Effects of date extract on adhesion of *Candida* species to human buccal epithelial cells *in vitro*

Khaled H. Abu-Elteen

Department of Biological Sciences, Faculty of Science, The Hashemite University, Zarqa, Jordan

Abstract: The adherence of three *Candida* species to human buccal epithelial cells (BEC) following treatment with different concentrations of date extract was investigated *in vitro*, as well as the effect of a mouth rinse with date extract on the adhesion of yeast to BEC. Adhesion of *C.albicans, C.tropicalis* and *C.kefyr* to BEC was significantly reduced after both short- and long-term periods of yeast exposure to various concentrations of date extract (reduction between 25% and 52% of the control value). A similar inhibition of adherence was observed upon pre-incubation of BEC with date extract. There was a significant reduction ($P<0.001$) in the adherence of yeast to BEC collected immediately or 5–20 min after an oral rinse with 10% date extract. No statistically significant difference was observed in the adhesion of BEC collected 30 min after an oral rinse with date extract and control BEC. In addition, pre-treatment of either *Candida* or BEC, or both, with date extract resulted in reduced adherence, the magnitude of which was largest when both types of cells were pre-treated. Date extract also inhibited germ-tube formation of *C. albicans* (56–85% inhibition), which might contribute to the effects on adherence.

Key words: adhesion; *Candida* species; date extract; mouth

*Candida albicans* is a communal fungus commonly found in the oral cavity, the respiratory, intestinal and genital tracts, and occasionally on the skin (1). The reported carrier rate of yeasts in the healthy oral cavity varies greatly (7–70%), probably depending on the sampling method, site and study population (1, 2). The incidence of candidosis in patients with diseases such as diabetes, AIDS, leukemia, and cancer may be due to a decrease in the host immune system, facilitating infection by opportunistic yeasts (3, 4). In addition, the use of immune-suppressing agents, certain antibiotics and corticosteroids creates an environment in which *C. albicans* and other yeast strains can thrive (1, 3). Adherence of *Candida* cells to the host surface is thought to be a crucial step in the pathogenic process and a prerequisite for colonization of these surfaces (5, 6).
Dates are regarded as high-energy food due to their sugar content (70–75%), protein (1.9–2.8%) and lipids (0.5–2.5%), on a dry weight basis (7). They also contain vitamins (1.5–5 mg/100 g) and significant amounts of certain minerals such as iron and potassium (1.5–2.5%) (7). Dates have been reported to have antibacterial effects, especially on the growth and spore germination of Bacillus subtilis (8). Date extract was also found to inhibit the growth and cause extensive leakage of cytoplasmic contents from C. albicans (9). Earlier studies showed that exposure of C. albicans to increasing concentrations of date extract led to drastic damage to the yeast, with cell lysis and concurrent leakage of cytoplasmic material and eventual cell death, as observed by scanning electron microscopy (10). Furthermore, ultrastructural results showed irregular shapes with prominent effects on the cell walls. Loss of cell membrane integrity, aggregation of the cytoplasmic contents and detachment of plasmalemma from the cell wall were also observed (10). Moreover, date extract has been found to inhibit the hemolytic activity of Cerastes cerastes and Leiurus quinquestriatus venoms, both in vivo and in vitro (11).

The outermost layer of the C. albicans cell wall plays an important role in pathogenesis, mainly because it possesses macromolecules that adhere to host tissue (4). In this study, we investigated the adhesion of Candida spp. to buccal epithelial cells (BEC) after exposure to various concentrations of aqueous date extract.

Material and methods

Organisms

Yeast species used were: C. albicans ATCC 10231, C. albicans JCC 1428, C. tropicalis JCC 1360 and C. kefyr JCC 1269. JCC are clinical strains obtained from the oral cavity of patients with denture stomatitis at Jordan University Hospital and identified as described previously (2). Stock culture was maintained on Sabouraud’s dextrose agar (SDA) medium (Difco Laboratories, Detroit, MI, USA), stored at 4°C and subcultured routinely.

