

ANTIFUNGAL ACTIVITY OF THE ESSENTIAL OIL FROM *CALENDULA OFFICINALIS* L. (ASTERACEAE) GROWING IN BRAZIL

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SHORT COMMUNICATION

ABSTRACT

This study tested *in vitro* activity of the essential oil from flowers of *Calendula officinalis* using disk-diffusion techniques. The antifungal assay results showed for the first time that the essential oil has good potential antifungal activity: it was effective against all 23 clinical fungi strains tested.

Key words: *Calendula officinalis*; essential oil; antifungal activity

Calendula officinalis L. (Asteraceae) is an annual herb with yellow to orange flowers, native to the Mediterranean region. It is also known as pot marigold, a name historically associated with its use in soups and stews to combat illnesses (1) and has a long history of safe use as a medicine in the treatment of inflammation and skin wounds (2). The plant contains sesquiterpenes glycosides, saponins, xanthophylls, triol triterpenes, flavonoids and volatiles. Chalchat and Cols (3) studied the essential oil of *C. officinalis* flowers cultivated in the Massif Central, France, and obtained sesquiterpene alcohol and mainly α -cadinol by using steam distillation. Radulescu and Cols (4) studied flowers from Romania by headspace and steam distillation, and found δ -cadinene plus 1,3,5-cadinatriene and α -muurolol as the major compounds, respectively. Because of the economic value of *C. officinalis* as an herbal medicine and its wide use in cosmetics, perfumes, pharmaceutical preparations and food, we decided to study the acclimatization of *C. officinalis* in southeast Brazil. The aim of the present work was to study *in vitro* antifungal activity of the essential oil from *C. officinalis* flowers, as determined by agar disk diffusion, on 23 clinical fungal strains.

Plant material

The *Calendula officinalis* flowers were collected from an experimental plot in the Medicinal Botanical Garden of the Universidade Paranaense in Umuarama, state of Paraná, southeastern Brazil (S23° 46.225' and W 53° 16.730', altitude 391 m). The flowers were dried at 25°C in a lighted room for 20 days. A voucher specimen, HEUP 1311, was deposited in the Educational Herbarium of the Universidade Paranaense (HEUP). The flowers were collected on 30 April 2004 (onset of winter).

Steam distillation

The essential oil was obtained in a Clevenger apparatus by steam distillation. After 3 hours of steam distillation, 150 g of dried sample was extracted with 500 ml of water. The water collected was re-extracted with 3 x 50 ml hexane. After drying in anhydrous Na₂SO₄, hexane was concentrated in a vacuum rotator evaporator apparatus to 47 mg to yield 0.1% w/w by weight of dry material (5).

Microorganisms used and growth conditions

The antimicrobial activity of the essential oil from *Calendula officinalis* flowers was evaluated using a panel which included

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laboratory control strains from the American Type Culture Collection (Rockville, MD, USA): fungal microorganisms: *Candida albicans* (ATCC 64548), *Candida dubliniensis* (ATCC 777), *Candida parapsilosis* (ATCC 22019), *Candida glabrata* (ATCC 90030) and *Candida krusei* (ATCC 6258); and the following yeasts clinically isolated from humans: *Candida albicans*, *Candida dubliniensis*, *Candida parapsilosis*, *Candida glabrata*, *Candida tropicalis*, *Candida guilliermondii*, *Candida krusei* and *Rhodotorulla* sp. The yeasts were cultured at 25°C in Sabouraud dextrose agar.

Disc diffusion method

In vitro antifungal activity of the *C. officinalis* essential oil was determined by the agar disk diffusion method according to Rubio *et al.* 2003 (6). Briefly, a suspension of each tested microorganism (2.0 ml of 10⁵ cells per ml) was carefully mixed in a tube with 18 ml of Mueller Hinton Agar (MHA), and then poured on Petri plates. Sterile filter-paper discs (Whatman No. 1, 6.0 mm in diameter) were impregnated with 15 µl of the oil and placed on the inoculated plates. Control disks containing 15 µl of the physiological saline and Nystatin (100 U.I. or 20 µg/disc, Cecon, São Paulo, Brazil) were used. These plates were allowed to dry at room temperature for 2 h, and were incubated at 25°C for 48 h. The diameters of the inhibition zones were measured in millimeters and their means were calculated. All the tests were performed in duplicate (7). Twenty-three yeast strains were tested, as listed in Table 1.

The main constituents of the essential oil were the following: sesquiterpene hydrocarbons (68.0%) and sesquiterpenols (27.0%). δ-cadinene (22.53%), α-cadinol (20.40%) and epi-α-muurolol (12.87%). The analyses were performed by GC and GC-MS as described by Gazim *et al.* (8).

