INTRODUCTION

Fungal infections now contribute more significantly to microbe-related morbidity and mortality because of the limited number of available antifungals, their inherent toxicity and the development of resistance to them, and the increased number of people living in fungus-friendly environments (Ghannoum & Rice, 1999; Nucci & Marr, 2005). Of the two prominent classes of antifungals, polyenes cause serious host toxicity (Kauffman & Carver, 1997; Cohen, 1998), whereas azoles are fungistatic and their prolonged use contributes to the development of drug resistance in Candida albicans and other species (Dupont et al., 1996; Sanglard et al., 2003). This has prompted the search for novel, less toxic molecules that can be developed as antifungals.

The antifungal effect of essential oils of many aromatic plants has been described in several studies (Knobloch et al., 2003). This has prompted the search for novel, less toxic antifungals. The antifungal effect of essential oils of many aromatic molecules that can be developed as antifungals.

The antifungal effects of cinnamaldehyde, 4-hydroxy-3-methoxycinnamaldehyde (coniferyl aldehyde) and 3,5-dimethoxy-4-hydroxycinnamaldehyde (sinapaldehyde) were investigated against 65 strains of Candida (six standard, 39 flucanazole-sensitive and 20 flucanazole-resistant). MICs of cinnamaldehyde, coniferyl aldehyde and sinapaldehyde ranged from 100 to 500 μg ml⁻¹, 100 to 300 μg ml⁻¹ and 100 to 200 μg ml⁻¹, respectively. All tested isolates showed a marked sensitivity towards these aldehydes in spot and time–kill assays. Sinapaldehyde was found to be the most effective, followed by coniferyl aldehyde and cinnamaldehyde. At their respective MIC₉₀ values, the three compounds caused mean inhibition levels of glucose-stimulated H⁺-efflux of 36, 34 and 41 % (cinnamaldehyde), 41, 42 and 47 % (coniferyl aldehyde) and 43, 45 and 51 % (sinapaldehyde) for standard-sensitive, clinical-sensitive and clinical-resistant isolates, respectively. Inhibition levels of H⁺-efflux caused by plasma membrane ATPase inhibitors Na⁺/K⁺-dicyclohexylcarbodiimide (100 μM) and diethylstilbestrol (10 μM) were 34, 45 and 44 %, and 57, 39 and 35 %, for standard-sensitive, clinical-sensitive and clinical-resistant isolates, respectively. Intracellular pH (pHi) was found to decrease by 0.34, 0.42 and 0.50 units following incubation with three tested aldehydes from the control pHi of 6.70. Scanning electron microscopy and transmission electron microscopy analysis was performed on a representative strain, C. albicans 10261, showing alterations in morphology, cell wall, plasma membrane damage and lysis. Haemolytic activity of the three compounds varied from 10 to 15 % at their highest MIC compared to an activity level of 20 % shown by fluconazole at 30 μg ml⁻¹. In conclusion, this study shows significant activity of cinnamaldehydes against Candida, including azole-resistant strains, suggesting that these molecules can be developed as antifungals.

Abbreviations: pHi, intracellular pH; PM, plasma membrane; SEM, scanning electron microscopy; TEM, transmission electron microscopy.
and its synthesized derivative affect proton extrusion and ergosterol biosynthesis (Shreaz et al., 2010, 2011).

In the present study, we analysed the effect of cinnamaldehyde (Fig. 1a), along with two of its natural derivatives, coniferyl aldehyde (Fig. 1b) and sinapaldehyde (Fig. 1c), against 65 strains of Candida; six of these were standard strains, 39 were fluconazole-sensitive and 20 were fluconazole-resistant. Insight into the mechanisms of the antifungal action of these cinnamic aldehydes has been gained through H+ extrusion studies on plasma membrane ATPase and ultrastructural studies using scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

METHODS

Candida isolates. Isolates used in this study are listed in Table 1. The standard laboratory strains were obtained from Dr I. A. Khan, IIIM, Jammu, India. Clinical isolates were collected from the Regional STD Centre, Safdarjung Hospital and Institute of Pathology, New Delhi, India. Ethical clearance for sampling was obtained from Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. (no. 1/12/08 S). The strains were grown in YPD media containing (w/v): 1% yeast extract, 2% peptone and 2% glucose. Strains were maintained on YPD plates with 2.5% (w/v) agar. Cinnamaldehyde, coniferyl aldehyde, sinapaldehyde and N,N’-dicyclohexylcarbodiimide were purchased from Aldrich. Diethylstilbestrol was obtained from ICN Biomedicals. All media and inorganic chemicals were of analytical grade and procured from Merck.