Preparation of date extract

Berhi date, Phoenix dactylifera, was used in its ripe stage. Brain-heart infusion (BHI) medium containing 5, 10 and 20% (w/v) date extract was prepared as follows: 50, 100 and 200 g amounts of date were suspended in 500 ml sterile distilled water for 24 h, and then homogenized in a Waring blender at maximum speed. The homogenized extracts were filtered through a double layer of cheesecloth. Then, 37 g of BHI broth was added to each filtrate and the final volume was made up to 1 liter with distilled water. All media were sterilized at 121°C for 10 min. Freshly prepared extract was used throughout.

Treatment of Candida species with date extract

For the adherence assay, overnight cultures of Candida spp. were grown at 37°C in yeast nitrogen base (Difco) supplemented with 2.5% (w/v) glucose (YNBG), and adjusted to pH 6.0. Flasks containing 50 ml of the same medium were inoculated with 1 ml of the overnight culture in the presence and absence of 5, 10 and 20% (w/v) date extract. The yeast was incubated in a shaking bath (180 r.p.m.) for 24 h at 37°C. Yeast cells were then harvested, washed twice with Hank’s balanced salt solution (HBSS), and standardized to 1×10⁷ cells/ml. These cells were then used in the adherence assay.

In other experiments, date extract was diluted in overnight cultures of yeast cells in YNBG and 5, 10, and 20% (w/v) date extract. These suspensions were incubated in a shaking bath at 37°C for 60 min. Thereafter, yeast cells were washed with HBSS, standardized and used for adherence assays.

Preparation of buccal epithelial cells for adherence assays

Buccal epithelial cells were collected from six healthy adult males by rubbing gently the mucosal surface of the cheeks with a sterile tongue depressor. The epithelial cells were washed twice with HBSS and collected by centrifugation (500 g × 10 min). This step was intended to wash away saliva and other contaminating oral secretions. These cells were then used to study the adhesion of Candida species to BEC following exposure of yeast to date extract. A final suspension of 2×10⁸ BEC/ml was prepared by appropriate dilution in HBSS after haemocytometer counting.

Effect of treating BECs with date extract

Buccal epithelial cells were collected as described above and suspended in 8 ml of HBSS. This cell suspension was divided into four equal samples of 2 ml. One sample was then exposed, for 5 min, to increasing concentrations of date extract (0.0, 5, 10 and 20% (w/v)). Cells were washed twice and suspended in HBSS buffer. Date extract-treated BECs were then tested for their adherence abilities following standardization (2×10⁶ BEC/ml).

The effect of a mouth rinse with date extract on the adherence of yeast cells was studied using the method described previously (12, 13). Buccal epithelial cells were collected from healthy adult males by gently rubbing the left cheek (control cells) with a sterile
tongue depressor, and then agitated in 5 ml HBSS. The same individuals had rinsed their mouth with 5 ml 10% (w/v) date extract for 5 min, followed once by a 5 ml tap water rinse. Thereafter, BECs were immediately collected from the right cheek (test cells) and suspended in 5 ml HBSS buffer. The experiment was repeated on separate days, but for different rinsing periods (10, 20 and 30 min).

In contrast, mouth rinsing was done using different concentrations of dates, 5, 10 and 20% (w/v), but for 1 min, followed by a tap-water rinse for 5 s. Then BECs were collected and assayed for adherence.

**Effect of pre-treatment of yeast and BEC with date extract**

Cells of exponentially growing *C. albicans* JCC 1428 and freshly collected BEC were suspended separately at 1×10⁶ and 2×10⁶ cells/ml in HBSS, respectively, in the absence or presence of 10% (w/v) date extract. The mixtures were incubated at 37°C for 30 min in a shaking bath (180 r.p.m.). Cells were harvested, washed twice in HBSS and used in the adherence assays.

**Candidal adhesion assay**

The candidal adhesion assay was conducted as described by Abu-Elteen et al. (14). Briefly, a mixture of equal volumes of BEC (2×10⁵ cells/ml) and yeast cells (1×10⁷ yeast/ml), treated as described above, was incubated at 37°C for 2 h in a shaking bath at 180 r.p.m. Cells were filtered through a 20 µm pore size filter (Retsch, Idar-Oberstein, Germany) to remove non-adherent yeast cells. The epithelial cells on the filter were washed twice with 5 ml portions of HBSS and finally suspended in 5 ml of the same buffer. A drop of this suspension was mounted on a glass slide, air-dried, heat-fixed and stained with crystal violet for 1 min. The number of yeasts adhering to 100 epithelial cells was counted microscopically at a magnification of ×400. Counting was undertaken randomly without prior knowledge of the source of the sample, and only uniform, unfolded epithelial cells were included. Each assay was carried out in duplicate and on two different occasions.

