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Antimicrobial potential of *Coriandrum sativum* L. against different *Candida* species *in vitro*

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ABSTRACT

The aim of this study was to evaluate the chemical and antifungal activity of the essential oil from *Coriandrum sativum* L. (Apiaceae) against different *Candida* species. The essential oil (EO) was obtained by hydrodistillation and submitted to dry-column chromatography, resulting in six fractions, which were then submitted to TLC and GC–MS analysis. The main compounds identified were alcohols: 1-decanol (24.20%); 2E-decenol (18.00%); 2Z-dodecenol (17.60%); and aldehydes (89%). Antibacterial activity of the EO and its fractions was tested against five species of *Candida albicans*. The EO showed antimicrobial activity against all the species of *Candida* tested, except for *Candida tropicalis* CBS 94. Fractions 4 and 6 had a greater antibiotic spectrum, probably due to the presence of such alcohols as 3-hexenol, 1-decanol, 2E-decenol and 2Z-dodecenol. In conclusion, the EO and its fractions could be used as potential antimicrobial agents to treat or prevent *Candida* yeast infections.

1. Introduction

There has been a high incidence of nosocomial infections caused by opportunistic microorganisms, especially species of *Candida;* the therapeutic approach to such infections is a great challenge due to the resistance developed by pathogens towards a number of widely-used drugs (Chopra, 2007). *Candida* spp may cause several infections involving oral mucosal lesions (candidiasis or candidosis) and potential systemic dissemination, which might be due to antibiotics use, as they kill the competing bacterial flora, leading to an overgrowth of yeasts (Koneman, Allen, Janda, Schreckenberger, & Winn, 2001; Xu, Samaranayake, & Samaranayake, 1999).

A growing demand for natural antimicrobial agents has been observed in the past few years. Several studies have investigated the antimicrobial activity against *Candida albicans* of the essential oil and other substances extracted from medicinal plants; however, no phototherapeutic products with antimicrobial activity have been developed for human or animal use so far (Duarte, Figueira, Sartoratto, Rehder, & Delarmelina, 2005; Rosato, Vitalli, Gallo, Balenzano, & Mallamaci, 2008; Runyoro, Matee, Ngassapa, Joseph, & Mbwambo, 2006).

Coriandrum sativum L. (Umbelliferae/Apiaceae), popularly known as coriander, is commonly found in Brazilian cuisine and medicinally believed to have several therapeutic properties: hypoglycaemic (Otoom, Al-Safi, Kerem, & Alkofani, 2006; Waheed, Miana, Ahmad, & Khan, 2006), anti-inflammatory and hypolipidaemic (Chithra & Leelamma, 1997; Chithra & Leelamma, 2000; Lal, Tkumar, & Pillai, 2004), analgesic and sedative (Chaudhry & Tariq, 2006; Emamghoreishi & Heidari-Hamedani, 2006), anxiolytic (Emamghoreishi, Khasaki, & Aazam, 2005), antimutagenic (Cortes-Eslava, Gomez-Arroyo, Villalobos-Pietrini, & Espinosa-Aguirre, 2004), antihypertensive (Medhim, Hadharzy, Bakos, & Verzar-Petri,1986), diuretic (Benjumea, Abdala, Hernandez-Luiz, Pérez-Paz, & Martin-Herrera, 2005; Maghrani, Zwggwagh, Haloui, & Eddouks, 2005), antioxidant (Melo, Bion, Filho, & Guerra, 2003; Ramadan, Kroh, & Morsel, 2003), antimicrobial (Kubo, Fujita, Kubo, Hihei, & Ogura, 2004; Lo Cantore, Iacobelli, Marco, Capasso, & Senatore, 2004), and carminative, antispasmodic and relaxant (Vejdani et al., 2006).

Antimicrobial activity has been reported for the essential oil (EO) extracted from *C. sativum* seeds against different species of *Candida*, Gram-positive/negative bacteria, and fungi (Elgayyar, Draughon, Golden, & Mount, 2001; Hammer, Carson, & Riley, 1998; Lo Cantore et al., 2004).

Matasyoh, Maiyo, and Ngure (2008) reported antibacterial activity for the EO obtained from *C. sativum* leaves against Gram-positive (*Staphylococcus aureus, Bacillus* spp.) and Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomona aeruginosa*), as well as fungi (*C. albicans*). Duarte, Figueira, Delarmelina, and Sartoratto (2007) showed antimicrobial activity of *C. sativum* leaves against *Pityrosporum ovale*, while Wong and Kitts (2006) demonstrated antimicrobial activity for the ethanolic and aqueous extracts of *C. sativum* against *Bacillus subtilis* and *E. coli*.

