



The effect of Marigold flower extracts on growth of *Leishmania*.

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ABSTRACT

This study searched in the inhibiting potency of different concentrations (12.5, 25, 50 and 100 µg/mL) of both aqueous and methanol extracts of marigold plant flower *Calendula officinalis* against the promastigote of *Leishmania major*, *L. donovani* and *L. mexicana*. Both flower extracts approved inhibiting potency against the growth of promastigotes of *Leishmania major* with rates 51% and 62%, respectively but showed only a slight effect on *L. donovani* and *L. mexicana*. It is concluded that the aqueous and methanolic marigold extracts have selective inhibiting potency of *Leishmania major*.

Keywords: Inhibitory, marigold plant extract, *Calendula officinalis*, *Leishmania* sp.

تأثير مستخلص أزهار Marigold على نمو اللشمانيا *Leishmania*

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الملخص

بحثت هذه الدراسة في فعالية تثبيط تراكيز مختلفة (12.5 و 25 و 50 و 100 ميكروغرام / مل) كل من المستخلصات المائية والميثانول من نبات أزهار Marigold *Calendula officinalis* ضد الطور امامي السوط لـ *Leishmania major*, *L. donovani* and *L. mexicana*. أظهرت النتائج ان المستخلص المائي والكحولي عند التركيز 50 مايكروغرام تثبط نمو اللشمانيا الجلدية بنسبة 51% و 62% على التوالي ولكن أظهرت تأثير طفيف ضد اللشمانيا الحشوية واللشمانيا المكسيكية. ويستنتج من ذلك أن المستخلصات المائية والمثانولية لها قدرة تثبيط ضد اللشمانيا الجلدية.

Keywords: Inhibitory, marigold plant extract, *Calendula officinalis*, *Leishmania* sp.

1. Introduction

Currently no vaccine available to protect people against infection, therefore the main methods used to control it is vector-control, drug treatment of clinical cases or control of reservoir hosts, e.g. culling dogs infected with VL [1,2]. The organic pentavalent antimonials, introduced in the 1940s, are still generally used as the first-line drug for some species of *Leishmania*, but they are potentially toxic, and require painful multiple injections by the parenteral route. Resistance in the endemic parasite population has negated their use in some parts of the world e.g. their use was associated with treatment failure in 60% cases in Bihar, India [3]. There are a limited number of alternative drugs and most possess toxic side effects, have to be given by the parental route, or are too expensive for large-scale use [4]. Miltefosine was the first anti-leishmanial drug which could be by the oral route. However there is emerging evidence suggesting that increased resistance to it may be developing in endemic areas, as there has been an increase in the drug concentration required to kill 90% parasites in in vitro tests [5]. At present, the only way to improve treatment outcome is to use combinations of drugs to cure infections and therefore there is a great need to develop new anti-leishmanial drugs. Natural products have been used to identify new chemotherapeutic agents, some of which have activity against *Leishmania* species [6,7]. Amongst the natural products are some plant extracts that demonstrate some potency to ameliorate, to certain extent, the infection. *Calendula officinalis* (Asteraceae family) also known as marigold or pot marigold is an annual or biennial aromatic herb with soft glandular leaves and attractive yellow or orange heads. It grows wild in the Southern, Eastern, and Central Europe [8]. The annual form is more widely grown and is usually multi-stemmed with a strong taproot. In Europe, the leaves are considered resolvent and diaphoretic while the flowers are used as a stimulant, antispasmodic and emmenagogue [2, 9]. However, in England, the decoction of the flowers was used as a posset drink for the treatment of measles and smallpox, and the fresh juice as a remedy for jaundice, costiveness (constipation) and suppression of menstrual flow [10]. In India, the florets are used in ointments for treating wounds, herpes, ulcers, frostbite, skin damage, scars and blood purification while the leaves, in infusion, are used for treating varicose veins externally [2,10]. The marigold is grown in Iraq too but its curing potency is not assessed yet. The current study was undertaken to assess the potency of the marigold both

aqueous extract and methanol used for treatment of *Leishmania major* promastigotes effect on growth of parasite.