**Statistical analysis**

Student’s *t*-test was used to evaluate the differences in the adherence values. A *P* value of <0.05 was considered to be significant. The percentage reduction in candidal adherence was calculated as follows:

\[
\% \text{ reduction in candidal adherence} = \frac{A_c - A_t}{A_c}
\]

where *A*<sub>c</sub>=mean number of yeasts adherent to 100 BECs prepared for control cells, and *A*<sub>t</sub>=mean number of yeasts adherent to 100 BECs prepared for treated cells.

**Effect of date extract on germ-tube formation**

*Candida albicans* JCC 1428 cells grown in the presence [5, 10 and 20% (w/v)] and absence of date extract were washed with phosphate buffered saline (PBS 0.1 M, pH 7.2), diluted in heat-inactivated

---

**Table 1. Adherence of *Candida* spp. to human buccal epithelial cells after incubation of yeasts in media containing date extract for 1 h and 24 h**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Control</th>
<th>5%</th>
<th>Reduction (%)</th>
<th>10%</th>
<th>Reduction (%)</th>
<th>20%</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 10231</td>
<td>513±27</td>
<td>410±35</td>
<td>0.05</td>
<td>20</td>
<td>390±29</td>
<td>0.05</td>
<td>24</td>
</tr>
<tr>
<td><em>C. albicans</em> JCC 1428</td>
<td>554±25</td>
<td>406±39</td>
<td>0.05</td>
<td>27</td>
<td>351±30</td>
<td>0.001</td>
<td>37</td>
</tr>
<tr>
<td><em>C. tropicalis</em> JCC 1360</td>
<td>356±18</td>
<td>282±17</td>
<td>0.05</td>
<td>21</td>
<td>260±24</td>
<td>0.05</td>
<td>27</td>
</tr>
<tr>
<td><em>C. kefyr</em> JCC 1269</td>
<td>283±24</td>
<td>246±21</td>
<td>NS</td>
<td>–</td>
<td>221±17</td>
<td>0.05</td>
<td>22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>Control</th>
<th>5%</th>
<th>Reduction (%)</th>
<th>10%</th>
<th>Reduction (%)</th>
<th>20%</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> ATCC 10231</td>
<td>582±29</td>
<td>386±32</td>
<td>0.05</td>
<td>34</td>
<td>332±25</td>
<td>0.001</td>
<td>43</td>
</tr>
<tr>
<td><em>C. albicans</em> JCC 1428</td>
<td>607±34</td>
<td>398±31</td>
<td>&lt;0.001</td>
<td>34</td>
<td>313±26</td>
<td>&lt;0.001</td>
<td>48</td>
</tr>
<tr>
<td><em>C. tropicalis</em> JCC 1360</td>
<td>385±24</td>
<td>290±16</td>
<td>&lt;0.001</td>
<td>25</td>
<td>211±21</td>
<td>&lt;0.001</td>
<td>45</td>
</tr>
<tr>
<td><em>C. kefyr</em> JCC 1269</td>
<td>289±23</td>
<td>217±14</td>
<td>&lt;0.001</td>
<td>25</td>
<td>181±14</td>
<td>&lt;0.001</td>
<td>37</td>
</tr>
</tbody>
</table>

* Probability values compared with control; NS=not significant.
new-born calf serum (Gibco, Paisley, Scotland) and incubated at 37°C in an orbital shaker. At 0, 60, 120 and 180 min, samples were removed and added to an equal volume of 1% glutaraldehyde in PBS for fixation. Germ-tube formation was determined microscopically (15). Numbers of cells showing germ-tubes/300 cells were recorded in triplicate. Mean values (±SE) were calculated and rounded to whole numbers.

