

## Antileishmanial Activity of Ferula Assa Foetida Oleo Resin Gum against *Leishmania (L) tropica*- An *in vitro* Study

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### ABSTRACT

Ferula assafoetida is an oleo-gum-resin collected from the exudates of the roots of the Iranian endemic medicinal plant. It traditionally used for the treatment of different diseases, such as, intestinal parasites. Recent medicinal and biological sciences studies have also shown several activities, such as antioxidant, antiviral, antifungal and anti- inflammation. Leishmaniasis currently is common in the most countries and a serious health problem in the world. The most appropriate drug immediately is the need for disease control. The aim of the study was Antileishmanial activity of Ferula assa foetida oleo resin gum against of *Leishmania (L) tropica*-an in vitro study. The gum parts of FAG prepared differ concentration 2.5, 5, 10 and 20 mg/ml it. Then *Leishmania (L) tropica* promastigotes cultured transferred to 5 ml of RPMI<sub>1640</sub> media to which differ concentration 2.5, 5, 10 and 20 mg FAG and Meglamine Antimonite, Glucantime(MA). Each run also included control on two methods, the slide and the ELISA. The results were statistically showed that FAG significantly reduced the growth of parasites in comparison with the control group. To study the effect of various concentrations of FAG on viability *Leishmania (L) tropica* results with four different concentrations of the test without a control group were treated with 2.5mg (P= 0.07), 5 mg (P=0.005), 10 mg (P=0.0010 and 20 mg (P=0.006) showed significant increase in the drug (FAG). Parasite development is decreased and the concentration is greater inhibitory effect on parasite growth is greater. Then comparison of optical density mean in the case of parasite in the culture among different densities and it showed (P=0.008, P=0.002, P=0.134, P=0.000) in which it was a statistically significance among different densities and controlling group. The results showed that the parasite *Leishmania* promastigotes are sensitive to FAG and its respective ED<sub>50</sub> was lower compared to the MA.

**KEYWORDS:** Ferula assafoetida, urban cutaneous Leishmaniasis, In Vitro, viability.

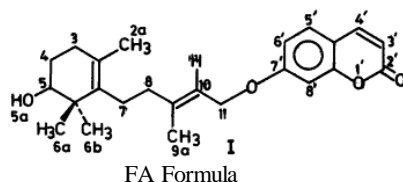
**Running Title:** Antileishmanial activity of Ferula assafoetida gum against on *Leishmania (L) tropica*.

### INTRODUCTION

Ferula assafoetida (FA) is an oleo-gum-resin procured from the Iranian endemic medicinal plant. It is often envisaged to be the main source of assafoetida. The oleo-gum-resin assafoetida is nominated “Khorakoma” or “Anghouzeh” in Iran. Assafoetida has been benefiting as a spice and folk phytomedicine for decades. It is utilized as a flavoring spice in a foods variety, particularly in India and Nepali people regularly expenditure it in their daily diets, and it is believed asfoetida has aphrodisiac, sedative, and diuretic properties. It has traditionally been applying for the different diseases treatment, such as intestinal parasites, weak digestion, and influenza. Recent pharmacological and biological studies have also specified many activities like antioxidant, antiviral, antifungal, cancer, anti-inflammation chemo preventive, ant diabetic, antispasmodic, hypertensive, and molluscicidal extracted from this oleo-gum-resin.



FA



FA Formula



FA Gum

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There is a major portion in acetone plant gum containing and several fractions such as gum fraction (25%, including glucose, galactose, l-arabinose, rhamnose and glucuronic acid), resin (40–64%, which contains ferulic acid esters (60%), free ferulic acid (1.3%), coumarin derivatives (e.g. umbelliferone), volatile oils (3–17%) including sulphur-containing compounds, and various monoterpenes have been isolated from this plant<sup>1-4</sup>. MA has been recommended for CL treatment by World Health Organization, there are some restrictions in this case including high expense, side effects, frequent injections need, and incomplete efficacy. Leishmaniasis is a complicated disease induced by an obligate intracellular parasite from the genus *Leishmania* and the major infectious diseases affecting the low economic populations throughout the world and there are two million annual new reports in 88 countries. In the endemic area, the parasite is transmitted via the bite of a sand fly which and leads to Visceral or Cutaneous Leishmaniasis. The world burden of this disease has remained stable for some years, causing 2.4 million disability adjusted life years and 59 000 deaths in 2001<sup>5-7</sup>. The aim of the present study was Antileishmanial activity *Ferula assa foetida* oleo resin gum against of *Leishmania (L) tropica*- an in vitro study.