2. Materials and Methods:

2.1 Preparation of plant extract:

The *Calendula officinalis* flowers were dried in an oven with ventilation system at 30 C°. Then, the fluid extract was prepared by maceration for seven days using 80% methanol as a solvent and 20% water. Solvent was evaporated, and the extract was lyophilized, dissolved in dimethyl-sulfoxide (DMSO, BDH, England) at 20mg/ml and stored at 4C°.

2.2 Parasite culture (Promastigote)

Cultures of *Leishmania major* (MHOH/IR/75/SP6), *Leishmania donovani* (MHOH/IR/75/SP6) and *Leishmania maxicana* (MHOH/MX/M379/SP2) promastigotes were maintained by RPMI-1640 medium supplemented with 10% bovine serum (FBS), 100 µg of Streptomycin/ml, and 100U of Penicillin/ml, with passage each 3 or 4 days at 26 °C. Promastogotes of *L. major*, *L. donovani* and *L. mexicana* (1×10⁶ parasites/ml) were incubated at 26 °C for 24, 48 and 72 hours in fresh medium, in absence or presence of different concentrations (12.5, 25, 50, 100 µg/ml) of the methanol extract and aqueous of *Calendula officinalis*. The parasite density for each concentration was determined by counting in a hemocytometer. The results were expressed by IC₅₀ (concentration that inhibited 50% parasite growth).

3. Results and Discussion

This study demonstrated that crude extracts from *Calendula officinalis* inhibited the growth of *Leishmania major*, *L. donovani* and *L. Mexicana*. The leishmanicidal activity of of both aqueous and methanol extracts of marigold plant flower *Calendula officinalis* was tested against three leishmanial species. The viable parasites were counted in the culture media, to calculate the percentage of parasitic death at different concentrations of crude extracts from *Calendula officinalis* used to test the leshmanicidal activity. The effect of the different concentrations (12.5, 25, 50 and 100 µg/ml) were almost ineffective against cultured promastigotes of *L. major*, *L. donovani* and *L. mexicana*. As may be seen from [Table\(1\)](#) aqueous extracts of flower *Calendula officinalis* are inhibitory to the growth of *leishmania*

with 50 percent inhibition occurring at a concentration of 50, >100 and >100 µg/ml, respectively. Methanol extracts of flower *Calendula officinalis* at concentration of 50 µg/ml inhibited the growth of *L. major* promastigotes by 3 days by 62% Table (2), on the other hand, the methanol extracts of *Calendula officinalis* were found to be barely active against *L. donovani* and *L. mexicana* only when at high concentration and had no effect. The major active constituents in marigold include saponins, triterpendiol esters and flavonoids including hyperoside and rutin. [11,12,13]. In present study, it was concluded that the crude extract from *Calendula officinalis* which parasite growth inhibition was greater than saponins Dasyscyphin C isolated from *Ecliptaprostrate* and Gymnemagenol isolated from *Gymnemasyvestre* (IC₅₀ = 450 µg/ml and IC₅₀ = 1000µg/ml, respectively on *L. major* promastigote [14].

