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Effect of *Kelussia odoratissima* Mozaff essential oil on promastigot form of *Leishmania major* (*in vitro*)

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ABSTRACT

Introduction: Leishmaniasis is a zoonotic disease caused by a protozoan of the genus *Leishmania*. In this study, the effects of *Kelussia odoratissima* Mozaff essential oil on the promastigot form of *Leishmania* major were studied.

Methods: In this study, the effects of *Kelussia odoratissima* Mozaff essential oil on the promastigot form of *Leishmania major* were assessed by calculating the average number of surviving promastigots after exposure to different concentrations of essential oil, relative to the control Glucantime, at different time intervals. To achieve this, various essential oil concentrations (7.5 µl, 15 µl, 25 µl, 35.25 µl, 50 µl) were added to parasites. Different groups in this study were kept in a 26°C incubator under identical conditions. 24, 48 and 72 hours after incubation, living promastigots were counted.

Results: The effect of the essential oil of *Kelussia odoratissima* Mozaff differed from the negative and positive controls and depended on the concentration: higher concentrations (35.25 µl, 50 µl) had a stronger effect on promastigots, causing total mortality.

Conclusion: This study showed that *Kelussia odoratissima* Mozaff essential oil had effects on promastigot form of *Leishmania major.* So it might be possible to use the essential oil of *Kelussia odoratissima* instead of chemical drugs.

Implication for health policy/practice/research/medical education:
It is possible to use the essential oil of *Kelussia odoratissima* instead of chemical drugs. The use of any concentration of essential oil of *Kelussia odoratissima* controlled *L. major* more effectively by increasing in essential oil concentration. Future research would assess the effect of this essential oil on the amastigote form of *L. major.*

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Introduction

Medicinal herbs contain physiologically active principles that have been exploited in traditional medicine for the treatment of various ailments due to their anti-microbial properties (1,2). The antifungal and antibacterial activity exhibited by extracts and essential oils of medicinal plants is well proved (3).

Kelussia odoratissima Mozaff. (wild celery; Apiaceae) is a sweet-smelling, glabrous, perennial aromatic herb, which is traditionally consumed in Iran and grows to a height of 120 to 200 cm. The flowers are 1-2 mm in diameter and all are hermaphroditic. *K. odoratissima* is an Iranian endemic plant native to Zagros (central region of Iran) (4).

Kelussia grows in April and its aerial parts are harvested (5,6). Flavonoids have anti-inflammatory (7), anti-allergy (8), anti-diabetes, antihyperlipidemic and anti-hypercholesterolemic effects (9,10) and improves spatial memory (11). Phthalides constitute 70% of the *Kelussia* essence with several effects: liver protection, prostaglandin F2α inhibition, prevention of cancer tumors, treatment of epilepsy and liver disorders and reduction in blood viscosity (5,12,13). Despite this, there are no study related to the antileishmanial effect of the essential oil of *K. odoratissima*, this being the focus of our study.

Leishmaniasis affects more than 12 million people in 88 countries (14). Cutaneous leishmaniasis (CL), with 1 to 1.5 million annual cases, is the most common form of this disease (15). There is no vaccine available against any form of leishmaniasis and chemotherapy is the only currently available treatment (16,17). The standard treatment for

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CL is antimoniate, which requires long-term injections, and resistance is emerging, which cause high dose usage, resulting side effects (17,18).

The search for new, safe and inexpensive drugs to treat and control CL, especially anthroponotic CL, is needed. Conventional healers are usually cheap and sometimes more effective than chemical drugs (19).

In this study, the effect of the essential oil of *K. odoratissima* on promastigot form of *Leishmania major* was studied. The results obtained from this study help us to find the alternative natural drug instead of chemical ones.

Materials and Methods

Plant material

A wild-growing plant from Kogiluye and Buyer Ahmad province located in the southwest of Iran, was collected in April and May, 2012. The samples, which were identified and confirmed as *K. odoratissima* Mozaff, were deposited in the Herbarium of the Research Institute of Jahade Keshavarzi of Shahrekord, Iran.

