Journal of Clinical Laboratory Analysis 30: 513–516 (2016)

Antifungal Activity of Propolis Against Yeasts Isolated From Blood Culture: In Vitro Evaluation

Fatma Mutlu Sariguzel,[1]* Elife Berk,[2] Ayes Nedret Koc,[2] Hafize Sav,[2] and Gonca Demir[2]

[1]*Department of Microbiology, Kayseri Education and Research Hospital, Kayseri, Turkey*

[2]*Department of Microbiology, Erciyes University Medical School, Kayseri, Turkey*

Background: Due to the failure of available antifungal agents in the treatment of candidemia and the toxic activities of these drugs, a lot of researches are being conducted to develop new nontoxic and effective antifungal agents for optimal control of fungal pathogens. The aim of this study is to evaluate the in vitro antifungal activity of propolis against yeasts isolated from the blood cultures of intensive care unit patients. *Methods*: Seventy-six strains were included in this study. The in vitro antifungal activity of propolis, fluconazole (FLU), and itraconazole (ITR) was investigated by the microdilution broth methods (CLSI guidelines M27-A3 for yeast). The propolis sample was collected from Kayseri, Turkey. *Results*: Of the 76 isolates, 33 were identified as *Candida albicans* while 37 were *C. parapsilosis*, three were *C. tropicalis*, and three were identified as *C. glabrata*. The geometric mean range for MIC (μg/ml) with regard to all isolates was 0.077 to 3 μg/ml for FLU and ITR, and 0.375 to 0.70 μg/ml for propolis. It was shown that propolis had significant antifungal activity against all *Candida* strains and the MIC range of propolis was determined as 0185 to 3 μg/ml. *Conclusion*: This study demonstrated that propolis had significant antifungal activity against yeasts isolated from blood culture compared with FLU and ITR. The propolis MIC in azole-resistant strains such as *C. glabrata* was found lower than the FLU MIC. J. Clin. Lab. Anal. **30**:513–516, 2016. © 2015 Wiley Periodicals, Inc.

Key words: *Candida* spp.; fluconazole; in vitro antifungal activity; itraconazole; propolis

INTRODUCTION

The incidence of bloodstream infections caused by yeasts is increasing because of the wide spread use of broad-spectrum antibiotics, corticosteroids, and invasive device or procedures in intensive care unit (ICU) patients. Although *Candida albicans* is the most common cause of candidemia, non-*albicans Candida* species such as *C. glabrata*, *C. krusei*, and *C. tropicalis* are emerging as opportunistic pathogens. *C. parapsilosis* has become the second most frequently isolated *Candida* species from blood cultures in Europe, Latin America, and Canada (1–6). The long-term therapy with antifungal agents is widely used for these infections, but non-*albicans Candida* strains have been reported to be less susceptible to antifungal agents and even agents toxic to patients (7). For this reason, immediate accurate diagnosis and treatment are very crucial for the prognosis of candidemia.

Propolis, a natural product of honey bee, has been attracting the attention of researchers due to its various biological activities and therapeutic properties. Flavonoids, aromatic acids, diterpenic acids, and phenolic compounds appear as the principal components that are responsible for the biological activities of propolis samples. The ethanolic extract of Turkish propolis samples collected in various areas exhibited antibacterial, antifungal, antioxidant, and anticarcinogenic properties (8–21). Also, the ethanolic extract of propolis has been reported to possess biological activities such as antiinflammatory and immunostimulating (22, 23).

Many authors have reported the inhibitory effect of the ethanolic extract of propolis on yeasts isolated from patients with superficial mycoses (14, 17). There is one report in the literature about the use of antifungal activities of

*Correspondence to: Fatma Mutlu Sariguzel, Department of Microbiology, Kayseri Education and Research Hospital, TR-38010 Kayseri, Turkey. E-mail: fmutluguzel@gmail.com

Received 24 February 2014; Accepted 30 July 2015
DOI 10.1002/jcla.21889
Published online in Wiley Online Library (wileyonlinelibrary.com).

Turkish propolis against yeasts isolated from blood culture (16). However, in their study, the number of strains isolated from blood cultures was restricted to eight *C. albicans*. The aim of this study is to evaluate the in vitro antifungal activity of propolis, fluconazole (FLU), and itraconazole (ITR) against yeasts isolated from the blood cultures of adult ICU patients.