Assessment of MIC<sub>90</sub>. MIC<sub>90</sub> defined as the lowest concentration of a test entity that causes a 90% decrease in absorbance compared with that of the control, was as determined as per NCCLS document M27-A2, 2002 (NCCLS, 2002) in microtitre plates.

Spot assay. Yeast cells were grown overnight on yeast nitrogen base (YNB) medium containing 2% glucose. The cells were then suspended in normal saline to an OD<sub>600</sub> of 0.1. Five microlitres of fivefold diluted yeast culture was spotted onto YNB plates in the absence (control) and in the presence of different concentrations of the test compounds. Growth differences were recorded following incubation of the plates for 48 h at 30°C (Mukhopadhyay et al., 2002).

Time-kill studies. Candida isolates were subcultured at least twice and grown for 24 h at 35°C on Sabouraud dextrose (glucose) agar (SDA) plates as described previously (Ahmad et al., 2010). An adjusted inoculum suspension of 5 × 10<sup>6</sup> c.f.u. ml<sup>-1</sup> was diluted 10-fold in media. Each concentration of test compound was diluted 1:10 in media containing 5 × 10<sup>5</sup> c.f.u. ml<sup>-1</sup>. This procedure yielded an initial inoculum of 4.5 × 10<sup>5</sup> c.f.u. ml<sup>-1</sup>. The final concentrations of the test compounds were equivalent to 1, 0.5 and 0.33 × MIC for each Candida isolate. Cultures (5 ml final volume) were incubated at 35°C with agitation (200 r.p.m.). At pre-determined time points (0, 2, 4, 8, 12 and 24 h), 100 µl aliquots were removed and transferred to Eppendorf tubes, centrifuged (3900 g at 4°C for 1 min) and rinsed twice with 0.9 ml sterile distilled water to obtain compound-free cells. Pellets were suspended in 100 µl of sterile distilled water and were serially diluted as required. Twenty microlitre volumes of diluted culture spread were spotted onto SDA plates and incubated at 35°C for 48 h until colonies could be seen on the plates, to determine the numbers of c.f.u. ml<sup>-1</sup>.

Proton efflux measurements. The proton pumping activity of Candida isolates was determined as described previously (Manzoor et al., 2002; Rashid et al., 2007; Khan et al., 2011). Briefly, mid-exponential-phase cells, harvested from YPD medium, were washed twice with distilled water and 100 mg cells was routinely suspended in 5 ml of a solution containing 0.1 M KCl and 0.1 mM CaCl<sub>2</sub> in distilled water. The suspension was kept in a double-jacketed glass container with constant stirring. The container was connected to a water circulator at 25°C. The initial pH was adjusted to 7.0 using 0.01 M HCl/NaOH. Test compounds were added to achieve the desired concentrations (MIC<sub>90</sub>) in 5 ml volumes. For glucose stimulation experiments, 100 µl glucose was added to achieve a final concentration of 5 mM. The pH<sup>+</sup> extrusion rate was calculated from the volume of 0.01 N NaOH consumed.

Measurement of intracellular pH. Intracellular pH (pHi) was measured following the methods of Kaur et al., 1988 and Manzoor et al., 2002, with minor modifications. Mid-exponential-phase cells grown in YPD medium were harvested and washed twice with distilled water. The cells (100 mg) were suspended in 5 ml solution containing 0.1 M KCl and 0.1 mM CaCl<sub>2</sub>. Desired concentrations of test compounds (their respective MIC<sub>90</sub> values) were added to the suspensions and pH was adjusted to 7.0 in each case. Following incubation for 30 min at 37°C with constant shaking at 200 r.p.m., the pH was readjusted to 7.0. Nystatin (20 µM) dissolved in DMSO was added to the unbuffered cell suspension and incubated at 37°C for 1 h. Any changes in pH were followed with constant stirring and the external pH at which nystatin permeabilization induced no further shift was taken as an estimate of pHi.

