

1 **In vitro contribution of herbal products on the**
2 **activity of antifungal drugs against clinical**
3 **Candida isolates**

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17 **ABSTRACT**
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Aims: The contribution of natural compounds may provide a significant progress in the discovery of new antifungal drugs. We aimed to investigate the contribution of carvacrol, farnesol, epigallocatechin gallate, ginger, naringenin and curcumin on the activity of antifungals, voriconazole, caspofungin and amphotericin B against clinical *Candida* isolates.

Methodology: Eight clinical *Candida* isolates were included in this study. The MIC values of each herbal compound and each antifungal were determined using reference broth microdilution method. The interactions of herbal compounds and antifungal drugs were assessed by checkerboard microdilution method.

Results: The MIC values ranged from 60 to 160 µg/mL for carvacrol, from 16 to 64 µg/mL for epigallocatechin gallate, from 80 to 320 µg/mL for ginger and 800 µg/mL for curcumin. Any MIC value was not detected for farnesol and naringenin in concentrations tested. Although the most common interaction type was indifference, farnesol, carvacrol and ginger reduced the MICs of all three antifungals against most isolates. Voriconazole and amphotericin B MICs decreased in combinations with epigallocatechin gallate, whereas naringenin and curcumin did not show any conspicuous effect on antifungal drug activities.

Conclusion: We showed that carvacrol, epigallocatechin gallate, curcumin and ginger enhanced the activity of voriconazole, caspofungin and amphotericin B against *Candida* isolates in vitro. These compounds may represent novel agents to be used in combination with available antifungal drugs to lower the doses of antifungals, thus toxic side effects and treatment costs may decrease.

19
20 *Keywords: Antifungal, Candida, carvacrol, farnesol, epigallocatechin gallate, ginger*

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27 **1. INTRODUCTION**

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29 Invasive fungal infections (IFIs) are increasingly **threats for** critically ill patients and
30 significant **causes** of morbidity and mortality for them. *Candida* spp are one the most
31 common agents of IFIs and still the most common fungi isolated from blood stream
32 infections. Although new antifungal agents are being developed, there is an increasing
33 resistance to standard antifungal therapy, and no new classes of antifungal agents have
34 been approved since 2006 [1]. Currently, three antifungal drug classes including triazoles,
35 polyenes and echinocandins are available to use in treatment of IFIs. However, treatment is
36 often complicated due to their high toxicity, low tolerability, drug interactions and limited
37 spectrums of activities. Moreover, some fungi are intrinsic resistant to these antifungal
38 agents. Therefore, the requirement of new drug or treatment alternatives especially those
39 with a wider spectrum, lower toxicity and cheaper are increasing day by day.

40 In recent years, the interest to studies related with therapeutic use of natural products is
41 increasing. Essential oils (EOs) are aromatic oily liquids obtained from plant material [2].
42 There are many studies investigating the antibacterial, antiviral, antifungal and antiparasitic
43 activity of various herbal oils or their components [3-5]. However, the acts of such
44 compounds on the antifungal activity of available drugs in treatment of *Candida* infections
45 have not been researched so much. **Carvacrol, farnesol, epigallocatechin gallate, ginger,**
46 **naringenin and curcumin** are herbal products that have been shown to have some antifungal
47 effects; carvacrol alone was almost more effective than fluconazole against oral *Candida*
48 isolates [6], and the combination of carvacrol with voriconazole exhibited synergistic or
49 additive activity against drug-resistant *Candida* spp [7]; the combinations of farnesol with
50 fluconazole and micafungin showed synergistic interactions against *C. albicans* biofilm [8];
51 epigallocatechin gallate enhanced the activity of miconazole, fluconazole or amphotericin B
52 against *Candida* isolates [9]; antifungal activity of ginger was stronger than those of
53 fluconazole and nystatin against *C. albicans* [10]; synergistic interactions were detected in
54 the combinations of curcumin with azoles and polyenes against clinical *Candida* isolates
55 [11]. In this study, we investigated the contribution of natural compounds carvacrol, farnesol,
56 epigallocatechin gallate, ginger, naringenin and curcumin on the activity of antifungals such
57 as voriconazole, caspofungin and amphotericin B against clinical *Candida* isolates.