Many antifungal agents are available for the treatment of candidal infections, and these are available in several pharmaceutical forms for either topical or systemic use. The major agents belong either to the polyenes, such as amphotericin B and nystatin; or to the azoles, such as itraconazole and fluconazole. However, because of the need for extended treatment, the high cost, toxicity and limited action of the classic drugs, new and effective products are desirable to treat these fungal infections. The antifungal effect of essential oils (EO) of many aromatic plants has been described in several studies (9). The

essential oils rich in cadinene isomers are widely reported to possess high levels of anticandidal activity (10). Our data indicate that the oil of *C. officinalis* flowers exhibited antifungal activity against all investigated yeasts, including the clinical pathogens obtained from reference stocks as well as those recently isolated. This oil provided more interesting results than Nystatin, a fungicide used for topical treatment of mucocutaneous candidiasis.

As seen in Table 1, discs impregnated with 15 µg of *C. officinalis* flower oil produced inhibition zones ranging from 11 to 30 mm of the diameter. The widest (28-30 mm) were obtained against *Candida parapsilosis* (isolates 11 and 12), *Candida glabrata* (isolate 15) and *Rhodotorulla* sp. (isolate 23). The oil also showed high activity, with inhibition zones of 20-27 mm, against *Candida albicans* (Isolates 3 and 7), *Candida*

Table 1. Antifungal activities of the essential oil of flowers of *Calendula officinalis*.

Isolate	Microorganisms	Origin*	Mean zone of inhibition a(mm)	
			Calendula oil 15 µl/disc	Nystatin 20 µg/disc
1	<i>C. albicans</i>	ATCC 64548	16	12
2	<i>C. albicans</i>	orotracheal tube	11	13
3	<i>C. albicans</i>	OC – HIV	26	11
4	<i>C. albicans</i>	VVC	18	12
5	<i>C. albicans</i>	VVC	15	12
6	<i>C. albicans</i>	VVC	15	12
7	<i>C. albicans</i>	Urine	27	11
8	<i>C. dubliniensis</i>	ATCC 777	24	11
9	<i>C. parapsilosis</i>	ATCC 22019	20	12
10	<i>C. parapsilosis</i>	Onychomycosis	14	13
11	<i>C. parapsilosis</i>	Paronychia	30	11
12	<i>C. parapsilosis</i>	Blood	30	11
13	<i>C. glabrata</i>	ATCC 90030	15	12
14	<i>C. glabrata</i>	Hands colonization	23	11
15	<i>C. glabrata</i>	Hands colonization	28	11
16	<i>C. tropicalis</i>	Urine	11	13
17	<i>C. tropicalis</i>	Granulomatous lesion	15	12
18	<i>C. tropicalis</i>	Urine	21	12
19	<i>C. tropicalis</i>	Urine	22	11
20	<i>C. guilliermondii</i>	Hands colonization	25	11
21	<i>C. guilliermondii</i>	Hands colonization	24	11
22	<i>C. krusei</i>	ATCC 6258	15	12
23	<i>Rhodotorulla</i> sp	Hands colonization	30	11

* Except to ATCC microorganisms all of others are human clinical isolates OC – HIV: oral candidiasis; VVC: vulvovaginal candidiasis. Mean of inhibition zone by oil of flowers of *Calendula officinalis*: Good activity (11 -18 mm); high activity (20-27 mm); highest activity (28-30 mm).

dublinskiensis ATCC 777, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* (Isolates 18 and 19), *Candida guilliermondii* (Isolates 20 and 21) and *Candida glabrata* (Isolate 14). For ten isolates: *Candida albicans* ATCC 64548 and isolates 2, 4, 5 and 6, *Candida parapsilosis* (Isolate 10), *Candida glabrata* ATCC 90030, *Candida tropicalis* (Isolates 16 and 17) and *Candida krusei* ATCC 6258, this oil also showed good antifungal activity (11-18mm).

We note that according to the manufacturer of the Nystatin disks, all 23 samples of yeasts tested were sensitive to Nystatin (inhibition diameter above 10 mm). However, the oil of *C. officinalis* flowers showed greater variability among the different isolates than did Nystatin, which ranged between 11 and 13 mm. It is possible that the wider range in the sensitivity profile shown by the oil of *C. officinalis* flowers may be advantageous, because these are widely available and demonstrate a wide action spectrum against pathogenic fungi. Moreover, the human therapeutic response to medicine is not uniform, as suggested by the in vitro assays with Nystatin.

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RESUMO

Atividade antifúngica do óleo essencial da *Calendula officinalis* cultivada no Brasil

Neste estudo foi avaliada a atividade antifúngica do óleo essencial, obtido das flores de *Calendula officinalis* utilizando-se técnica de difusão em discos. Os resultados dos ensaios antifúngicos mostraram pela primeira vez, que o óleo essencial

tem um grande potencial antifúngico, pois foi efetivo contra todas as 23 amostras clínicas de fungos testados.

Palavras-chave: *Calendula officinalis*; óleo essencial; atividade antifúngica

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