Electron microscopy of Candida cells. Test compounds equivalent to MIC<sub>90</sub> were added to the cell suspensions (~1 × 10<sup>6</sup> c.f.u. ml<sup>-1</sup>) and incubated for 14 h at 30°C. The cells were then prepared for electron microscopy. All Candida cells were fixed with 2%
Table 1. Isolates used in this study

<table>
<thead>
<tr>
<th>Classification of isolates/strain</th>
<th>Species (number of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive (standard, n=6)</td>
<td></td>
</tr>
<tr>
<td>ATCC 10261, 90028, 44829</td>
<td>C. albicans</td>
</tr>
<tr>
<td>ATCC 750</td>
<td></td>
</tr>
<tr>
<td>ATCC 6258</td>
<td>C. tropicalis</td>
</tr>
<tr>
<td>ATCC 90030</td>
<td>C. krusei</td>
</tr>
<tr>
<td>Sensitive (clinical, n=39)</td>
<td></td>
</tr>
<tr>
<td>Invasive (n=9)</td>
<td>C. albicans (4), C. glabrata (3), C. krusei (1), C. tropicalis (1)</td>
</tr>
<tr>
<td>Cutaneous (n=11)</td>
<td>C. albicans (7), C. glabrata (3), C. krusei (1)</td>
</tr>
<tr>
<td>Respiratory; bronchoalveolar (n=8)</td>
<td>C. glabrata (4), C. albicans (2), C. krusei (2)</td>
</tr>
<tr>
<td>Respiratory; tracheal (n=5)</td>
<td>C. albicans (3), C. tropicalis (2)</td>
</tr>
<tr>
<td>Oropharyngeal (n=6)</td>
<td>C. albicans (1), C. krusei (1), C. glabrata (2), C. tropicalis (1), C. guilliermondii (1)</td>
</tr>
<tr>
<td>Resistant (clinical, n=20)*</td>
<td></td>
</tr>
<tr>
<td>Invasive (n=7)</td>
<td>C. albicans (4), C. krusei (1), C. glabrata (1), C. tropicalis (1)</td>
</tr>
<tr>
<td>Cutaneous (n=7)</td>
<td>C. tropicalis (2), C. glabrata (2), C. guilliermondii (2), C. krusei (1)</td>
</tr>
<tr>
<td>Respiratory (n=6)</td>
<td>C. albicans (2), C. tropicalis (2), C. glabrata (2)</td>
</tr>
</tbody>
</table>

*Considered as resistant to fluconazole at MIC ≥ 64 µg ml⁻¹.

glutaraldehyde in 0.1 M phosphate buffer for 1 h at 20 °C (Mares, 1989; Kaneshima et al., 1977). Cells were washed with 0.1 M phosphate buffer (pH 7.2) and post-fixed by using 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h at 4°C. For SEM, samples were dehydrated in acetone and dropped on round glass coverslips with HMDS and dried at room temperature, then sputter coated with gold and observed under the microscope (Zeiss EV040). For ultrastructure studies, samples were dehydrated with graded acetone, cleared with toluene and infiltrated with a toluene and araldite mixture at room temperature then finally in pure araldite at 50°C and embedded in an Eppendorf tube (1.5 ml) with pure araldite mixture at 60°C. Samples were prepared using a sectioning ultramicrotome (Lecia EM UC6) and were observed using TEM. Other details of the procedure were as described by Mares, 1989 and Borgers et al., 1989.

Haemolytic activity. The haemolytic activities of the test compounds were assessed as described earlier (Ahmad et al., 2011). Samples were obtained in EDTA tubes and erythrocytes were harvested by centrifugation for 10 min at 2000 r.p.m. at 20 °C, washed in PBS and diluted to yield a 10% erythrocyte suspension. The 10% suspension was then diluted 1:10 in PBS. For each sample, 100 µl of the suspension was added, in triplicate, to 100 µl volumes of the test compounds (or fluconazole) at different concentrations using the same buffer in Eppendorf tubes. Results were recorded using an iMark microplate absorbance reader (Bio-Rad), at 450 nm. The haemolysis percentage was calculated by comparing lysis caused by Triton X-100.

RESULTS

MIC
Table 2 summarizes the in vitro susceptibilities of the six standard Candida strains and 59 clinical Candida isolates, 39 of which were fluconazole-susceptible and 20 were fluconazole-resistant. Data are reported as the MIC required to inhibit 90% growth of the isolates (MIC90).