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60 **2. MATERIAL AND METHODS**

61

62 **2.1 Isolates and Media.**

63

64 Clinical *Candida* isolates, *C. albicans* (n=3), *C. parapsilosis* (n=3) and *C. glabrata* (n=2),
65 were used in this study. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used
66 as quality-control isolates for antifungal susceptibility testing. All isolates were subcultured
67 onto Sabouraud dextrose agar (SDA, Merck, Darmstadt, Germany) at 37°C for 24-48 h prior
68 to testing. RPMI-1640 medium (Merck, Darmstadt, Germany) buffered to pH 7.0 with MOPS
69 (3-N-morpholinopropanesulfonic acid) was used for broth microdilution testing and
70 checkerboard method.

71

72 **2.2 Antifungals and herbal compounds**

73

74 Stock solutions of voriconazole (VOR) and amphotericin B (AmB) (Sigma Chemical Co., St
75 Louis, MO, USA) in dimethyl sulfoxide (DMSO), caspofungin (CAS, Sigma Chemical Co, St
76 Louis, MO, USA) in distilled water were prepared at the concentrations of 6400 µg/mL and
77 1600 µg/mL, respectively. Antifungal stock solutions were dispensed into 1 mL tubes and
78 stored at -70 °C until they were used. Carvacrol (CRV), naringenin (NAR), epigallocatechin

79 gallate (EPG), curcumin (CUR), ginger (GGR) and farnesol (FAR) were commercially
80 obtained (Sigma Chemical Co.) and solved in DMSO, to be at concentrations at least 100
81 times higher than the desired highest test concentration, prior to each experiment [12].

83 2.3 Determination of minimum inhibitory concentration (MIC)

84
85 The MIC values for each of antifungal drugs and herbal compounds were determined
86 against all *Candida* isolates using broth microdilution method according to Clinical and
87 Laboratory Standards Institute (CLSI) M27-A3 guideline [12]. For MIC testing, U bottom
88 well microplates were used and serial twofold dilutions ranging 0.0313 to 16 µg/mL for VOR
89 and AmB, 0.015 to 8 µg/mL for CAS, 10 to 1280 µg/mL for CRV, 3.12 to 1600 µg/mL for
90 NAR, 0.06 to 64 µg/mL for EPG, 1.56 to 1600 µg/mL for CUR, 12.5 to 6400 µg/mL for GGR
91 and 6 to 6000 µM for FAR were prepared in RPMI 1640 medium and stored at -70°C until
92 use. A standard 0.5 McFarland fungal suspension was prepared with sterile 0.85% saline by
93 a spectrophotometer at 530 nm wavelength. This procedure yielded a yeast stock
94 suspension of $1-5 \times 10^6$ cells per mL. It was diluted with RPMI 1640 broth medium to obtain
95 a starting inoculum of $1-5 \times 10^3$ cells per mL. Microplates were inoculated and incubated at
96 35°C. The MICs were visually read after both 24 and 48 h. Endpoints for azoles,
97 echinocandins and herbal compounds were defined as the lowest concentration of drug that
98 resulted in a prominent reduction (approximately 50% inhibition) of growth compared with
99 the growth control wells, and MICs of AmB were defined as the lowest concentration of drug
100 which resulted in total inhibition of growth [12].