## MATERIALS AND METHODS

The present probe design was experimental (laboratory-trial); therefore, Iranian endemic species including *Leishmania (L) tropica* was proliferated, and maintained in the standard culture.

### Preparation of *Ferula assafoetida* oleo Gum

*Ferula asafetida* was collected from Tabas region (Yazd province, Iran) during the summer and the plant spices was botanically identified by the botanist in Yazd Agricultural Research Center and voucher number of the specimen was 2365. The whole dried *Ferula assa-foetida* oleo gum resin was powdered (10 g) and dissolved in distilled water (100 ml) for overnight at room temperature and the yielded suspension was used. Concentrations and dosages of asafetida were expressed as crude amount of the dried oleo gum resin used in preparing the stock solution

### Source of parasites

*Leishmania (L) tropica* promastigotes were obtained from the Tehran Tarbiat Modares University. *Leishmania (L) tropica* had maintained in BALB/c mice. After culturing in NNN media supplemented with streptomycin, penicillin and 20% heat-inactivated fetal calf serum (FCS) at 25°C. Subsequently, the third passage promastigotes from NNN medium were progressively adjusted to RPMI<sub>1640</sub> media, MA and FCS<sup>8-9</sup>.

### The slide method

A fixed initial density of the parasites was transferred to 5 ml of RPMI<sub>1640</sub> media to which differ concentrations 2.5, 5, 10 and 20 mg FAG and MA were added and each concentration was done in triplicates. Each run also included control. The vials were then incubated at 26°C for promastigotes, on the next four days, the culture counted. A 1:10 dilution in saline together with the appropriate dye was prepared. The dye for promastigotes was 0.4% Trepan blue. The chamber of a Neubauer slide is charged and the number of organisms in 16 small corners square is counted. The total number per ml = N (counted) x 10 (number in 1 mm<sup>3</sup> x 10<sup>3</sup> number 1 ml) x 10 (dilution factor). The LD<sub>50</sub> was calculated according to the method of Hearly. This method was first described by Sharquie. A modified method was used in the present work and obtained results compared with previous ones. Promastigotes of *Leishmania (L) tropica* were used. One drop containing the parasites was put on a slide together with a drop of a drug solution and covered by a cover slip. The slides were examined under the microscope and the percentage of stained parasites was noted and normal saline was used as a control<sup>10-12</sup>.

### The cell proliferation ELISA

The cell proliferation ELISA was performed as (Version march 2005, Cat. No. 11 669 915 001) that in brief is: a. A fixed initial density of the parasites was transferred to 5 ml of liquid medium to which different concentrations 2.5, 5, 10, and 20 mg FAG and MA were added and each concentration was done in triplicates. Each run also included control.

- b. After stimulation with acetone in the period
- c. Dioxy bromouridin was added and incubated at 37°C for 8 hours.
- d. Supernatant is removed.
- e. Fixation added to the membrane is permeable.
- f. Anti-oxibromouridin conjugated with POD is added and incubated for 3 hours.
- g. Chromogen is added and incubated
- h. And finally, stop and read at 450 nm.

### Statistics

Data are expressed as Mean ± SEM of lesion's diameter and animal's weight, and were statistically analyzed by multivariate nonparametric test (Kruskal-Wallis Test) for analyzing the effect of time within-subjects and multiple comparisons test for comparing the variables between groups.  $p < 0.05$  was considered as the significant level.

## RESULTS

Lethal Dose<sub>50</sub> (LD<sub>50</sub>) FAG and MA against logarithmic and stationary phase's promastigotes calculated. Furthermore, the sensitivities of *Leishmania (L) tropica* were tested using a simple slide method and compared to results of the standard method the in vitro sensitivities of promastigotes *Leishmania (L) tropica* (Table 1).

Table 1: Calculation of the LD<sub>50</sub> of FAG and MA against logarithmic and stationary phases promastigotes *Leishmania (L) tropica*.

Organism	FAG (mg/ml)	MA (mg/ml)
Logarithmic Phase Promastigotes	34.2	334.6
Stationary Phase Promastigotes	37.4	339.0

**The FAG effect on viability of old world cutaneous leishmaniasis urban strain in vitro media by handheld (the slide method):**

The effect of FAG against *Leishmania (L) tropica* of logarithmic phase PMs Figure 1 shows the results of FAG logarithmic phase PMs of *Leishmania (L) tropica*. In geometrically increasing concentrations, dose was dependently inhibited the growth of *Leishmania (L) tropica* PMs according to hours. Also the results show strain of leishmania (L) tropica was sensitive to FAG, increasing concentrations, and dose dependently inhibited the growth of parasite. FAG with concentrations 2.5, 5, 10 and 20 mg was added to cultured parasite and counted pomastigotes with Neubauer slide method, per hour lasted 72 hours. The inhibitory effect of FAG on parasite was dose dependent and most inhibition effect was seen with 20 mg concentration. There was statistically significant difference between FAG groups and control (P=0.007, P=0.005, P=0.001, P=0.006) (figure 1).