Spot assay
Susceptibility testing by spot assay revealed that all the three compounds were highly potent against all tested Candida isolates. Fig. 2 shows the spot assay results of the three compounds against C. albicans ATCC 10261 (standard, sensitive), C. glabrata STD no. 1121 (clinical, resistant) and C. tropicalis STD no. 36 (clinical, sensitive). A pronounced effect on growth reduction was observed at the MIC and 0.5 MIC of the three compounds against all three Candida isolates. Similar results were obtained against other isolates (data not shown). Sinapaldehyde appears to have been most effective, based on these results. These results correlate well with the MIC90 values determined by microtitre assay.

Time–kill curves
Fig. 3 shows the killing activity of cinnamaldehyde, coniferyl aldehyde and sinapaldehyde against C. albicans ATCC 10261, C. glabrata STD no. 1121 and C. tropicalis STD no. 36, respectively. At their respective MIC and 0.5 × MIC values, the fungicidal activity of the three tested compounds was fast against all the tested Candida isolates. Similar results were obtained for other fluconazole-resistant and fluconazole-susceptible Candida isolates used in this study (data not shown). Full fungicidal end points were reached in a mean time of 12 h following incubation with three cinnamic aldehydes. No systematic difference was observed between fluconazole-susceptible and -resistant isolates or between isolates from different locations.

Proton efflux measurements
Table 3 gives the relative rates of H⁺-efflux by Candida isolates in the presence of an MIC90 of cinnamaldehyde,
coniferyl aldehyde and sinapaldehyde. N,N'-dicyclohexylcarbodiimide (100 μM) and diethylstilbestrol (10 μM) were used as positive controls (Luo et al., 2002; Arai et al., 1987). No systematic or significant difference was observed between various isolates. Accordingly, Table 3 gives the mean values of inhibition of H⁺ extrusion by the three different class of strains; standard-sensitive, clinical-sensitive and clinical-resistant. In the absence of glucose, the mean inhibition rates caused by cinnamaldehyde against the three classes of Candida strains, standard-sensitive, clinical-sensitive and clinical-resistant, were 53, 50 and 61 %, respectively; similar inhibition rates were caused by coniferyl aldehyde and sinapaldehyde: 63, 62 and 65 % and 66, 70 and 71 %, respectively, against three classes of strains. Glucose (5 mM) increased activation of H⁺ extrusion by 3.96-, 3.72- and 3.66-fold in the three classes of strains. Levels of inhibition of glucose-stimulated H⁺ extrusion in standard-sensitive, clinical-sensitive and clinical-resistant isolates, respectively. This correlates well with the levels of inhibition caused by the three cinnamic aldehydes in the presence of glucose, suggesting that inhibition of PM-ATPase-mediated H⁺ extrusion is an immediate target of cinnamic aldehydes.

**Intracellular pH**

Fig. 4 shows an estimate of pHi of control- and treated cells exposed for 30 min to the MIC₉₀ of the three cinnamic aldehydes. Yeast control cells maintained a mean pHi of 6.70, while the cells exposed to cinnamaldehyde, coniferyl aldehyde and sinapaldehyde showed a decrease in pHi of 0.34, 0.42 and 0.50 units, respectively. In terms of magnitude, no strict correlation could be established between inhibition observed for each compound against all types of strains in the presence of glucose. Respective inhibition levels of H⁺-efflux in the presence of glucose, caused by known plasma membrane (PM)-ATPase inhibitors N,N'-dicyclohexylcarbodiimide (100 μM) and diethylstilbestrol (10 μM), were 34, 45 and 44 % and 57, 39 and 35 % for standard-sensitive, clinical-sensitive and clinical-resistant isolates, respectively. This correlates well with the levels of inhibition caused by the three cinnamic aldehydes in the presence of glucose, suggesting that inhibition of PM-ATPase-mediated H⁺ extrusion is an immediate target of cinnamic aldehydes.

### Table 2. MIC₉₀ of cinnamaldehyde, coniferyl aldehyde and sinapaldehyde against various Candida isolates.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC₉₀ range (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fluconazole-sensitive (n=45)</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>150–500</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>150–300</td>
</tr>
<tr>
<td>Sinapaldehyde</td>
<td>100–200</td>
</tr>
</tbody>
</table>

![Fig. 2. Spot assay profile of Candida cells in presence of different concentrations of cinnamaldehyde (a), coniferyl aldehyde (b) and sinapaldehyde (c).](http://jmm.sgmjournals.org)
of proton extrusion and pH for the various compounds and
strains tested. There was a universal correlation that could be
established, in that inhibition of proton extrusion leads to a
significant decrease in pH. Those compounds that inhibit
H⁺-efflux more, lead to a greater decrease in pH.

