Short Communication

*In vitro* activity of 23 tea extractions and epigallocatechin gallate against *Candida species*

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Abstract

In this study, we investigate the susceptibility of *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida tropicalis*, and *Aspergillus fumigatus* using the EUCAST microdilution minimum inhibitory concentration (MIC) method (final tea supernatant concentration range 5.0–0.005 mg/ml) to 23 different teas and tea catechins including epigallocatechin gallate (EGCG) isolated from green tea. All teas exhibited potent *in vitro* antifungal activity against *C. glabrata*. Six out of nine green teas and three of eight black teas had an MIC of 0.078 mg/ml, one white tea had an MIC of 0.156 mg/ml, and finally three of five oolong teas had an MIC of 0.156 mg/ml. Three teas exhibited activity against *C. albicans* (MIC 1.25 mg/ml), one green tea was active against *C. parapsilosis* (MIC 1.25 mg/ml), but none were effective against *C. krusei*, *C. tropicalis* or *A. fumigatus* at the concentrations tested. The MIC of EGCG was 0.3125 μg/ml against *C. glabrata* and 5.0 μg/ml against *C. albicans* and *C. parapsilosis*. The effect was fungicidal against *C. glabrata* at higher concentrations. In conclusion, EGCG and other yet undefined substances in tea have differential antifungal activity *in vitro* against *C. glabrata*, *C. albicans* and *C. parapsilosis*. These data indicate that components of tea and EGCG might be useful particularly for the treatment of *C. glabrata* infections and warrants further investigations.

Key words: antifungal activity, *Aspergillus*, *Candida*, epigallocatechin gallate (EGCG), tea.

Introduction

*Candida* and *Aspergillus* are the most common human fungal pathogens causing an increasing number of invasive infections worldwide. *C. albicans* remains the predominant *Candida* species associated with clinical cases, with *C. glabrata* the second most common in clinical laboratories in the northern hemisphere, while *C. parapsilosis* and *C. tropicalis* are more common in the southern and eastern parts of the world [1–3].

Although there are a number of antifungal drugs available, such as azoles and echinocandins, decreased azole susceptibility *Candida* species has been reported, and echinocandin resistance has been increasingly described among fluconazole resistant *C. glabrata* which causes obvious therapeutic challenges [4–7]. Moreover, the number
of cases involvingazole resistant *Aspergillus fumigatus* has increased recent years [8,9]. Therefore, there is still a great need for the development of new antifungal agents.

It has been reported that tea and tea catechins, such as epigallocatechin gallate (EGCG) have antioxidant activity [10], anticancer activity [11], and antimicrobial activity including antibacterial and antiviral activity [12–15], but there are few studies on antifungal activity of tea extracts and tea catechins. However, Hiratsatomo and Takada reported that green tea catechins, including EGCG, have potent antifungal activity against *C. albicans* [16], and Sirtheeque et al. noted that black tea polyphenols (catechins and theaflavins) showed anti-Candida activity against *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis* [17].

The purpose of this study was to investigate the susceptibility of *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *A. fumigatus* to different sorts of tea and to EGCG isolated from green tea.

## Materials and Methods

### Microorganisms and culture conditions

*C. albicans* ATCC2091, *C. glabrata* ATCC2001, *C. krusei* ATCC6258, *C. parapsilosis* ATCC22019, *C. tropicalis* CBS94629, and *A. fumigatus* NCPF2109 were used in this study. All strains were stored in glycerol broth at −80 °C. The Candida strains were subcultured on CHROMagar (SSI Diagnostika, Hillerød, Denmark) and the *A. fumigatus* strain on YGCagar (SSI Diagnostika) before use in the studies.

### Teas, tea catechins, and antimycotics

Twenty-three teas (nine green teas, eight black teas, five oolong teas, and one white tea) were used in this study (20 were purchased in China while three (one green and two black teas) were obtained from Denmark). The name of the teas was given an abbreviation and listed in Table 1. Epigallocatechin gallate (EGCG) isolated from green tea was purchased from Sigma-Aldrich, Vallensbæk Strand, Denmark (Lot number 108K1564).