102 2.4 Checkerboard microdilution tests

103
104 The interaction of each herbal compound with each of all three antifungals was assessed
105 against all isolates by the checkerboard method using CLSI document M27-A3. The
106 concentrations of antifungal agents ranged from 1/32 to 8xMIC and herbal compounds
107 ranged from 1/8 to 8xMIC. Antifungal and herbal compound dilutions were prepared in RPMI
108 1640 medium to be 4-fold of the final concentrations in the microplates. Each of them, 50 µL
109 were dispensed to be antifungal in rows and compound in columns of 96-well microplate.
110 Fungal inoculums were prepared and inoculated to all wells as described in antifungal
111 susceptibility testing. After incubation at 35°C, results were visually read at 24 h and when
112 the absence of adequate growth in growth control well, the incubation was extended more
113 24 h. The interactions of drug and herbal compound were evaluated based on the fractional
114 inhibitor concentration index (FICI). The FICI was obtained by summing the FIC values of
115 each drug; the FIC was calculated for each agent by dividing the inhibitory concentration of
116 each antifungal or compound when used in combination by its MIC. Synergy was defined as
117 a FICI of ≤ 0.5 ; no interaction was defined as a FICI > 0.5 but < 4 ; and antagonism was
118 defined as a FICI ≥ 4 [13]. Off-scale MIC values were converted to the next highest two-fold
119 concentration.

121 3. RESULTS AND DISCUSSION

122
123 Susceptibility testing results of all antifungal agents and herbal compounds were
124 summarized in Table 1. Any MIC value was not detected for FAR and NAR in concentrations
125 tested against *Candida* isolates in this study. However, the MIC values ranged from 60 to
126 160 µg/mL for CRV, from 16 to 64 µg/mL for EPG, from 80 to 320 µg/mL for GGR and 800
127 µg/mL for CUR. The lowest MICs were obtained with EPG.

128
129 The results of interactions between antifungal drugs and herbal compounds were exhibited
130 in Table 2. The most obvious positive interaction was observed between FAR and all three
131 antifungal drugs. FAR caused a prominent decreasing in the MICs of antifungal drugs (Table

132 3). For other combinations, although the most common interaction type was no interaction,
 133 CRV and GGR reduced the MICs of all three antifungals against most isolates (Table 3).
 134 VOR and AmB MICs decreased in combinations with EPG, whereas NAR and CUR did not
 135 show any conspicuous effect on antifungal drug activities.

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 137

138 **Table 1. The antifungal susceptibility testing results for all antifungals and herbal**
 139 **compounds**

Isolates	MIC values ($\mu\text{g/mL}$; μM for FAR)								
	VOR	CAS	AmB	CRV	FAR	NAR	EPG	CUR	GGR
<i>C. albicans-1</i>	0.03	0.125	1	120	>6000	1600	32	800	320
<i>C. albicans-2</i>	0.015	0.125	1	120	>6000	>1600	64	800	240
<i>C. albicans-3</i>	0.015	0.125	0.5	120	>6000	>1600	32	800	160
<i>C. glabrata-1</i>	0.25	0.125	2	120	>6000	>1600	32	800	160
<i>C. glabrata-2</i>	0.03	0.125	2	160	>6000	>1600	64	800	120
<i>C. parapsilosis-1</i>	0.06	0.5	1	60	>6000	>1600	32	800	80
<i>C. parapsilosis-2</i>	0.03	0.5	0.5	60	>6000	>1600	16	800	320
<i>C. parapsilosis-3</i>	0.5	0.5	1	60	>6000	>1600	32	800	160

140 VOR, voriconazole; CAS, caspofungin; AmB, amphotericin B; CRV, carvacrol; FAR, farnesol; NAR,
 141 naringenin; EPG, epigallocatechin gallate; CUR, curcumin; GGR, ginger.