Electron microscopy
To verify the effect of cinnamic aldehydes, we looked for
morphological changes in Candida cells by using SEM. Cells
treated with cinnamaldehyde and its natural derivatives at
their respective MIC₉₀ values for 14 h showed wrinkling of
the cell surface and oozing out of the intracellular contents
in large quantities (Fig. 5b–d), compared with the smooth
surface of untreated cells (Fig. 5a). Alterations in the cell
walls of treated cells were also clearly visible. TEM images of
ultrathin sections of treated cells also showed breakage in
the cell wall and cell membrane (Fig. 6b, c) in comparison to
untreated Candida cells (Fig. 6a). Significant morphological
changes in cell shape were also noticed in sinapaldehyde-
treated Candida cells (Fig. 6d).

Haemolysis
Toxicity of the tested compounds was checked on fresh
human red blood cells. Table 4 shows the levels of
haemolysis caused by the compounds tested and flucona-
zole at their highest MIC₉₀ values. The levels of haemolysis
were 10.10, 13.10 and 14.50 % for cinnamaldehyde,
coniferaldehyde and sinapaldehyde, respectively. Flucona-
zole at an MIC of 30 µg ml⁻¹ caused 20.0 % haemolysis.

DISCUSSION
Cinnamaldehyde and its derivatives are employed as
flavouring agents and preservatives and some of them have
Table 3. Relative H⁺-efflux and percentage inhibition caused by MIC₉₀ of cinnamaldehyde, coniferyl aldehyde and sinapaldehyde on various Candida isolates at pH 7.0. Diethylstilbestrol (10 µM) and N,N’,dicyclohexylcarbodiimide (100 µM) were employed as positive controls of PM-ATPase inhibitors.

Control cells were suspended in 0.1 mM CaCl₂ and 0.1 M KCl at 25 °C. Control standard, clinical and resistant isolates had mean (from four independent recordings) H⁺-efflux rates of 5.63, 5.54 and 5.65 nmol min⁻¹ mg⁻¹ cells, respectively. Glucose (5 mM) caused mean stimulation rates of 3.96, 3.72 and 3.66-fold for control standard, clinical and resistant isolates, respectively. Values in parentheses are mean percentages of inhibition of H⁺-efflux of isolates in the group, with respect to the control.

<table>
<thead>
<tr>
<th>Glucose with compound</th>
<th>Standard</th>
<th>Clinical</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cinnamaldehyde (CD)</td>
<td>0.45 ± 0.10 (53.86)</td>
<td>0.48 ± 0.05 (50.74)</td>
<td>0.37 ± 0.14 (61.79)</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>0.36 ± 0.09 (63.21)</td>
<td>0.36 ± 0.05 (62.58)</td>
<td>0.33 ± 0.10 (65.70)</td>
</tr>
<tr>
<td>Sinapaldehyde</td>
<td>0.32 ± 0.10 (66.91)</td>
<td>0.28 ± 0.07 (70.85)</td>
<td>0.27 ± 0.10 (71.77)</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>0.29 ± 0.01 (71.00)</td>
<td>0.38 ± 0.08 (61.00)</td>
<td>0.51 ± 0.01 (49.00)</td>
</tr>
<tr>
<td>Dicyclohexylcarbodiimide</td>
<td>0.53 ± 0.05 (46.00)</td>
<td>0.45 ± 0.02 (54.00)</td>
<td>0.46 ± 0.03 (52.00)</td>
</tr>
<tr>
<td>Glucose present (5 mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.96 ± 0.70</td>
<td>3.72 ± 0.47</td>
<td>3.66 ± 0.28</td>
</tr>
<tr>
<td>Cinnamaldehyde (CD)</td>
<td>2.52 ± 0.66 (36.76)</td>
<td>2.44 ± 0.34 (34.20)</td>
<td>2.15 ± 0.44 (41.05)</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>2.35 ± 0.73 (41.51)</td>
<td>2.13 ± 0.32 (42.71)</td>
<td>1.93 ± 0.27 (47.01)</td>
</tr>
<tr>
<td>Sinapaldehyde</td>
<td>2.25 ± 0.72 (43.85)</td>
<td>2.02 ± 0.32 (45.55)</td>
<td>1.77 ± 0.26 (51.81)</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>1.67 ± 0.13 (57.00)</td>
<td>2.29 ± 0.10 (39.00)</td>
<td>2.36 ± 0.36 (35.00)</td>
</tr>
<tr>
<td>Dicyclohexylcarbodiimide</td>
<td>2.61 ± 0.20 (34.00)</td>
<td>2.18 ± 0.40 (45.00)</td>
<td>2.03 ± 0.12 (44.00)</td>
</tr>
</tbody>
</table>