Isolation of the volatile oil

20 kg from aerial parts of the plant was dried in an oven equipped with warm air circulation. One hundred grams of the air-dried material was ground and powdered. The powder was subjected to hydro-distillation for 3 hours using a Clevenger-type system. Finally, 30 ml essential oil extracted. The oil was kept at 2°C to 4°C in a sealed brown vial.

Chemical analysis of the oil

Analysis of the essential oil was performed on a Hewlett-Packard Model 6890 GC-MS using a HP-5MS capillary column (30 m×0.25 mm i.d., 0.25 m film thickness). The injector temperature and detector temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.00 ml/min. The column temperature was programmed from 40°C to 280°C at a rate of 4°C/min. The EI mode was at 70 eV, while the mass spectra were recorded from 30 to 450 m/z. Confirmation of identity was carried out by comparing their retention indices with the Wiley 275 library data of the GC–MS system and with the mass spectra literature data (20).

Preparing the promastigot form of Leishmania major

The promastigot form of *Leishmania major* strain MRHO/IR/ER/75 was prepared from department of Parasitology Research, University of Medical Sciences of Isfahan, Iran. The promastigots were transferred to RPMI 1640 medium and incubated at 26°C for 72 hours.

Preparing the different concentrations and control groups

After the promastigots increased in the medium, they were counted using a Neobar chamber (0.100 mm, Tiefe, Depth, Profondeur, Neubauer Improved, Precicolor, HBG, Germany) in which 10 µl of this medium was mixed with 10 µl of formalin, which was used to kill the promastigots. 150 µl of medium contained 10^6 promastigots. Next, different concentrations of *K. odoratissima* essential oil (15, 30, 50, 75 and 100 mg/ml) were prepared, to achieve this, various essential oil concentrations (7.5 µl, 15 µl, 25 µl, 35.25 µl, 50 µl) were added to parasites. There was a negative control (no essential oil) and a positive control (Glucantime®, Specia, Paris, France) by mixing with sterile RPMI 1640 medium in different tubes. 10^6 promastigots (i.e., 150 µl of medium containing promastigots) were added to each tubes, as presented in Table 1. The total volume of all tubes was 500 µl after mixing.

Counting the number of living promastigots after the mixing

After mixing the promastigots with different concentrations of *K. odoratissima* essential oil, those tubes, as well as the negative and positive controls, were incubated in dark incubator and in 26°C for 72 hours. The number of living promastigots was counted at 24, 48 and 72 hours after incubation.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and significant differences ($P<0.05$) between means were assessed by Tukey's test using SPSS software version 19.0.

Results

Composition of essential oil

In the essential oil of *K. odoratissima*, the sesquiterpene hydrocarbons were the most abundant compounds (57.77%). In addition, the least quantitative percentage (2.7%) was belonged to monoterpene hydrocarbons. The major components were *Z*-ligustilide (34.5%), *E*-ligustilide (11.79%), 3-*Z*-butylidenephthalide (8.81%) and Dec-9-en-1-ol (5.86%).

24 and 48 hours after incubation

Effects of essential oil of *Kelussia odoratissima* Mozaff on promastigot form of *Lishmania major* differenced from

Table 1. Volume of materials in each tube prepared for different concentrations of essential oil

Tubes Materials	15 mg/ml	30 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml	Negative control	Positive control (Glucantime)
Promastigots	150 µl	150 µl	150 µl	150 µl	150 µl	150 µl	150 µl
RPMI 1640	342.5 µl	335 µl	325 µl	314.75 µl	300 µl	350 µl	181 µl
Glucantime	-	-	-	-	-	-	169 µl
Essential oil	7.5 µl	15 µl	25 µl	35.25 µl	50 µl	-	-
Total volume	500 µl	500 µl	500 µl	500 µl	500 µl	500 µl	500 µl

Table 2. Mean number of living promastigots ± SEM 24, 48 and 72 hours after incubation

