, 15]. Recent studies have shown that frankincense has anti-inflammatory, antifungal, antimicrobial and, antioxidant properties, and no side effects or toxicity have been reported in humans [16]. The efficiency of the immune system was also increased by consuming this compound [17]. In this study, the viability of *Leishmania major* exposed to different concentrations of *B. sacra* gum resin extract was investigated due to the antimicrobial effects of frankincense resin.

2. Materials and methods

2.1. Parasite Culture

Leishmania major strain (MHOM/IR/75/ER) was provided by the Pasteur Institute, Tehran, Iran. Promastigote were cultured in RPMI-1640 medium (containing 10 % FBS and 100IU/ml penicillin & 100µg/ml streptomycin antibiotics) by incubation at 24 ± 1 °C (permission number: IR.BASU.REC.1403.055). Logarithmic phases

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from promastigote were used for this investigation.

2.2. Preparation of B. sacra gum resin (frankincense) extract

The dried resin was ground into powder according to the herbarium confirmation (Herbarium Code: BASU-HBI). 200g of *B. sacra* powder was dissolved in 2000ml of distilled water (D.W) and stored at 4°C for 24 hours. The soaked powder was incubated at 60°C until dissolved. Then the solution was filtered through filter paper (Whatman) and made up to a volume of 1000 ml with D.W. The extract solution was sterilized using a 22 µm syringe filter and stored at 4 °C [18].

2.3. Anti-promastigote activity assay

Promastigote forms (2×10^5 cells/well) in the logarithmic phase were cultured in plastic tissue culture (96-well plates). Promastigotes were treated for 24 hours at 24 ± 1 °C with different doses of *B. sacra* resin extract (15, 25, 50, 70, 100, 200, 400, 800, 1600 and, 3200 µg/ml) and RPMI-1640 medium as a control. After incubation, the MTT method was used to determine the survival rate of parasites exposed to the *B. sacra* gum resin extract. For this purpose, 20 µl of MTT solution (5 mg/ml) was added to each well, the plates were incubated again for 4 h at 24 ± 1 °C, then the optical density was measured and the reduction of MTT dye (tetrazolium) to formazan was determined by adding isopropanol solution(0.04 M HCL) to the treated and untreated samples (450 nm) [19]. Finally, the cell viability rate was calculated using the following formula [20]:

$$\text{Cell viability (\%)} = \frac{(absorbance\ of\ treated\ well)}{(absorbance\ of\ control\ well)} \times 100$$

2.4. Statistical analysis

Data were statistically analyzed using SPSS version 18. One-way (ANOVAs) analyses followed by Tukey tests were used ($P \leq 0.05$ & $P \leq 0.01$) to determine significant differences. The results were visualized using GraphPad Prism 8.

3. Results

3.1. Parasite survival rate

The gum resin extract of *B. sacra* was prepared and, the anti-leishmaniasis effect of different concentrations on promastigotes were measured using the MTT method. Cell viability was evaluated with different concentrations of the extract (Table 1) (Fig. 1A, 2A).

Table 1. Survival percentage of parasites exposed to different doses of *B. sacra* gum resin extract

Concentration (µg/ml)	Mean ± SD
0 (Control)	100.69 ± 1.32
15	90.21 ± 3.35
25	87.32 ± 1.26
50	87.21 ± 6.54
75	84.29 ± 2.62
100	81.71 ± 3.96
200	81.67 ± 3.85
400	82.93 ± 7.37
800*	81.15 ± 2.24
1600*	77.18 ± 2.23
3200*	75.58 ± 0.60