**Results**

The effects of pre-incubating Candida spp. for a short (60 min) or a long (24 h) period in the presence of various concentrations of date extract on adherence to BEC is shown in Table 1. Long exposure reduced adherence of all Candida spp. to between 25–34% of the control value when using 5% (w/v) date extract. Adherence reductions between 37–48% and 43–52% were obtained using 10 and 20% (w/v) date extract, respectively. Short exposure of yeast cells to date extract similarly reduced the adherence, albeit to a lesser extent. However, C. kefyr JCC 1269 showed no significant reduction in adherence when incubated with 5% (w/v) date extract for 60 min (P >0.05).

The effect of pre-incubating BEC in vitro in various concentrations of date extract on adherence of C. albicans JCC 1428 is shown in Table 2. Pre-incubation of BEC with increasing concentrations of date extract for 5 min resulted in a significant reduction (27–42%) in yeast adherence to BEC. This reduction was concentration-dependent, since higher concentrations resulted in higher adherence blockage (between 35% to 46% reduction as compared with control value).

The effect of a mouth rinse with various concentrations of date extract on the adherence of C. albicans JCC 1428 to BEC is shown in Table 3. A significant reduction (27–42%) in yeast adherence to BEC was observed with an increase in the concentration of date extract used for the mouth rinse.

The adherence of C. albicans to BEC, collected immediately, 5, 10 or 20 min after an oral rinse with 10% (w/v) date extract, was significantly reduced (P<0.05) (Table 4). Delay of 30 min in collection of BEC after a rinse with date extract resulted in an increase in yeast adhesion with no significant difference (P>0.05) as compared to the control.

The effects of pre-treatment of yeast and buccal cells for 30 min with 10% (w/v) date extract before assay are presented in Table 5. A reduction in adherence can be achieved by pre-treatment of either partner. When yeast cells alone were pre-treated, a 23% reduction in adherence was achieved, while 25% was achieved when BECs were pre-treated. However, 38% reduction in adherence was obtained when both types of cells, yeast cells and BECs, were pre-treated at the same time.

### Table 2. The effect of pre-incubation (5 min) of BEC with various concentrations of date extract on adherence of C. albicans JCC 1428

<table>
<thead>
<tr>
<th>Date extract concentration (%)</th>
<th>Adherent yeasts/100 BEC (mean±SE)</th>
<th>P*</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)b</td>
<td>557±34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>359±24</td>
<td>&lt;0.001</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>339±20</td>
<td>&lt;0.001</td>
<td>39</td>
</tr>
<tr>
<td>20</td>
<td>299±23</td>
<td>&lt;0.001</td>
<td>46</td>
</tr>
</tbody>
</table>

* C. albicans was not exposed to date extract.
* P values compared to control.
* BEC not exposed to date extract.

### Table 3. Adherence of C. albicans JCC 1428 to BEC collected after an oral rinse with various concentrations of date extract for 1 min

<table>
<thead>
<tr>
<th>Date extract concentration (%)</th>
<th>Adherent yeasts/100 BEC (mean±SE)</th>
<th>P*</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)b</td>
<td>560±37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>530±32</td>
<td>NS</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>410±24</td>
<td>&lt;0.001</td>
<td>27</td>
</tr>
<tr>
<td>20</td>
<td>325±21</td>
<td>&lt;0.001</td>
<td>42</td>
</tr>
</tbody>
</table>

* P values compared to control; b BEC not exposed to date extract; NS, not significant.

### Table 4. Adhesion of C. albicans JCC 1428 to BEC collected at different times following an oral rinse with 10% (w/v) date extract

<table>
<thead>
<tr>
<th>Time elapsed after oral rinse (min)</th>
<th>Adherent yeasts/100 BEC (mean±SE)</th>
<th>Control</th>
<th>Test</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>520±30</td>
<td>298±22*</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>534±28</td>
<td>339±26*</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>548±36</td>
<td>426±21*</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>559±29</td>
<td>480±28*</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>568±32</td>
<td>540±32*</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference from control (P<0.05).