142 Although there are several classes of antifungal drugs at the present time, treatment and
 143 prophylaxis of invasive fungal infections continue to be a significant clinical problem.
 144 Because, treatment is often complicated due to their high toxicity, low tolerability, drug
 145 interactions and limited spectrums of activities, alongside with higher treatment costs.
 146 Moreover, some fungi are intrinsic resistant to these antifungal agents. Therefore, new drug
 147 or treatment alternatives especially those with a wider spectrum, lower toxicity and cheaper
 148 are needed. The most important challenge to developing a new antifungal drug is the
 149 eukaryotic nature of fungal cell similar to mammalian host cell. Natural products are unique
 150 chemicals with different biological activities and the potential antimicrobial effects of certain
 151 natural compounds have attracted serious attention within the scientific area. Therefore, it
 152 has been estimated that significant progress may be observed in the discovery of new
 153 antifungal drugs with the contribution of inexpensive, natural, nontoxic and easily accessible
 154 natural compounds. This study aimed to investigate the antifungal activities of several herbal
 155 products that are widely consumed in the diet worldwide and their contribution on the
 156 efficacy of antifungal drugs against *Candida* spp.

157

158 Carvacrol is a monoterpene phenol derivative extracted from the herb thyme (*Thymus*) and
 159 its many pharmacological properties, including the antimicrobial activity, have been
 160 investigated [2, 3, 6, 14]. It has been shown that CRV is almost more effective than
 161 fluconazole against oral *Candida* isolates; the range of MICs was 0.03-0.5% at 24 h [6].
 162 There are many studies evaluating the antifungal activity of CRV, its activity in combination
 163 with antifungal drugs or its contribution on the activity of antifungal drugs has not been
 164 investigated adequately. Recently, Sharifzadeh et al. [7] investigated the interaction of CRV
 165 and VOR against drug-resistant *Candida* spp. They reported that MIC values of CRV were \leq
 166 125 $\mu\text{g/mL}$ for all isolates and they detected synergistic or additive interactions in this
 167 combination against all *Candida* isolates; FICI values were \leq 0.853 and no antagonistic
 168 activity was seen in the strains tested [7]. In other two studies, CRV MICs were \leq 100 $\mu\text{g/mL}$
 169 and synergic interactions were reported in combination of CRV with fluconazole [14, 15]. The
 170 MIC values of CRV were 60-160 $\mu\text{g/mL}$ against all *Candida* isolates in our study. Although
 171 we didn't detect any prominent synergistic interaction in combinations with CRV and VOR,
 172 CAS or AmB (FICI=0.5-2, 0.58-2, 0.75-1, respectively), CRV caused significant reductions in

173 MICs of all three antifungal drugs; usually four-fold reduction. Although the exact mechanism
 174 is still unclear, it has been reported that the action of CRV based on the inhibition of
 175 ergosterol biosynthesis and the disruption of fungal cell membrane integrity similarly to
 176 azoles and polyenes [16]. The contribution of CRV on the MICs of antifungals may be
 177 explained by; i) these compounds affect simultaneously the same target on fungal cell
 178 resulting with enhanced strength in effectiveness; ii) these compounds show sequentially
 179 effects on the different targets on fungal cell and the disruption of cell wall by a drug (i.e. an
 180 echinocandin) makes it easy to reach of other compound the membrane target. As a result,
 181 simultaneously or sequentially effects of antifungals with herbal compounds can provide the
 182 reduction in the dose of the administered antifungal.
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Isolates	Fractional Inhibitory Concentration (FIC) index (interaction)																	
	CRV			FAR			NAR			EPG			CUR			GGR		
	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB
<i>C. albicans-1</i>	0.91 (I)	0.58 (I)	0.75 (I)	0.5 (S)	0.26 (S)	0.25 (S)	2 (I)	2 (I)	2 (I)	0.5 (S)	2 (I)	1.25 (I)	2 (I)	2 (I)	2 (I)	0.49 (S)	1.12 (I)	0.75 (I)
<i>C. albicans-2</i>	0.75 (I)	2 (I)	0.91 (I)	0.5 (S)	0.09 (S)	0.26 (S)	1 (I)	1.5 (I)	1.5 (I)	0.38 (S)	2 (I)	0.75 (I)	2 (I)	2 (I)	3 (I)	0.75 (I)	0.9 (I)	0.58 (I)
<i>C. albicans-3</i>	0.75 (I)	0.78 (I)	0.91 (I)	0.51 (I)	0.38 (S)	0.25 (S)	1 (I)	1.5 (I)	0.75 (I)	0.75 (I)	2 (I)	0.75 (I)	2 (I)	2 (I)	2 (I)	1.25 (I)	0.62 (I)	0.75 (I)

