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Original Research

Pulicaria vulgaris Gaertn. essential oil: an alternative or complementary treatment for Leishmaniasis

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Abstract: Leishmaniasis is a neglected parasitic protozoal disease that affects approximately 12 million people and represents a public health problem in Iran. The objectives of this study were to obtain the essential oil (EO) from *Pulicaria vulgaris* Gaertn. growing in Iran and to carry out *in-vitro* antileishmanial screening of the EO against promastigotes of *Leishmania major* and *Leishmania infantum*. The EO from the aerial parts of *P. vulgaris* was extracted by hydrodistillation. Serial dilutions of the EO were screened for *in-vitro* antileishmanial activity using 96-well microtiter plates. The *P. vulgaris* EO was active against the promastigote forms of *L. major* and *L. infantum*, with IC_{50} values of 244.70 and 233.65 µg/mL, respectively. *Pulicaria vulgaris* EO may serve as an alternative or complementary treatment for leishmaniasis.

Key words: Protozoal diseases; Parasites; Thymol; Iranian plants.

Introduction

An estimated two million people per year are afflicted by the parasitic protozoal disease leishmaniasis (1). Leishmaniasis presents a wide spectrum of clinical manifestations from cutaneous lesions to visceral distress (2-4). Visceral leishmaniasis is a serious threat to children's health. In endemic regions, children are at much greater risk compared to adults. Paediatric leishmaniasis presents symptoms that include paleness, intermittent fever, tendency to anorexia, abdominal distension, and weight loss, as well as hepatomegaly, splenomegaly, lymph node enlargement, anaemia, thrombocytopaenia, hypergammaglobulinemia and leukopaenia (5).

Protocols for the treatment of leishmaniasis remain problematic; currently available chemotherapeutics are associated with various limitations including the need for long-term treatments, adverse side effects, and limited or reduced efficacy (4). As a result, there is an urgent need to find novel therapeutic agents or treatment regimens that are effective in treating leishmaniasis.

In recent years, phytotherapy has been shown to be useful for treatment of many human and animal diseases (6-18). However, plants have been immensely utilized in traditional healing systems, and in only a few cases have their curative potentials in human diseases been confirmed (19-27). Essential oils (EOs) are one of the options that have been recently used to treat a variety of diseases. EOs are complex mixtures of aromatic plant secondary metabolites, volatile, and lipophilic (28-30). Health care researchers and practitioners increasingly consider use of EO-bearing plants.

The genus *Pulicaria* Gaertn. (Asteraceae), according to Iranica flora, includes five species that exist in Iran: *P. dysenterica* (L.) Bernh., *P. arabica* (L.) Cass., *P. gnaphalodes* (Vent.) Boiss., *P. salvifolia* Bunge and *P. vulgaris* Gaertn. *P. vulgaris* is an annual plant that has many branched reddish stems and small (6-12 mm) yellow flower heads. To our knowledge, there have been no previous studies on the anti-leishmanial activity *P. vulgaris* EO.

Materials and Methods

Pulicaria vulgaris Gaertn. was collected during the flowering period, March 2014, from the area surrounding Hamun Lake, Zabol, in Sistan and Baluchestan Province of Iran. The plant species was identified at Ferdowsi University, where a voucher specimen (no. 26432) was deposited in Mashhad Herbarium. For the EO extraction, the dried aerial parts (stems, leaves, and flowers) (200 g) of *P. vulgaris* were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus based on method described by the British Pharmacopoeia (31). The EO obtained was dried using anhydrous sodium sulphate (Sigma-Aldrich Corp., St. Louis, MO, USA) and the EO obtained was stored at 4°C until analysis and further assays.

The EO of *P. vulgaris* initially was dissolved in 5% dimethyl sulphoxide (DMSO) (Sigma-Aldrich Corp., St. Louis, MO, USA)/95% water and further diluted

Table 1. Percent survival of *Leishmania major* and *Leishmania infantum* promastigotes after 72 hours treatment with different concentrations of *Pulicaria vulgaris* essential oil.

	Survival (%)	
Concentration (µg/mL)	***Leishmania major***	***Leishmania infantum***
0	100 ± 00§ a	100 ± 0.0 a
5	100 ± 00 a	95.56 ± 0.11 b
10	90.54 ± 1.25 b	94.34 ± 0.23 b
25	89.32 ± 0.28 c	90.56 ± 0.23 c
50	79.93 ± 1.32 d	85.55 ± 0.44 d
100	74.23 ± 1.11 d	79.45 ± 0.13 e
150	60.52 ± 1.22 e	69.59 ± 0.45 f
300	45.62 ± 2.35 f	38.95 ± 1.22 g
600	25.64 ± 1.52 g	18.54 ± 0.32 h
DMSO	-	-

§Data are expressed as mean ± SD of % viability for different concentrations of EO and controls. Significant differences of EO effects on survival of *Leishmania major* and *Leishmania infantum* promastigotes are indicated with different letters for each *Leishmania* species. That is, in each column, the values that have given different letter were significantly different in comparison with other values in the column, while the values with same letters in the each column represent those values having no significant difference with other values in the column at $P < 0.05$.