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Therefore, the aim of the present study was to evaluate the antimicrobial activity of the EO extracted from *C. sativum* leaves and its fractions against different species of *Candida* (*C. albicans* CBS 562, *Candida tropicalis* CDS 94, *C. parapsilosis* CBS 604, *C. dubliniensis* CBS 7987, and *C. krusei* CBS 573), as well as to identify the chemical constituents responsible for such activity.

2. Materials and methods

2.1. Plant material

Leaves of *C. sativum* L. were obtained at CEASA (grocery wholesalers and retailers) in Campinas, January 2007.

2.2. Microorganisms

Microorganisms were obtained from the Department of Oral Diagnosis, Division of Microbiology and Immunology, at Piracicaba Dental School, University of Campinas (UNICAMP). Antibacterial assays involved such *Candida* species, as *C. albicans* CBS 562, *C. tropicalis* CDS 94, *C. parapsilosis* CBS 604, *C. dubliniensis* CBS 7987, and *C. krusei* CBS 573.

2.3. Distillation of essential oil and fractionation

The essential oil was obtained by the hydrodistillation of fresh leaves (7.5 kg) using a Clevenger-type apparatus for 4 h. The resulting oil/water mixture obtained was extracted using dichloromethane. The organic layer was then separated, dried over Na_2SO_4, filtered and the solvent removed by means of vacuum evaporation at room temperature, resulting in *C. sativum* essential oil (yield: 0.03% w/w).

The EO of the *C. sativum* (0.5 g) was further fractionated by dried column chromatography (cellulose 2 × 30 cm) using Si gel 60 (Merck; Darmstadt, Germany) and dichloromethane as the eluent. The columns were cut into six parts and extracted using dichloromethane, yielding the following fractions: **1** (84.3 mg), **2** (49.5 mg), **3** (21.6 mg), **4** (82.4 mg), **5** (164.8 mg), and **6** (50.9 mg). The fractions were analysed by thin layer chromatography and gas chromatography–mass spectrometry (GC–MS) and then submitted to antimicrobial assays.

2.4. Analysis

The EO and its fractions were analysed through thin layer chromatography (TLC) using silica gel 60 F_{254} layers (Merck) eluted with dichloromethane and visualised under UV 254 nm, following anisaldehyde solution application and drying at 105 °C for 5 min. The samples of the EO and its fractions were diluted in ethyl acetate (10 mg/ml). GC–MS analyses were carried out using gas chromatography (Agilent 6890), with mass selective detector (Agilent 5975; Agilent; Santa Clara, CA), using an HP-5 MS capillary column (25 m × 0.25 mm i.d. × 1.0 μm d.f.). Injection temperature was 220 °C, detector temperature was 250 °C, column temperature was increased from 60 °C to 240 °C at 3 °C per min. Carrier gas was He at 1.0 ml/min and split injection was used.

The programmed temperature retention index of each compound was determined in relation to *n*-alkanes. The MS was operated in the EI mode at 70 eV in the *m*/*z* range from 42 to 350. Compounds were identified by comparing the mass spectra with those in a mass spectral library database (NIST 05), co-injection of hydrocarbon standards to calculate the retention indices (RI's), and analysis of data described by Adams (2007). The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalisation; all relative response factors were taken as one.

2.5. Anti-Candida assay – minimal inhibitory concentration (MIC) test

The yeast was grown overnight at 36 °C in Sabouraud dextrose agar (Merck) plates. Inocula for antimicrobial assays were prepared by diluting the scraped cell mass in 0.85% NaCl solution, adjusted to 0.5 McFarland scale and confirmed by spectrophotometric readings at 580 nm.

Cell suspensions were finally diluted to 10^4 CFU ml^{-1} (colony forming units) for use in the assays. Minimum inhibitory concentration (*MIC*) tests were carried out in RPMI-1640 medium according to NCCLS. (2002) using a tissue culture test plate (96 wells). The EO was diluted in 0.1% Tween 80 solution in sterile water and the stock solution transferred into the first well, and serial dilutions were performed, to obtain concentrations ranging between 0.003 and 2 mg/ml.

Nystatin (Merck) was used as the reference antimycotic control (5–60 μg/ml), the yeast inoculum was added to all wells and the plates were incubated at 36 °C for 24 h.

Table 1
Chemical composition of *Coriandrum sativum* leaves essential oil and fractions.