184
 185 **Table 2. The combination results of antifungal drugs and herbal compounds**
 186 *CRV, carvacrol; FAR, farnesol; NAR, naringenin; EPG, epigallocatechin gallate; CUR, curcumin; GGR,*
 187 *ginger; VOR, voriconazol; CAS, caspofungin; AmB, amphotericin B; S, synergic interaction; I,*
 188 *indifference; A, antagonistic interaction.*
 189
 190

<i>C. glabrata-1</i>	0.75 (I)	0.71 (I)	1 (I)	0.14 (S)	0.75 (I)	0.25 (S)	1.5 (I)	1.5 (I)	1.5 (I)	2 (I)	2 (I)	0.75 (I)	2 (I)	3 (I)	2 (I)	0.75 (I)	0.74 (I)	0.75 (I)
<i>C. glabrata-2</i>	0.5 (S)	0.78 (I)	0.75 (I)	0.5 (S)	0.27 (S)	0.25 (S)	1.5 (I)	1.5 (I)	1.5 (I)	0.37 (S)	2 (I)	0.75 (I)	2 (I)	2 (I)	2 (I)	0.58 (I)	0.58 (I)	1 (I)
<i>C. parapsilosis-1</i>	2 (I)	1.25 (I)	1 (I)	0.05 (S)	0.09 (S)	0.26 (S)	1.5 (I)	1.5 (I)	1.5 (I)	0.75 (I)	2 (I)	0.75 (I)	2 (I)	2 (I)	2 (I)	2 (I)	0.5 (S)	0.53 (I)
<i>C. parapsilosis-2</i>	0.75 (I)	2 (I)	0.75 (I)	0.14 (S)	2 (I)	0.27 (S)	1.5 (I)	1.5 (I)	1.5 (I)	0.63 (I)	2 (I)	2 (I)	2 (I)	2 (I)	3 (I)	1.5 (I)	1.03 (I)	0.75 (I)
<i>C. parapsilosis-3</i>	0.67 (I)	2 (I)	0.91 (I)	0.13 (S)	2 (I)	0.26 (S)	1.5 (I)	0.75 (I)	1.5 (I)	2 (I)	2 (I)	1.5 (I)	2 (I)	2 (I)	2 (I)	0.75 (I)	1 (I)	0.38 (S)

191 Farnesol is an extracellular quorum-sensing molecule producing by *C. albicans* and inhibits
192 the yeast-to-hypha transition in *C. albicans* and consequently blocks biofilm formation [17].
193 FAR is also a sesquiterpene alcohol existing in many herbal products. It has been shown
194 that exogenously FAR inhibits the conidiation in *Aspergillus niger* and the germination of
195 macroconidia in *Fusarium graminearum* [18, 19]. However, the number of studies assessing
196 the antifungal efficacy of FAR with standardised methods is limited. In a study evaluating the
197 combinations of FAR with fluconazole, micafungin and AmB against *C. albicans* biofilm,
198 synergic interactions were observed for FAR with fluconazole and micafungin combinations,
199 and no interaction for FAR with AmB combination according to FIC indexes [8]. Cordeiro et
200 al. [20] evaluated the antifungal activity of farnesol and its interaction with fluconazole,
201 itraconazole, AmB and CAS against drug-resistant strains of *Candida* species (n=45); the
202 MICs of FAR ranged 4.68-150 µM and FAR significantly reduced the MICs of all antifungals
203 against all isolates. Furthermore, they observed significant rates of synergic interactions
204 without any antagonistic interactions in all combinations [20]. We didn't detect any MIC value
205 in concentrations tested for FAR against the isolates in this study. However, synergistic
206 interactions and MIC reductions in antifungal MICs were conspicuous in all three
207 combinations with FAR. The action of FAR on the fungal cell is largely unclear, probably; it is
208 effective with several mechanisms including growth-inhibitory and apoptosis-promoting
209 effects [8].