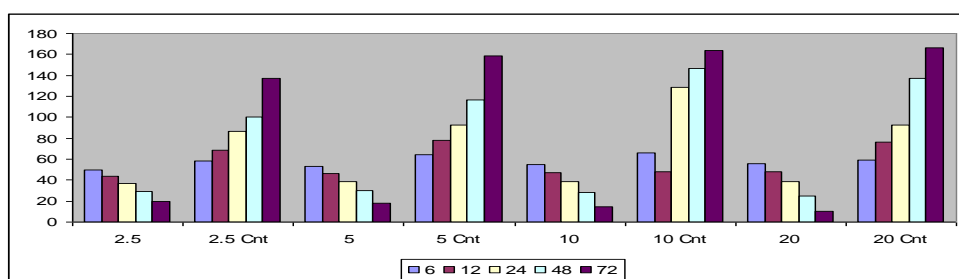


Figure. 1: Average number of live Logarithmic Phase PM *Leishmania (L) tropical* in concentrations of 2.5 (P=0.007), 5 (P=0.005), 10 (P=0.001) and 20 (P=0.006) mg of FAG and Control on 6-48 hours.

Effect of FAG against *Leishmania (L) tropica* of stationary phase PMs (Figure 2) shows the results of different concentrations of FAG on stationary phase PMs of *Leishmania (L) tropica*. In geometrically increasing concentrations, dose dependently inhibited the growth of *Leishmania (L) tropica* PMs according to hours. The results show strain of *Leishmania (L) tropica* was sensitive to FAG, increasing concentrations; dose dependently inhibited the growth of parasite. FAG concentrations 2.5, 5, 10 and 20 mg was added to cultured parasite and counted pomastigotes with Neubauer slide method, per hour lasted 72 hours. The inhibitory effect of FAG on parasite was dose dependent and most inhibition effect was seen with 20 mg concentration. There was statistically significant difference between FAG groups and control (P=0.008, P=0.002, P=0.134, P=0.000) (figure 2).

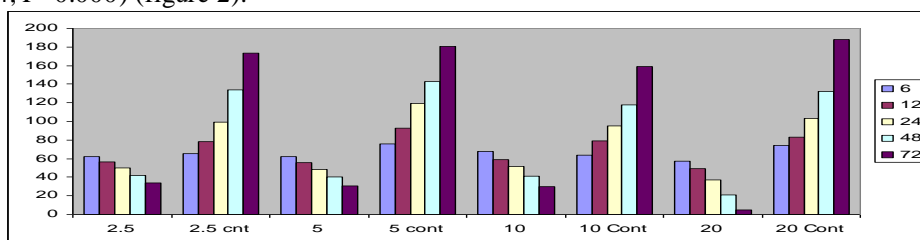


Figure. 2: Average number of live Stationary Phase PM *Leishmania (L) tropical* in concentrations of 2.5(P=0.008), 5(P=0.002), 10 (P=0.134) and 20 (P=0.000) mg of FAG and Control on 6-48 hours.

**The FAG effect on Viability of logarithmic & stationary phases urban strain of old world Cutaneous Leishmaniasis**  
 In second stage 24 hours after adding 2.5, 5, 10 and 20 mg of FAG to media was counted parasite with cell proliferation ELISA kit, the result showed statistically significant difference between FAG groups and control group. (Control group, Log Phase 9P=0.0011), Control group, Stationary Phase (P=0.009), Logarithmic Phase, Stationary Phase (P=0.0016).

**Table 2:** Mean number of live Logarithmic and Stationary Phases Promastigotes *Leishmania (L) tropica* in Concentrations of 2.5, 5, 10 and 20 mg FAG and Control on 6-48 hours.