Compounds	Relative amount (%)						
	Oil	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
Alcohols							
3-Hexenol	10.30	–	–	–	0.70	10.50	16.30
2-Hexenol	3.80	–	–	–	0.40	4.40	6.20
2E-Hecenol	18.00	–	–	–	30.10	35.00	
1-Decanol	24.10	2.20	–	–	11.40	22.40	54.80
1-Undecanol	–	–	–	–	0.60	1.10	1.10
2Z-Dodecenol	17.60	–	–	–	39.90	20.70	6.20
2E-Tetradecenol	3.10	–	26.30	27.00	8.30	2.90	
	77.00	2.20	26.30	27.00	91.40	97.00	84.60
Aldehydes							
2-Hexenal	0.40			2.60	–	–	0.40
Decanal	4.80	24.80	24.90	4.40	–	–	–
2-Decenal	–	–	3.10	15.90	–	–	–
Dodecanal	–	2.90	1.90	–	–	–	–
Tridecanal	3.00	29.30	14.40	2.40	–	–	–
2-Dodecenal	2.90	0.60	14.90	30.40	–	–	–
Tetradecanal	0.90	13.00	3.30	0.50	–	–	–
	12.00	70.00	62.50	56.20	–	–	–
Total	89.00	72.20	88.80	83.20	91.40	97.00	85.00

Table 2
Antimicrobial activity of essential oil and its fractions from *Coriandrum sativum* against different species of *Candida*.

	MIC (μg/ml)								
Candida spp.	Oil	Fraction 1	Fraction 2	Fraction 3**	Fraction 4	Fraction 5	Fraction 6	Nistatin	Fluconazole
C. albicans CBS 562	500	*	1000	–	31	63	31	2	63
C. krusei CBS 573	250	*	125	–	63	250	63	0,5	15
C. parapsilosis CBS 604	125	31	1000	–	7	10	7	8	7
C. dubliniensis CBS 7987	250	*	500	–	7	31	15	1	7
C. tropicalis CBS 94	*	*	*	–	63	125	63	16	125

* MIC > 1000 μg/ml.
** The essential oil fraction three was not subjected to the tests of antimicrobial activity due the insufficient mass.

Minimal inhibitory concentration was determined as the lowest concentration of the EO to inhibit visible growth of yeast (RPMI medium is known to change colour, pink to yellow, as microbial growth occurs).

3. Results and discussion

The EO from the C. *sativum* obtained through hydrodistillation was submitted to GC–MS analysis. The main constituents identified were alcohols (78.13%) and aldehydes (11.96%), with linear chains ranging from 6 to 14 carbon atoms (Table 1). The major alcohols were 1-decanol (24.17%), 2E-decenol (18.05%), 2Z-dodecenol (17.55%) and 3-hexenol (10.34%), while the major aldehydes were decanal (4.76%), dodecanal (3.02%) and 2-dodecenal (2.88%).

These results were observed to be different from those concerning the EO of *C. sativum* leaves collected in Kenya (Matasyoh et al., 2008) and Tunisia (Msaada, Hosni, Ben Taarit, Chahed, & Marzouk, 2007), where the main compounds identified were the aldehydes (E)-2-decenal and (*E*)-2-dodecenal.

In the present study, the EO (*C. sativum*) showed antimicrobial activity, varying from 125 μg/ml (*C. parapsilosis* CBS 604) to 500 μg/ml (*C. albicans* CBS 562), against most of the Candida species tested, except for *C. tropicalis* CBS 94. These results showed a greater antimicrobial potential of the EO, when compared to those obtained by Matasyoh et al. (2008), reporting an *MIC* value of 163 mg/ml for *C. albicans*.

Fractionation of the EO through dry-column chromatography resulted in 6 fractions with different polarities and their main constituents were identified by GC–MS (Table 1).

Aldehydes were the main compounds observed in fractions **1**, **2** and **3** (56–71%) while alcohols were found in fractions **4**, **5** and **6** (85–97%). Results showed potential bacterial inhibition for fractions **4**, **5** and **6,** with *MIC* values varying from 7 to 250 μg/ml. The other fractions showed higher *MIC* values and lower antimicrobial activity, which might be explained by the greater concentration of aldehydes found.

Fractions **4**–**6** and the antibiotics tested showed similar antimicrobial activity against *C. albicans* CBS 562, *C. parapsilosis* CBS 604, *C. dubliniensis* CBS 7987, and *C. tropicalis* with *MIC* values ranging from 7 to 63 μg/ml. These values showed potential antimicrobial activity when compared to those obtained for the EO (125–500 μg/ml), which might be explained by the higher concentration of alcohols found (Table 2).

4. Conclusion

A high concentration of alcohols and aldehydes was observed for the EO of C. *sativum* fresh leaves, showing antimicrobial activity against different species of *Candida*, except for *C. tropicalis* CBS 94.

Chemical fractions of the EO possessed greater antimicrobial activity (*MIC*: 7–63 μg/ml), similar to that of standard antibiotics. The high concentration of alcohols found in these fractions might be responsible for such activity.

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