210 Epigallocatechin 3-O-gallate, the main polyphenol component of green tea, has been
211 extensively investigated for antioxidant, anticancer, antibacterial and antiviral effects [21-23].
212 In a study evaluating many different teas, although EPG didn't have any effect against *C.*
213 *krusei*, *C. tropicalis*, or *A. fumigatus* at the concentrations tested, the MICs of EPG were
214 0.3125 µg/mL against *C. glabrata*, and 5.0 µg/mL against *C. albicans* and *C. parapsilosis*
215 [24]. Ning et al. [9] reported that synergistic interaction was observed between EPG and
216 miconazole, fluconazole or amphotericin B against most of the planktonic and biofilm cells of
217 seven *Candida* isolates and EPG enhanced the activity of these antifungals. In our study,
218 EPG alone had the MICs between 16-64 µg/mL, the combinations of EPG with antifungals
219 were frequently resulted indifference, however EPG caused the reductions at MICs of VOR
220 and AmB. Navarro-Martinez et al. [25] reported that the combination of EPG with azoles
221 showed synergistic interactions against *C. albicans* and the mechanism of this effect could
222 be explained by disturbing the folate metabolism and inhibiting of ergosterol production,
223 because EPG affects the folic acid metabolism by inhibiting dihydrofolate reductase.

224 GGR is used as a spice derived from the roots of ginger all over the world and it contains
225 more than 60 active compounds [26]. In a study evaluated the antibacterial, antifungal and
226 anti-biofilm activity of GGR, MICs of GGR were 20–40 µg/mL for bacteria, 5 µg/mL for *C.*
227 *krusei* and 10 µg/mL for *C. albicans*. Antifungal activity of GGR was stronger than those of
228 fluconazole and nystatin against *C. albicans* [10]. Whereas, Soares et al. [27] did not detect

229 any inhibitory effect on the growth of *C. glabrata* isolates at the tested concentrations (GGR
 230 MIC > 3200 µg/mL). We detected MICs ≤ 320 µg/mL for GGR against our *Candida* isolates,

Isolates	CRV			FAR			NAR			EPG			CUR			GGR		
	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB	VOR	CAS	AmB
<i>C. albicans-1</i>	4	4	4	2	4	4	1	1	1	4	1	4	1	1	1	4	8	4
	↓	↓	↓	↓	↓	↓	↔	↔	↔	↓	↔	↓	↔	↔	↔	↓	↓	↓

231 while we didn't observe significant synergy, GGR reduced the MICs of antifungals for
 232 especially CAS and AmB in combination tests.

233 Naringenin is a flavonoid derivative from citrus fruits. It has been showed that although NAR
 234 and its derivatives had significant antimicrobial activity against *Staphylococcus aureus*, it had
 235 no antimicrobial efficacy against Gram-negative bacteria and fungi including *Alternaria* sp.,
 236 *Rhodotorula rubra* and *C. albicans* even at the high concentrations [28]. Similarly, we
 237 detected any activity of NAR neither alone nor in combination against our *Candida* isolates;
 238 NAR did not exhibit any decreasing effect on the antifungal MICs.