	Mean number of living promastigots ± SEM						
Tube	**15 mg/ml**	**30 mg/ml**	**50 mg/ml**	**75 mg/ml**	**100 mg/ml**	**Negative Control**	**Positive Control**
24 h	127.25±3.12 a	119.11±4.54 a	116.63±3.96 a	111.76±4.32 a	103.51±3.81 a	125.3±1.96 b	79.47±0.44 c
48 h	124.12±2.68 a	117.63±2.96 a	110.17±4.23 a	104.00±3.69 a	98.99±1.99 a	148.25±2.25 b	74.23±0.97 c
72 h	119.08±1.75 a	102.45±1.85 a	93.27±2.17 a	87.00±4.44 a	73.93±3.12 d	211.63±2.19 b	67.56±0.82 c
Mean of 3 Times	123.48±2.51 a	113.06±3.11 b	106.69±3.45 c	100.92±4.15 d	92.14±2.97 e	161.72±2.13 f	73.75±0.74 g

Different letters within each row (i.e., within each time interval) indicate significant differences (P<0.05) between mean of living promastigots number in each tube according to Tukey's test.

negative control and positive controls. However, there were no significant differences between groups with different concentrations of essential oil (Table 2).

72 hours after incubation

In this time decreasing in living promastigots in different tubes were more than past times. Same as past times, effect of essential oil of *Kelussia odoratissima* Mozaff on promastigot form of *Lishmania major* was differenced from negative control and positive control (Table 2).

Totally the *K. odoratissima* essential oil had a different effect on the promastigot form of *L. major* relative to the negative and positive control, but this depended on the essential oil concentration; higher concentrations (35.25 µl, 50 µl) had a stronger effect on promastigots, causing total mortality (Table 2). In all three-time intervals, the use of any concentration of essential oil of *Kelussia odoratissima* controlled *L. major* more effectively by increasing in essential oil concentration (Table 2).

Discussion

The application of natural plant products for control of bacterial, fungal and parasitic bee and brood diseases has several advantages over conventional means. In the specialized literature resistance of bacteria to essential oils has not yet been documented. In addition, natural substances in bee products decompose rapidly, their quantity in honey is low and they do not have an adverse effect on the health of consumers (21). Although in small amounts, essential oils and organic acids are normally contained in various types of honey. According to some authors their use as alternative means for prevention and control of bee diseases guarantees ecologically clean bee products (22).

This study showed antileishmanial effects of *Kelussia odoratissima* Mozaff essential oil on promastigot form of *Leishmania major.* The current treatment regimens for leishmaniasis, which are based on chemotherapy, are limited and are not ideal because they are often associated with severe side effects (23). In a case report from France, successful treatment of a 10-year-old boy with cutaneous leishmaniasis (*L. major*) was reported with oral azithromycin (24).

Pentavalent antimonials remain the drug of choice for the treatment of cutaneous and mucocutaneous leishmaniasis. Despite its availability in health facilities, its high toxicity and its parenteral use are limiting factors for the proper treatment of cases of CL. It should be noted that most of these cases occur in areas of difficult access, in rural areas, which complicates the parenteral application of the drug and monitoring of its side effects. Treatment of CL with the drugs currently available represents an obstacle to proper clinical handling of the cases of leishmaniasis, and efforts should be made in order to add the promising drugs to clinical trials and investigate the use of new alternative drugs (25)

Traditional alternatives to antimonials used in unresponsive cases are amphotericin B, which cause serious, toxic side-effects (26,27), and the emergence of drug-resistant parasites is a major problem (17). These limitations and factors emphasize the urgent need for new, inexpensive, safe and easy-to-administer substances for the treatment of this infectious disease, possibly through the form of traditional medicines which represent the accumulation of practices based on theories, beliefs and experiences of different cultures over a wide period of time (28).

Amphotericin B is the second drug of choice when antimonials fail or cannot be used. However, they are toxic and expensive drugs, which often require hospitalization for their administration (29). An alternative to the treatment with conventional amphotericin B is the use of liposomal amphotericin B, which has higher peak plasma levels and lower toxicity (30).

Traditional medicines are used for the maintenance of health and in the prevention, diagnosis, improvement and treatment of illnesses (31). Based on traditional medicine, new drugs of herbal origins discovered through ethnopharmacological studies have shown promising results.

Conclusion

By considering an increase in drug resistance against leishmania, it is possible to use the essential oil of *Kelussia odoratissima* instead of chemical drugs. The use of any concentration of essential oil of *Kelussia odoratissima* controlled *L. major* more effectively by increasing in essential oil concentration. Future research would assess the effect of this essential oil on the amastigote form of *L. major*.

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All the authors wrote the manuscript equally.

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