* Significant values compared to the control

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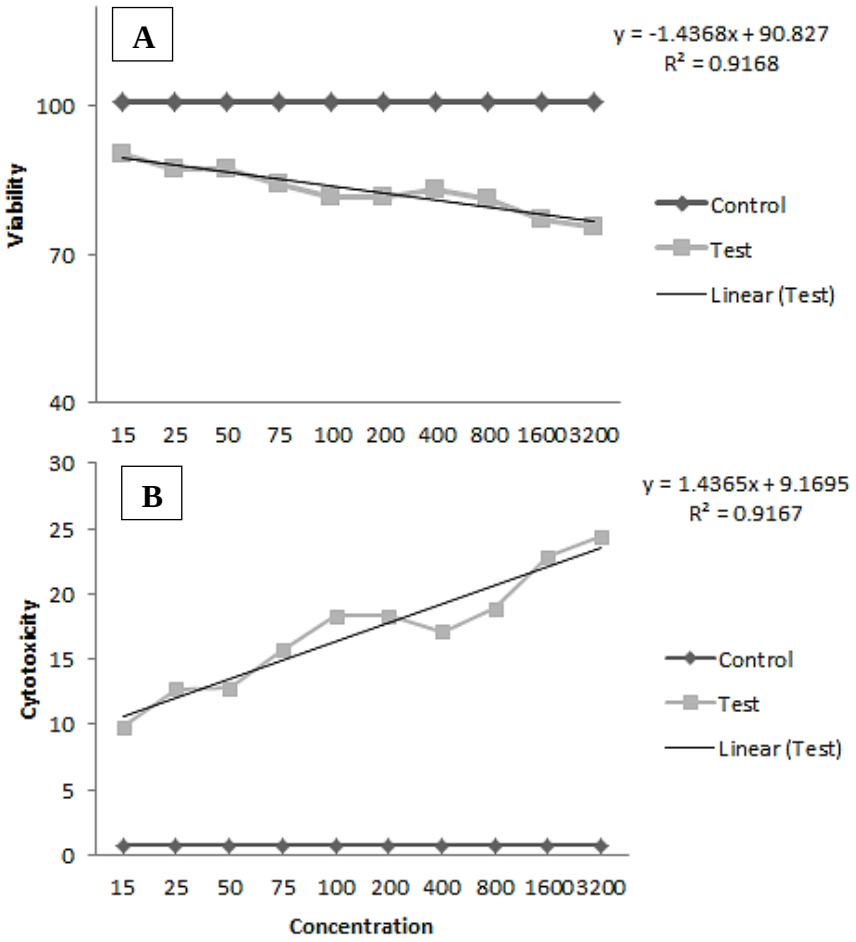


Fig. 1. A: Evaluation of *L. major* promastigote viability **B:** Cytotoxicity effect of *B. sacra* gum resin extract on *L. major* promastigotes

3.2. Inhibition of parasite growth

The gum resin extract at different concentrations (800, 1600 and, 3200 µg/ml) caused a reduction in live parasite cells compared to the control ($P < 0.05$). The gum resin of *B. sacra* significantly killed promastigotes at all doses except at the concentration of 15µg/ml compared to the control. This cytotoxicity effect was significant between (15vs.1600, 15vs.3200, 25vs.3200 & 50vs.3200) concentrations of extract treatment (Table 2) (Figure 1B, 2B) ($P < 0.01$).

The different concentrations of *B. sacra* gum resin extract killed a maximum of 25 % of promastigotes and a 50 % inhibitory dose (IC_{50}) could not be determined at these concentrations. Based on the linear equation, the percentage of parasite viability decreases with increasing concentration of *B. sacra* resin extract [see Figure 1(A)].

The survival rate of promastigotes of the parasite after the MTT test showed that the doses of 800, 1600 and, 3200 µg caused a significant decrease in the survival rate of the parasite during the 24-hour incubation (Figure 2A) ($P < 0.05$).