239 CUR, known as Indian saffron in Asia, is a polyphenolic compound and cause of the golden
 240 color in turmeric [29]. Martins et al. [29] detected the strong antifungal activity of CUR; it was
 241 a more potent antifungal than fluconazole against *Paracoccidioides brasiliensis* and 2.5-fold
 242 more potent than fluconazole at inhibition of the adhesion to buccal epithelial cells of *C.*
 243 *albicans* or *C. parapsilosis* [29]. Neelofar et al. [30] observed antifungal activity of CUR
 244 against 14 *Candida* strains, with high MICs varying 250-2000 µg/mL, but CUR was less
 245 effective than fluconazole. Sharma et al. [11] detected synergistic interactions along with a
 246 10-35-fold reductions in the MIC80 values of drugs in combinations of CUR with azoles and
 247 polyenes against clinical *Candida* isolates. In the present study, CUR had high MICs against
 248 *Candida* isolates tested, and no positive interaction was observed causing decreases in the
 249 MIC values of antifungals against our isolates.

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Table 3. The effects of herbal compounds on the antifungal MIC values

<i>C. albicans-2</i>	4	1	4	2	32	4	1	2	1	4	1	4	1	1	2	2	4	4
	↓	↔	↓	↓	↓	↓	↔	↓	↔	↓	↔	↓	↔	↔	↑	↓	↓	↓
<i>C. albicans-3</i>	4	8	4	2	4	4	1	2	4	2	1	4	1	1	1	1	8	4
	↓	↓	↓	↓	↓	↓	↔	↓	↓	↓	↔	↓	↔	↔	↔	↔	↓	↓
<i>C. glabrata-1</i>	4	36	4	8	4	4	1	1	1	1	1	4	1	2	1	4	4	4
	↓	↓	↓	↓	↓	↓	↔	↔	↔	↔	↔	↓	↔	↑	↔	↓	↓	↓
<i>C. glabrata-2</i>	4	8	4	2	4	4	1	1	1	8	1	4	1	1	1	4	4	4
	↓	↓	↓	↓	↓	↓	↔	↔	↔	↓	↔	↓	↔	↔	↔	↓	↓	↓
<i>C. parapsilosis-1</i>	1	4	4	16	32	4	1	1	1	4	1	4	1	1	1	1	4	32
	↔	↓	↓	↓	↓	↓	↔	↔	↔	↓	↔	↓	↔	↔	↔	↔	↓	↓
<i>C. parapsilosis-2</i>	4	1	4	4	1	4	1	1	1	2	1	1	1	1	2	1	32	2
	↓	↔	↓	↔	↓	↓	↔	↔	↔	↓	↔	↔	↔	↔	↑	↔	↓	↓
<i>C. parapsilosis-3</i>	140	1	4	16	1	4	4	1	1	1	1	1	1	1	1	4	4	4
	↓	↔	↓	↓	↔	↓	↓	↔	↔	↔	↔	↔	↔	↔	↔	↓	↓	↓

257 CRV, carvacrol; FAR, farnesol; NAR, naringenin; EPG, epigallocatechin gallate; CUR, curcumin; GGR,
258 ginger; VOR, voriconazole; CAS, caspofungin; AmB, amphotericin B; ↑, fold increase of MIC; ↓, fold
259 decrease of MIC; ↔, no change of MIC.

260

261

262 4. CONCLUSION

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264 We showed that some natural products such as carvacrol, epigallocatechin gallate,
265 curcumin, and ginger, acting like an adjuvant, enhanced the in vitro antifungal effects of
266 voriconazole, caspofungin and amphotericin B against *Candida* isolates. Although the exact
267 mechanism of action of these products is not clear, these compounds may represent novel
268 agents to be used in combination with available antifungal drugs to lower doses of
269 antifungal, thus toxic side effects may decrease and treatment costs may reduce. However,
270 further studies are necessary to clarify the mechanisms of action and also to prove in vivo
271 efficacies and present study may be a guide for them.

272

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277

278 COMPETING INTERESTS

279

280 Authors have declared that no competing interests exist.

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282 AUTHORS' CONTRIBUTIONS

283

284 All authors together designed the study, performed the tests, analyzed the results, and
285 managed the literature searches. Oz Y wrote the first draft of the manuscript. All authors
286 read and approved the final manuscript.

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