A wide range of biological activities have been determined for α-hederin, β-hederin and hederacolchiside A1 that isolated from *Hedera helix*, showed a significant inhibitory effect on *Leishmania donovani* promastigote [15]. These findings were consistent with the results obtained from two triterpenes obtained from the leaves of *Celaenododendron mexicanum* (Euphorbiaceae), (24Z)-3-oxotirucalla-7,24-dien-26-oic acid and epi-oleanolic acid, also showed exhibit leishmanicidal activity on promastigotes of *L. donovani* (IC₅₀ = 13.7 and 18.8 µg, respectively) [16]. Similarly, toxic activity were observed in some saponins isolated from the leaves of *H.helix* are α-hederin 67, β-hederin 68 and hederagenin 69 against promastigotes of *L. infantum* and *L. tropica* [17]. Literature review revealed that there are no studies on the effectiveness of *Calendula officinalis* against parasites. This crude of *Calendula officinalis* may be sources of new compounds that are clinically active against *leishmania*, but mechanism of the direct antileishmania effect awaits elucidation. Earlier reports from our laboratory revealed the antibacterial and antifungi activity of *Calendula officinalis*. Researchers from Venezuela examined extracts of dried flowers from *Calendula officinalis* for its inhibitory effects on the human immunodeficiency virus type1(HIV-1) [18] *Calendula officinalis* extracts show anti-cancer effects in vitro studies on tumor cell lines, derive from Leukemias, melanomas, fibrosacommas, breast, prostate, cervix, lung and pancreas [19]. To our Knowledge, this is the first report of marigold flower against *leishmania*. If a plant could be found with therapeutic activity against leishmanial species it would be useful as current therapy is unsatisfactory [20].

Such studies would identify whether these substances would be worth pursuing for therapeutic purposes, since cytotoxicity identified at this stage would rule out their use in pharmacology. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against *Leishmania*. Even if it had only local activity it would be of value since there is evidence that the incidence of visceral and cutaneous metastatic spread is low. [21] These data should not be interpreted as a comparison of crude plant extract and sodium stibogluconate (pentostam), due to the small number of observations. The value of these observations is to show that *L. major* do respond to these plant extracts. The mode of action of these plant extract is generally unknown.

Based on the results of the current study, extract marigold appear to be a future alternative to development of new antileishmanial chemotherapies. There is a need to develop new antiparasites drugs. Parasites are responsible for several diseases which are of worldwide importance, including giardiasis, amoebiasis, *Leishmaniasis* and malaria. Finally, Natural products continue to be an important source of chemotherapeutic agents.

Table (1): Effect of the *Calendula officinalis* aqueous extracts on the growth of *Leishmania major*, *Leishmania donovani* and *Leishmania mexicana*.

Species of Leishmania	Concentration µg/ml	% Growth	% inhibition
<i>Leishmania major</i>	Control	100	-
	12.5	75	25
	25	66	34
	50	49	51
	100	40	60
<i>Leishmania donovani</i>	Control	100	-
	12.5	90	10
	25	85	15
	50	80	20
	100	75	25
<i>Leishmania mexicana</i> .	Control	100	-
	12.5	100	-
	25	90	10
	50	85	25
	100	79	21

Table (2): Effect of the *Calendula officinalis* methanol extracts on the growth of *Leishmania major*, *Leishmaniadonovani* and *Leishmaniamexicana*.

Species of Leishmania	Concentration $\mu\text{g/ml}$	% Growth	% inhibition
<i>Leishmania major</i>	Control	100	-
	12.5	60	40
	25	55	45
	50	38	62
	100	30	70
<i>Leishmania donovani</i>	Control	100	-
	12.5	90	10
	25	80	20
	50	65	35
	100	50	50
<i>Leishmania mexicana</i> .	Control	100	-
	12.5	92	8
	25	90	10
	50	82	18
	100	75	25

Table (3): Effect of IC50 of *Calendula officinalis* dissolve in water and methanol against *Leishmania major*, *L. donovani* and *L. mexicana*.

Treatment	Species of leishmanial	IC50 ($\mu\text{g/ml}$)
aquious of <i>Calendula officinalis</i>	<i>Leishmania major</i>	51
	<i>Leishmania donovani</i>	>100
	<i>Leishmania mexicana</i>	>100
methanol of <i>Calendula officinalis</i>	<i>Leishmania major</i>	62
	<i>Leishmania donovani</i>	>100
	<i>Leishmania mexicana</i>	>100

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