Phases Concentration	Logarithmic Phase Mean $\pm$ SD	Stationary Phase Mean $\pm$ SD
Control	154 $\pm$ 0.005	175 $\pm$ 0.006
2.5	138 $\pm$ 0.003	151 $\pm$ 0.004
5	121 $\pm$ 0.006	131 $\pm$ 0.003
10	112 $\pm$ 0.004	119 $\pm$ 0.005
20	86 $\pm$ 0.002	94 $\pm$ 0.003

Control Group, Logarithmic Phase (P=0.0011), Control Group, Stationary Phase (P=0.00)  
 Logarithmic Phase, Stationary Phase (P=0.0016) Kruskal-Wallis Test

## DISCUSSION

The in vitro FAG Effect on Urban strain CL' Viability of old world was under investigation, and then in differ intervals, parasite numbers were counted in Mentioned culture through two methods including the slide and cell proliferation ELISA. Study indicates that FAG inhibits the growth of Promastigotes *Leishmania* in vitro. This study showed significant inhibitory effect of FAG with 2.5, 5, 10 and 20 mg concentrations on multiplicity and viability of Leishmania parasite with increasing concentration allegiance. And this raises the possibility that owing to drug intervention, different strains of the parasite in different geographical areas show various sensitivity. Leishmaniasis treatments mostly recommended the use of topical and systemic remedies. The Pentavalent Antimony compounds have been posed since 1940 for systemic treatment and the pentavalent Antimony compounds used as the first priority in treatment since 1984. The mentioned drug is relatively expensive and leads to severe side effects. Ignoring the considered issues, there are many cases of inefficacy in wound recovery. Owing to the reason, various drugs have been used<sup>14-17</sup>. The antioxidant activity of the aerial parts of FA was determined by employing various in vitro assay systems, The extract of FAG showed good nitric oxide-scavenging activity and it is indicated that the essential oil has a garlic-like flavor and it can be used instead of its gum which finds application in the preparation of some local dishes besides its unique medicinal value<sup>18-19</sup>.

The highest reduction in *Shistosoma haematobium* worm burden and egg counts was found with powder form of FA when compared to oil form as confirmed histopathologically and by ultrastructural profile alteration<sup>20</sup>. The antibacterial activity was comparable with reference antibiotics at same amount against some of the Gram positive and negative bacteria. At higher amounts (150/200  $\mu$ g) all the bacterial strains were more sensitive to oil than the reference antibiotics (100  $\mu$ g) except *Vibrio cholerae*. The MIC of oil ranged from 80-200  $\mu$ g/mL against the susceptible bacteria. All the MBC (Minimum Bactericidal Concentration) were the same as the MICs and so

Ethanollic extracts of *Ferula assafoetida* resin, *Grewia asiatica* leaves, *Ipomoea hederacea* seeds, *Lepidium sativum* seeds, *Nigella sativa* seeds and *Terminalia chebula* fruits were tested in vitro for their antibacterial and antifungal activities. The antibacterial study performed against eight bacterial species viz., *Escherichia coli*, *Citrobacter*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Micrococcus luteus*, *Proteus mirabilis* and *Bacillus subtilis* indicated that the investigated plants have potent activity against all the tested microorganisms. The antifungal activity of these extracts was performed against nine fungal strains, viz., *Aspergillus parasiticus*, *A. Niger*, *A. effusus*, *Candida albicans*, *Fusarium solani*, *Macrophomina phaseolina*, *Saccharomyces cerevisiae* and *Trichophyton rubrum*. The extracts showed moderate as well as significant activity against the different fungal strains. Essential oils extracted from the seeds of neem (*Azadirachta indica*), mustard (*Brassicacampestris*), black cumin (*Nigella sativa*) and asafoetida (*Ferula assafoetida*) were evaluated for their antifungal activity 0.5, 0.1 and 0.15% against eight borne fungi viz., *Aspergillus niger*, *A. flavus*, *Fusarium oxysporum*, *F. moniliforme*, *F. nivale*, *F. semitectum*, *Drechslera hawiinesis* and *Alternaria alternata*.

Randomly gold (MZ 68%WP) was used for comparison. All the oils extracted except mustard, showed fungicidal activity of varying degree against test species. Of these oils, Asafoetida oil 0.1% and 0.15% significantly inhibited the growth of all test fungi except *A. flavus* and *Nigella sativa* oil 0.15 was also effective but it showed little fungicidal activity against *A. niger* followed by neem, Ridomyl gold and mustard oils<sup>21-23</sup>.

## Conclusion

Summary, the results suggested low concentrations of FAG, high ability in inhibition of parasite replication in culture media. This effect was more regularly with concentrations; in this regard, the safety of this drug in comparison with MA possibility of using higher concentrations and to obtain better results is not unexpected. On the other hand, the results show the effectiveness of the drug culture on the parasite strain native to Iran, it may raise kind of hope that the studies on animal models and ultimately its impact on human disease can be reevaluated. With consideration of drug safety compared to MA, the use of FAG in the treatment of cutaneous leishmaniasis would be encouraged. Nevertheless, further studies are needed to increase the understanding of the physiological mechanisms, and then more animal and clinical studies required to obtain the best concentration.

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### Conflict of Interest

The authors have no conflict of interest, including specific financial interests, relationships, and/ or affiliations relevant to the subject matter or materials included.

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