MATERIALS AND METHODS

Propolis Sample

Propolis was collected in Kayseri, Central Anatolia, Turkey. An aliquot of crude propolis (7 g) was dissolved in 80% ethanol by shaking at 50°C for 3 days; it was protected from light. The resulting aqueous ethanol extract was filtered through Whatman No. 1 filter paper (Whatman International, Maidstone, UK) and concentrated at 50°C. The resin obtained was dissolved in 80% ethanol to a final concentration of 3 mg/ml. This final solution was used for the antifungal assays. The controls (80% ethanol) did not show an inhibitory effect on any of the test microorganisms. It had been detected in other studies in which the main compounds of propolis were flavonoids, aromatic and fatty acids, and also alcohol and ketones (8, 17).

Yeast Strains and Susceptibility Testing

A total of 76 nonrepetitive strains isolated from the positive blood cultures of ICU patients were included in the study. These strains were considered to be the agent responsible for infection. Strains were identified by the following assessments: the assimilation of carbohydrate with API AUX C 20 (bioMérieux, France) kits, the microscopic and macroscopic morphologies on corn meal agar, germ tube test, capability of growing at 37ºC, urea hydrolysis, and sensitivity for cycloheximide. The isolates were stored at –20ºC in tryptic soy broth containing 10% glycerin until used in this study. Prior to use, the yeasts were thawed and subcultured at least twice on Sabouraud's glucose agar plates. Quality control was performed by testing *C. albicans* ATCC 90028 according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) document M27-A3 (24).

FLU and ITR were obtained from the manufacturer as powders. The microdilution broth methods of the CLSI (previously the National Committee for Clinical Laboratory Standards) were used to determine Minimum Inhibitory Concentrations (MICs). The in vitro antifungal activity of propolis, FLU, and ITR was investigated by the following guidelines (M27-A3) for yeast. Stocks and dilutions of FLU and ITR were prepared in sterile distilled water. Final drug concentrations in the microdilution plates ranged from 0.125 to 64 μg/ml for FLU, from 0.03 to 16 μg/ml for ITR, and from 0.005 to 3 μg/ml for propolis. The microdilution plates were prepared using the synthetic medium Roswell Park Memorial Institute (RPMI) 1640 broth medium (Sigma Chemical, Madrid, Spain) with L-glutamine but without sodium bicarbonate and buffered at pH 7.0 with 0.165 mol/l morpholinepropansulfonic acid (Sigma Chemical).

Yeast inoculum suspensions were prepared as described in the CLSI M27-A3 document using sterile 0.85% saline. The cell density was adjusted with a spectrophotometer by adding sufficient saline to match the transmittance produced by a 0.5 McFarland density standard at a 530 nm wavelength, resulting in a concentration of 0.5×10^3 to 2.5×10^3 cells/ml. MICs were visually determined at 24 and 48 hr of incubation at 35ºC, and plates were observed for the presence or absence of growth at 24 and 48 hr (24).

The MIC for propolis was defined as the lowest concentration in which optical clarity was observed (16, 17). For FLU and ITR, MIC was defined as the lowest concentration in which 50% decrease in turbidity as visually is observed (24).

RESULTS

The 76 strains were identified as 37 (48.6%) *C. parapsilosis*, 33 (43.4%) *C. albicans*, 3 (3.9%) *C. glabrata*, and 3 (3.9%) *C. tropicalis. Candida parapsilosis* have become more frequent etiological agents for invasive fungal infections in our ICU. The geometric mean range for MIC (MIC GM) with regard to all isolates was 0.077 to 3 μg/ml for FLU and ITR, and 0.375 to 0.70 μg/ml for propolis. The significant antifungal activity of propolis against all *Candida* strains was shown and the MIC range of propolis was determined as 0.185 to 3 μg/ml.

After 24 hr of incubation, 75 strains were sensitive to FLU and 64 strains were sensitive to ITR. Twelve strains were dose-dependent sensitive to ITR and one strain was dose-dependent sensitive to FLU. The MIC values of propolis were 0.375 to 0.185 μg/ml against ITR and FLU dose-dependent sensitive strains.

At 48 hr of incubation, 74 strains were sensitive to FLU and 42 strains were sensitive to ITR. Thirty-three strains were dose-dependent sensitive to ITR and one strain was resistant. Two of all strains were dose-dependent sensitive to FLU. The MIC values of propolis were 0.375 to 0.185 μg/ml against ITR and FLU dose-dependent sensitive and resistant strains. The range of MICs and geometric means of FLU, ITR, and propolis for *Candida* spp., as well as the MICs at which 50% and 90% of the isolates were inhibited, are summarized in Table 1.

