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

A.F. Begnamia, M.C.T. Duarte, V. Furletti, V.L.G. Rehder

**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 development by pathogens towards a number of widely-used drugs. The essential oil (EO) extracted 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.
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 CBS 94, C. parapsilosis CBS 604, C. dubliniensis CBS 7987, and C. kruzei 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 CBS 94, C. parapsilosis CBS 604, C. dubliniensis CBS 7987, and C. kruzei 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, yielding the following fractions: 1 (21.6 mg), 2 (49.5 mg), 3 (50.9 mg), 4 (54.8 mg), 5 (35.0 mg), and 6 (7.5 kg) using a Clevenger-type apparatus for 4 h. The results from the assay involved such Candida species, as C. albicans CBS 562, C. tropicalis CBS 94, C. parapsilosis CBS 604, C. dubliniensis CBS 7987, and C. kruzei CBS 573.

2.4. Analysis

The EO and its fractions were analysed through thin layer chromatography (TLC) using silica gel 60 F254 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 antymycotic 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>
<thead>
<tr>
<th>Compounds</th>
<th>Relative amount (%)</th>
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<tbody>
<tr>
<td></td>
<td>Oil</td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
</tr>
<tr>
<td>3-Hexenol</td>
<td>10.30</td>
</tr>
<tr>
<td>2-Hexenol</td>
<td>3.80</td>
</tr>
<tr>
<td>2E-Hexenol</td>
<td>18.00</td>
</tr>
<tr>
<td>1-Decanol</td>
<td>24.10</td>
</tr>
<tr>
<td>1-Undecanol</td>
<td>–</td>
</tr>
<tr>
<td>2Z-Dodecanol</td>
<td>17.60</td>
</tr>
<tr>
<td>2E-Tetradecanol</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>77.00</td>
</tr>
<tr>
<td>Aldehydes</td>
<td></td>
</tr>
<tr>
<td>2-Hexenal</td>
<td>0.40</td>
</tr>
<tr>
<td>Decanal</td>
<td>4.80</td>
</tr>
<tr>
<td>2-Decanal</td>
<td>–</td>
</tr>
<tr>
<td>Dodecanol</td>
<td>–</td>
</tr>
<tr>
<td>Tridecanol</td>
<td>3.00</td>
</tr>
<tr>
<td>2,6-Dodecanol</td>
<td>2.90</td>
</tr>
<tr>
<td>Tetradecanol</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>12.00</td>
</tr>
<tr>
<td>Total</td>
<td>89.00</td>
</tr>
</tbody>
</table>
Minimal inhibitory concentration was determined as the lowest concentration of the EO to inhibit visible growth of yeast (RPMI medium is known to change color, 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-dodecenal (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-decanal and (E)-2-dodecanal.

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.

Acknowledgement

The authors thank CAPES (Brazilian Funding Agency) for the financial support.

References