Fluconazole (Sigma-Aldrich) was dissolved in dimethyl sulphoxide (DMSO) (Sigma-Aldrich) in stock solution (10,000 mg/l). Anidulafungin pure substance was kindly provided by Pfizer, Ballerup, Denmark. Stock solutions (5000 mg/l) were prepared in DMSO.

### Tea extractions

One gram of tea was incubated with 10 ml of boiled water (80–85 °C) for 10 min, centrifuged (3000 rpm, 10 min) and the supernatant filtered through a 0.8 μm filter. Final concentration of tea extractions was therefore 100 mg of solid tea/ml.

### Antifungal susceptibility testing

The antifungal activity of each tea (twofold dilutions, final tea extraction concentration range 5.0 mg/ml to 0.005 mg/ml) and EGCG (final concentration range 5.0 μg/ml to 0.039 μg/ml) was determined by the EUCAST EDef 7.2 standard [18] using fluconazole as comparator. Microtiter plates were read spectrophotometrically at 490 nm after 24 h, and the minimum inhibitory concentration (MIC) was determined using a 50% growth inhibition endpoint as recommended. All assays were performed in duplicate and repeated at least twice (on different days). MICs against *A. fumigatus* were determined similarly using the EUCAST E.DEF 9.1 standard and a visual endpoint reading as recommended [19].

### Time-kill studies against *C. glabrata*

Time-kill studies were performed using *C. glabrata* (ATCC2001). Overnight cultures were diluted to a concentration 1 × 10⁵ cfu/ml for the starting inoculum. The study was carried out in 5 ml EUCAST growth medium (RPMI 1640 medium with 2% glucose supplemented with MOPS). One green tea (EB), one black tea (HM), and EGCG were incubated with *C. glabrata* (ATCC2001) 1 × 10⁵ CFU/ml in concentrations of 1, 4, 16 and 32 × MIC using fluconazole and anidulafungin at a concentration of 1 × MIC as comparators [20]. One-tenth ml samples were removed from the cultures at 1, 3, 6, and 24 h and inoculated on Sabouraud dextrose agar (SSI Diagnostika, Hillerød, Denmark) to determine cfu after subsequent 10-fold dilutions. The plates were examined after 48 h at 35 °C. The stationary concentration (SC), defined as the lowest concentration for which the initial cfu count was higher than the cfu count at 24 h, and fungicidal concentrations defined as a 99.9% reduction in cfu pelliliter from the starting inoculum were determined. All assays were performed in duplicated and repeated twice (on different days).

### Results

#### MIC determinations

The individual MICs of the twenty-three teas used in these studies and EGCG against *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, and *A. fumigatus* are shown in Table 1. All teas and EGCG exhibited antifungal activity against *C. glabrata*, three were active against
Table 1. The MIC of water extracts of 24 different teas (mg solid tea/ml), Epigallocatechin gallate (EGCG) from green tea (μg/ml), and fluconazole (μg/ml) against Candida albicans (ATCC 2091), Candida glabrata (ATCC 2001), Candida krusei (ATCC 6258), Candida tropicalis (CBS 94 629) Candida parapsilosis (ATCC 22019), and Aspergillus fumigatus (NCPF 2109).

<table>
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<tr>
<th>Tea/compound</th>
<th>C. albicans</th>
<th>C. glabrata</th>
<th>C. krusei</th>
<th>C. parapsilosis</th>
<th>C. tropicalis</th>
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<td>&gt; 16</td>
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MIC, Minimum inhibitory concentration; ND, not done; NI, no inhibition.

C. albicans (one of nine green tea, one of eight black tea, and one of five oolong tea), and one of the green teas showed activity against C. parapsilosis. None of the tested teas had effect on C. krusei, C. tropicalis, or A. fumigatus at the concentrations tested. The MIC of EGCG against C. glabrata was 0.3125 μg/ml, and 5.0 μg/ml against C. albicans and C. parapsilosis, indicating that C. glabrata was more susceptible in vitro to EGCG than C. albicans and C. parapsilosis. EGCG has no activity against C. tropicalis (EGCG was not tested against C. krusei and A. fumigatus as these were not susceptible to any of the teas).