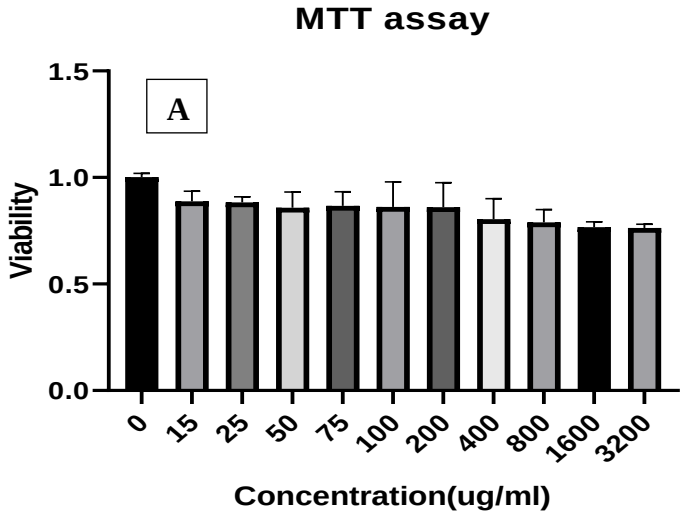
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Table 2. Evaluation of cytotoxicity effect of *B. sacra* gum resin extract on *L. major*

Concentration (µg/ml)	Mean ± SD
0 (control)	0.69 ± 1.32
15	9.78 ± 3.35
25*	12.67 ± 1.26
50*	12.78 ± 6.54
75**	15.70 ± 2.62
100***	18.28 ± 3.96
200***	18.32 ± 3.85
400**	17.06 ± 7.37
800***	18.84 ± 2.24
1600****	22.81 ± 2.23
3200****	24.41±0.60

* Significant values compared to the control



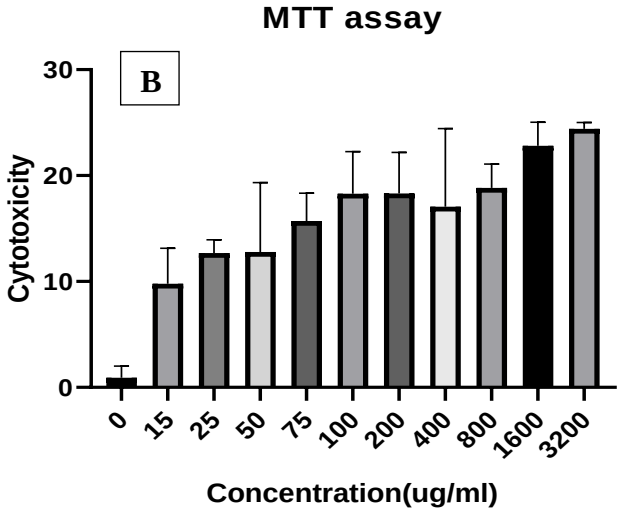


Fig. 2. Comparison of parasite viability (A) and, cytotoxicity effect of *B. sacra* gum resin extract (B)

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4. Discussion

In this study, the anti-leishmanial effect of *B. sacra* gum resin extract was investigated in various concentrations. The gum resin extract showed a significant effect against leishmaniasis at concentrations of 800 µg/ml and more. Treatments based on chemical drugs against the Leishmania parasite have side effects for patients in addition to their effectiveness. Recently, researchers have been trying to investigate herbal ingredients to replace chemical drugs especially in cases of antimicrobial drug resistance [21]. The antimicrobial properties of *B. sacra* gum resin extract have been studied [22]. Antioxidant properties of plants are an important feature for medicinal studies of plants, and the antioxidant properties of *B. sacra* resin have also been reported [21]. The properties of *B. sacra* resin have been studied in clinical trials and had a positive effect *in vivo* models such as anti-inflammatory and analgesic [23], the antiparasitic properties of this plant are related to the anti-inflammatory properties in animal models [23]. Although the information on the antiparasitic activity of *B. sacra* is not extensive, the effect of a non-aqueous *B. sacra* resin extract on the parasite *Plasmodium falciparum* (Tropical Malaria) has also been reported, in addition to the antiplasmodial effect the anti-*Trypanosoma brucei rhodesiense* (East African Human Trypanosomiasis) activity of plant compounds has also been demonstrated [24]. Relative effectiveness of pharmacological activity from *Boswellia* species has been reported at different doses in comparison with control drugs against protozoan human pathogens, including *Trypanosoma cruzi* (Chagas' disease) and *Leishmania donovani* (Kala-azar) [24]. Researchers believe that the mechanism of activity of *B. sacra* resin is related to immunomodulatory properties on macrophages, antigen-presenting cells, natural killer cells (NK), mast cells and, co-stimulatory molecules. This modulation may involve influencing cytokine production, cell activation and interaction between different immune cells [25]. The anti-leishmaniasis effect of *B. sacra* resin extract is the inhibition of *L. tropica* promastigotes proliferation by using *B. serrata* oil and its nanoliposomes [26]. Another study, indicated the *in vitro* anti-leishmanial activity of Boswellic acids against *Leishmania donovani* [27]. Boswellic acids are known for their anti-inflammatory and immune system modulating properties found in frankincense and, also exhibit anti-leishmanial activity in animal models [28]. The anti-inflammatory effect of *B. sacra* essential oil has been demonstrated by an increased Th1 cytokine profile and a decreased Th2 cytokine profile in mouse models of allergic asthma [29].