been reported to have strong antifungal activity (Kim et al., 2011; Usta et al., 2003; Gill & Holley, 2004). In this study, we explored the effect of hydroxy and methoxy derivatives of naturally occurring cinnamaldehyde, which has very little toxicity to the host, on standard and clinical isolates of Candida. Cinnamaldehyde is thought to act by inhibiting ATPases and cell-wall biosynthesis and by changing membrane structure and integrity. To our knowledge there are no other reports available that demonstrate the in vitro effects of coniferyl aldehyde and sinapaldehyde on isolates of Candida. Cinnamaldehyde and its derivatives are found to be effective against all tested Candida strains: standard-sensitive, clinical-sensitive and clinical-resistant. The range of MIC₉₀ decreased with the increase in methoxy groups: 100–500 µg ml⁻¹ for cinnamaldehyde, 100–300 µg ml⁻¹ for coniferyl aldehyde, and 100–200 µg ml⁻¹ for sinapaldehyde. Significantly, all three tested compounds were active against all fluconazole-resistant strains. The plasma membrane H⁺-ATPase of C. albicans actively extrudes H⁺ to generate an electrochemical gradient, which is employed for transport (Serrano, 1988). H⁺ extrusion is linked to pHi and other important fungal cell functions. It is of note that an inhibition of proton extrusion in range of 36–51 % was observed in the presence of glucose. Established inhibitors of fungal PM-ATPase, N,N’-dicyclohexylcarbodiimide and diethylstilbestrol, caused inhibition ranging from 34 to 57 %. This strong correlation suggests that PM-ATPase-mediated proton extrusion is an immediate target of these cinnamic aldehydes. Inhibition of proton extrusion is linked to pHi, which decreased following incubation of cells with these cinnamic aldehydes. Coniferyl aldehyde and sinapaldehydes act upon respective alcohol dehydrogenases, which deplete NADPH. Part of the effects caused by these cinnamic aldehydes come from alterations in the activity of dehydrogenases and depletion of NADPH. SEM and TEM analyses performed in this study, on a representative Candida strain ATCC 10261 cells treated with compounds for 14 h, clearly demonstrate the effects of cinnamic aldehydes on membrane- and cell-wall structure, as suggested by other investigators as an effect of cinnamaldehyde (Bang et al., 2000; Giordani et al., 2006; Xie et al., 2004; Di Pasqua et al., 2006, 2007).
To conclude, cinnamaldehyde, coniferyl aldehyde and sinapaldehyde are found to be effective antifungal agents against several fluconazole-sensitive and -resistant clinical isolates. The immediate effect of this antifungal activity may originate from the inhibition of PM-ATPase and decrease of pH. Another factor that may be contributing is the depletion of NADPH; this eventually leads to damage of the membrane and cell wall as is evident from the SEM and TEM results. The limited toxicity of these compounds towards human red blood cells (10–15% lysis) coupled with a broad range of activity suggests that these cinnamic aldehydes can be developed as antifungals.
Table 4. Haemolysis caused by different compounds at their highest MIC<sub>90</sub> value, data represent the means of three experiments.

<table>
<thead>
<tr>
<th>Test compounds</th>
<th>Concentration (µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Percentage haemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamaldehyde</td>
<td>500</td>
<td>10.10</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>300</td>
<td>13.10</td>
</tr>
<tr>
<td>Sinapaldehyde</td>
<td>200</td>
<td>14.50</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>30</td>
<td>20.00</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENTS

This work was supported by UGC grant no. 33–223/2007 to L A K. S was awarded a Senior Research Fellowship from the Indian Council of Medical Research. The authors would like to acknowledge Indresh Kumar Maurya and the Advanced Instrumentation Research Facility (Jawaharlal Nehru University, Delhi) for SEM and TEM analysis. Sagheer Ahmad, Laboratory Assistant in the Department of Biosciences at Jamia Millia Islamia, provided technical assistance.

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