Time-kill studies against C. glabrata

Figure 1 shows the antifungal activity of tested teas and EGCG was concentration- and time- dependent. A fungicidal activity was observed for HM (black tea) and EB (green tea) at 32 × MIC, as a 99.8% killing rate was reached for HM and a 98.0% killing rate was reached for EB at 32 × MIC. In addition, a 98.0% killing rate was reached for anidulafungin at 1 × MIC.

Discussion

Tea is the most widely drunk beverage in the world and is an infusion of variously processed leaves of one of the varieties of an evergreen shrub Camellia sinensis. It has been reported that tea has antimicrobial activities [12–17] and that EGCG isolated from green tea has antifungal activity against C. albicans [16,17].

In this study, we have shown a potent activity, particularly against C. glabrata, of as of yet unknown substances in various teas. The activity was strain specific as C. albicans and C. parapsilosis were less susceptible, and no inhibition...
could be demonstrated against \textit{C. krusei}, \textit{C. tropicalis}, and \textit{A. fumigatus} at the concentrations tested. Furthermore, this activity was fungicidal and concentration and time dependent. Our data also indicated that not only green tea but also other teas, such as black tea, oolong tea, and white tea, have anti-	extit{Candida} activity.

The chemical composition of tea includes proteins, minerals, amino and organic acids, alkaloids, polyphenols, and so forth. The predominant source of tea polyphenols is catechins, which constitute approximately 30% of dry leaf weight of \textit{C. sinensis} \cite{21}. The major green tea catechins are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). The latter makes up 40% of the total catechin content and is the major antioxidant ingredient in green tea \cite{14}. Black and oolong tea contain more EGC and gallic acid than green tea. It is possible that the majority of the antifungal activity of green tea is attributed to EGCG, but it is less clear which compounds contribute to the antifungal activity of the other tea sorts, which is warranted for further studies.

The mechanism of action of the antifungal activity of tea and tea polyphenols is not clear. Reports have shown that tea catechins damaged bacterial membranes of \textit{Staphylococcus aureus} and \textit{Escherichia coli} \cite{22}, attacked the cell membrane of \textit{Trichophyton mentagrophytes}, and caused lysis of the conidia and hyphae \cite{23}, affected folic acid metabolism by inhibiting dihydrofolate reductase \cite{15,24}, and inhibited biofilm formation and proteasome activity in \textit{C. albicans} \cite{25}. Our studies suggested that EGCG and the two teas tested were indeed fungicidal at higher concentrations, but further studies regarding the underlying mechanism of action of tea and tea catechins are warranted.

The antifungal activity was species specific with the highest activity observed against \textit{C. glabrata}. \textit{C. glabrata} is phylogenetically quite distinct from \textit{C. albicans}, but the underlying reason for this differential activity is unknown and will await characterisation of the active component and underlying mechanism of action. None of the tested teas or EGCG had effect on \textit{C. krusei}, \textit{C. tropicalis}, or \textit{A. fumigatus} at the concentrations tested, which might be due to that they are different from \textit{C. glabrata} phylogenetically \cite{26–28}.

Although this is a pilot study that only included reference strains, these data indicate that components of tea including EGCG might be useful particularly for the treatment of \textit{C. glabrata} infections. This is of obvious interest as \textit{C. glabrata} is an important pathogen causing difficult to treat invasive and mucosal infections \cite{29–32}. The emerging of echinocandin resistance of \textit{C. glabrata} is a serious problem as it could indicate resistance anidulafungin, caspofungin, and micafungin, and if that were the case, only amphotericin B would remain to treat the infection \cite{4–7,33}.

It remains to be understood if drinking tea or washing the mouth with tea may help to reduce oral fungal infections and if tea or concentrated tea components could be used for topical treatment of vulvovaginal candidiasis. We hope the findings will stimulate further research in this area.
Acknowledgments

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