with RPMI 1640 medium (GIBCO, Grand Island, New York, USA). The concentration of DMSO in the wells was not higher than 0.01%. For assessing the antileishmanial activity of the EO, logarithmic phase promastigotes of *Leishmania major* (MRHO/IR/75/ER) and *Leishmania infantum* (MCAN/IR/96/LON49) (1×10^6 cells/mL) were seeded in a 96-well microtiter plate along with serial dilutions (600, 300, 150, 100, 50, 25, 20, 10, 5, and 0 µg/mL w/v) of the EO and afterwards incubated at 24°C, for 72 hours. Antileishmanial activity was determined by light microscope and the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma-Aldrich Corp., St. Louis, MO, USA] assay. The concentration inhibiting parasite growth by 50% (IC_{50}) was calculated by using the formula: EXP (LN (conc >50%) - ((signal >50%-50)/ (signal >50%-signal<50%)*LN (conc >50%/conc <50%))); where EXP is exponential and LN is natural logarithm (32).

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) following a completely random design to determine the least significant difference (LSD) at $P < 0.05$ by SPSS v. 11.5. All assays were carried out in triplicate.

Results and Discussion

The % survival of *L. infantum* and *L. major* promastigotes after 72 hours treatment, with various concentrations of *P. vulgaris* EO is shown in Table 1.

The results of this study showed that *P. vulgaris* EO had anti-leishmanial effects. In particular, *P. vulgaris* EO was active against the promastigote forms of *L. major* and *L. infantum*, with IC_{50} values of 244.7 and 233.6 µg/mL, respectively. DMSO, used as the co-solvent for the EO, served as negative control, and had no effect on survival of *L. major* and *L. infantum* promastigotes. Torres-Santos *et al.* (33) have reported an IC_{50} value of 83 µg/mL for glucantime as antileishmanial drug.

Sharifi-Rad et al. (32) investigated the chemical composition of *P. vulgaris* EO from the Zabol region. They reported that the main components were thymol, *p*-menth-6-en-2-one (carvotanacetone), thymol isobutyrate, menthan-2-one, 1-methyl-1,2-propanedione, 2,5-dimethoxy-*p*-cymene, myrtenol, linalool, and β-myrcene with 50.2%, 20.2%, 16.9%, 4.3%, 4.1%, 4.0%, 1.2%, 1.1%, and 1.9% in the EO, respectively (Table 2).

The high thymol content in *P. vulgaris* EO is notable; thymol has shown *in vitro* and *in vivo* antileishmanial activity against *Leishmania panamensis* (34) and *L. infantum* ssp. *chagasi* (35). In terms of effectiveness, the EO of this plant is certainly more active than the main components alone. However, antileishmanial activity of *P. vulgaris* EO was not as active as glucantime, and several essential oils reported in the literature have shown leishmanicidal activities with IC_{50} in the range of 2 to 100 µg/mL (36). Therefore, for example, *Bursera graveolens* EO, rich in limonene (26.5%), *β*-elemene (14.1%), and (*E*)-*β*-ocimene (13.0%), had IC_{50} = 36.7 µg/mL against *Leishmania amazonensis* amastigotes (37), and *Artemisia absinthium* EO, rich in *trans*-sabinyl acetate, had IC_{50} = 13.4 µg/mL against *L. amazonensis* amastigotes (38). Nevertheless, the leishmanicidal activity of *P. vulgaris* EO is comparable to the activities of many other EOs (39). According to the results, it seems that *P. vulgaris* EO can be a promising candidate for dis-

Table 2. Major components of essential oil of *Pulicaria vulgaris*.

Plant name	Collection area	Major components	References
Pulicaria vulgaris Gaertn.	Hamun Lake, Zabol, Iran	Thymol (50.22%), *p*-menth-6-en-2-one (carvotanacetone, 20.2%), thymol isobutyrate (16.88%), menthan-2-one (4.31%), 1-methyl-1,2-propanedione (4.13%), 2,5-dimethoxy-*p*-cymene (4.01%), myrtenol (1.22%), linalool (1.1%), β-myrcene (1.9%).	Sharifi-Rad et. (32)

covery of new natural antileishmanial drugs, especially in terms of paediatric leishmaniasis.

Today, many classes of synthetic antileishmanial drugs are showing diminishing effectiveness because of the emergence of drug-resistant strains. Hence, using effective natural antileishmanial agents with fewer side effects is an encouraging approach to combat leishmaniasis. However, these preliminary antileishmanial screening used the promastigote (insect) form of the parasite rather than the intracellular (amastigote) form. Therefore, more studies are needed to examine *in vivo* antileishmanial effects of *P. vulgaris* EO, identify mechanism(s) of activion and investigate adverse effects.

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Conflict of Interest.
The authors declare no conflict of interest.

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