Our data are consistent with the results of the anti-leishmanial efficacy of *B. sacra* gum resin against *L. donovani* [27], in these studies, the survival rate of the parasite was also reduced when treated with *B. sacra* gum resin extract and active ingredient. Our study indicated an antiproliferative activity on the promastigotes of the parasite at all concentrations of *B. sacra* gum resin extract, the percentage of growth inhibition upregulated due to the increase in dose and the inhibitory effect was significant at high concentrations (800, 1600 and 3200 µg/ml). The cytotoxicity rate of the concentrations of 1600 and, 3200 µg/ml was significantly different from that of 15, 25 and 50 µg/ml and showed the positive effect of the extract after increasing the dose, there was no significant difference between the other concentrations. The determination of an effective dose of *B. sacra* resin extract with an

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inhibitory effect on 50 % of promastigotes was not determined in this study. This is one of the limitations of this study, as this inhibitory index can be only determined after an incubation period of 48 hours or more. Therefore, higher concentrations than 3200 µg should be tested or the duration of treatment with the same concentration should be tested at 48 and 72 hours. The antimicrobial and anti-inflammatory pharmacological activities of *B. sacra* has been investigated, and clinical trials indicated that the anti-inflammatory potential of this compound are related to the boswellic acids [30]. Due to the anti-inflammatory effects of this resin, it may be essential to study about use on microbial agents at *in vitro* and *in vivo* models [27]. Anti-inflammatory mechanisms from *B. sacra* resin extract are related to its boswellic acids, are the main active components responsible for the therapeutic effects of frankincense [31]. *B. sacra* gum resin contains terpenes such as boswellic acids and α-pinene which contribute to its antibacterial properties; these compounds not only prevent the growth of germs but also modulate the immune system [22, 32]. *In vitro* and *in vivo* investigation have shown that *B. sacra* resin can reduce the bacterial load in the affected tissues. However the efficacy may depend on the dose and the particular bacterial strains [11, 32]. In addition to the various therapeutic effects of *B. sacra* essential oil, the incorporating of nanoparticles enhances some pharmacological effects such as antimicrobial and antioxidant activity [33]. Our results and those of researchers on the antiparasitic effects of *B. sacra* resin extract have shown that the active and potent compounds of this plant have the antiproliferation potential against parasites, because of its anti-inflammatory and immune response regulatory properties, this agent can repair cutaneous lesions caused by CL in an *in vivo* model, by altering the profile of pro-inflammatory cytokines.

5. Conclusion

Our study suggests that *B. sacra* gum resin extract exhibits anti-leishmanial activity against *Leishmania major* promastigotes. The extract effectiveness might be amplified by its pharmacological effects such as anti-inflammatory and antioxidant properties, potentially leading to faster healing of treatment-resistant cutaneous leishmaniasis wounds in animal models.

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Author contributions

SH was involved in the study design, edited the manuscript and approving the version to be published. HR defined the intellectual content. FB, BB and MM contributed to the experimental studies.

Conflicts of interest

We declare that there is no conflict of interest.

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