### Table 5. Effect of pre-treatment of cells with 10% (w/v) date extract on the adherence of C. albicans JCC 1428 to BEC

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Yeast cells adhering to 100 Yeast BEC BEC (mean±SE)</th>
<th>Reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>– – (control)</td>
<td>490±21</td>
<td>–</td>
</tr>
<tr>
<td>+ –</td>
<td>379±18*</td>
<td>23</td>
</tr>
<tr>
<td>– +</td>
<td>368±17*</td>
<td>25</td>
</tr>
<tr>
<td>+ +</td>
<td>306±19*</td>
<td>38</td>
</tr>
</tbody>
</table>

* Significant difference from control (P<0.05).
**Fig. 1.** Effect of date extract on the germ-tube formation of *C. albicans* JCC 1428. (5% = 5% date extract, 10% = 10% date extract, 20% = 20% date extract)

The results shown in Fig. 1 demonstrate that germ-tube formation was significantly suppressed when *C. albicans* was incubated for 24 h in the presence of increasing concentrations of date extract. A 5–20% (w/v) date extract showed 61–82% inhibition of germ-tube formation after the first hour of incubation. A longer period (3 h) resulted in 56–85% inhibition in germ-tube formation.

**Discussion**

The results obtained in this study clearly show that in addition to the known antibacterial (8), and anticandidal (9, 10) effects, date extract interferes with the adherence of *Candida* spp. to buccal epithelial cells *in vitro*. Either short (60 min)- or long (24 h)-term pre-incubation of *Candida* spp. with different concentrations of date extract resulted in a significant reduction in adherence to BECs, although the longer term was generally more effective.

Mouth rinsing with date extract resulted in a significant reduction in yeast adhesion (42% reduction). There seems to be a reduction in the anti-adhesive effect, however, 30 min after the oral rinse. Shorter times did not reduce this, which may be due to the constant flushing action of saliva. McCourtie & Douglas (16) reported that adherence of *C. albicans* to acrylic was inhibited when saliva-treated acrylic strips were used in assays or when yeast cells were suspended in saliva. Similar results were reported by others and support the role of saliva on the adherence of *C. albicans* to human epithelial cells (17–19).

Date extract-treated *C. albicans* cells had a reduced ability for germ-tube formation. In this context, exposure to sub-inhibitory concentrations of garlic extract (20), octenidine and piritenidine (21), amphotericin B and nystatin (14, 22) and ketoconazole (23, 24) have been shown to inhibit *Candida* germination and consequently to reduce attachment to human epithelial cells. Thus, the inhibition of germ-tube formation by date extract is important, since it is well known that germ-tube and mycelial forms of *C. albicans* adhere more efficiently to host cells than do yeast form cells (25–28).

The mechanism(s) responsible for inhibition of adherence by date extract is/are still to be determined, but these could include alterations to cell surface features that could mask the adhesins present on the yeast or on the receptors present on the buccal cells. In a previous study we have shown that date extract treatment affected the structure and integrity of the outer surfaces of the yeast cells (10). It is possible that date extract may interfere with the synthesis of adhesins that may be involved in the adhesion process, or it may cause a mechanical distortion of the adhesins already present in the outer envelopes, thus blocking adherence.

Dates are regarded as high-energy food due to their sugar content, which includes glucose, galactose, sucrose, fructose, arabinose and rhamnose (7). Sucrose constitutes 60–80% of the total sugar at the ripe stage (7). Dates also contain significant amounts of pectin and certain minerals such as iron and potassium. Earlier studies have shown that maltose, sucrose, lactose, celllobiase and trehalose are highly effective in inhibiting the adherence of *C. albicans* to epithelial cells (6, 29). McCourtie & Douglas (16, 30) found that *C. albicans* grown on 500 mM galactose adhered to acrylic surfaces at a maximal linear rate throughout an incubation period of 1 h, whereas non-linear adhesion rates were observed with cells grown on 500 mM sucrose, 50 mM glucose, or 50 mM galactose. Similar results were reported by others and showed that the most effective sugar was maltose and the least effective was glucose (31). In these studies it was noted that adhesion of yeast cells was directly proportional to the sugar concentration.

In conclusion, these experiments demonstrate that date extract interferes with the adherence of *Candida* to BEC *in vitro*. Purification of the active ingredients against candidal adhesion is in progress. The combined anti-candidal and anti-adhesion properties of date extract suggest that it could be useful to consider the use of date extract as an effective measure in the treatment of *Candida*-induced denture stomatitis.
References


Acknowledgements

Hashemite University, Research Council, supported this work. The assistance of A. Nimer is appreciated.