DISCUSSION

Candidemia has recently emerged as an increasingly common problem in hospitalized patients, especially

TABLE 1. MICs Values Obtained for FLU, ITR, and Propolis at the End of 24- and 48-Hr Incubation of All Strains

		Incubation time (24 hr)				Incubation time (48 hr)			
		MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC GM (μg/ml)	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	MIC GM (μg/ml)
C. parapsilosis ($n = 37$)	FLU	0.125–8	1	4	0.768	0.25–16	1	4	1.056
	ITR	0.03–0.5	0.125	0.25	0.104	0.06–1	0.125	0.5	0.170
	PRO	0.185–0.75	0.375	0.75	0.403	0.185–3	0.75	0.75	0.514
C. albicans ($n = 33$)	FLU	0.125–4	0.5	2	0.543	0.125–4	1	2	0.777
	ITR	0.06–0.25	0.125	0.25	0.118	0.06–0.5	0.125	0.5	0.182
	PRO	0.185–0.75	0.375	0.75	0.415	0.185–3	0.75	0.75	0.523
C. tropicalis ($n = 3$)	FLU	1–8	1	-	2	1–8	2	-	2.519
	ITR	0.125–0.25	0.25	-	0.198	0.25	0.25	-	0.25
	PRO	0.375–0.375	0.375	-	0.375	0.375	0.75	-	0.595
C. glabrata ($n = 3$)	FLU	1–16	1	-	2.519	1–16	2	-	3.174
	ITR	0.03–0.125	0.125	-	0.077	0.06–0.5	0.125	-	0.155
	PRO	0.185–0.75	0.75	-	0.470	0.185–0.75	0.75	-	0.470

PRO = propolis.

among immunocompromized patients in ICUs. Toxicity concerns, limited spectrum of available antifungal agents, and the emergence of resistance to available antifungal agents in yeasts have created a need for new effective antifungal agents to be used in patients with candidemia (13, 25, 26).

The present study researches the in vitro antifungal activity of locally obtained propolis (in Kayseri, Central Anatolia, Turkey) against *Candida* isolated from blood culture. Propolis is presented as a new antifungal agent for the treatment of infections caused by yeasts. Reliable methods are available for testing of the in vitro antifungal activity of propolis. According to the CLSI (M27-A3), the highest agreement among laboratories was obtained with RPMI-1640 medium at 35°C and after a 24-hr incubation time with antifungal compounds (27, 28). Therefore, we preferred the RPMI-1640 medium at 35°C for our susceptibility study.

Ghisalbert (29) reported that the antifungal activity of propolis against yeasts is because of the phenols, flavonoids, and esters in its structure. The properties of propolis examined in our study are well known (17). Popova et al. (8) determined that propolis sample originating from Kayseri, Central Anatolia, Turkey, was determined to be rich in phenols, flavonoids, and esters.

Other researchers have found that propolis has antifungal activity against different *Candida* pathogens and other yeasts. Ota et al. (30) showed the antifungal activity of propolis against 75 *Candida* strains. The *Candida* strains showed a clear sensitivity against propolis with the following order of sensitivity: *C. albicans* > *C. tropicalis* > *C. krusei* > *C. guilliermondii*. Koc et al. (16) reported the antifungal activity of honeybee products (honey, royal jelly, pollen, and propolis) in 40 yeast strains with the broth microdilution method and the antifungal activities of each product decreased in the following order: propolis > pollen > royal jelly > honey. The MIC range values of propolis against nine *C. albicans* and ten *C. glabrata* strains were found as 0.006 to 0.05, 0.025 to 0.1, 0.0125 to 0.1, and 0.025 to 0.1 μg/ml at 24 and 48 hr of incubation, respectively. Also, in this study, the propolis MIC range of eight *C. albicans* strains isolated from blood culture was found as 0.006 to 0.5 μg/ml. Silici and Koc (17) reported that the antifungal activities (geometric mean) of propolis and ITR against 15 yeasts isolated from patients with superficial mycoses as 0.06 and 0.35 μg/ml by the broth microdilution method, respectively. In our study, the number of strains isolated from blood culture was higher compared with other studies. Although a limitation of our study was the small number of different species, this study was conducted on the major clinically important yeasts currently encountered and was also performed with the most commonly isolated strains in our adult ICU patients. Non-*albicans Candida* species was the most frequently causative agent for candidemia in our adult ICU patients. Propolis was shown to have significant antifungal activity against all *Candida* strains in our study. The geometric mean range for propolis (0.375–0.70 μg/ml) with regard to all isolates was lower than FLU and ITR (0.077–3 μg/ml). The MIC values of propolis did not change by the 24 or 48 hr of incubation.

In conclusion, this study demonstrated that propolis was found to have significant in vitro antifungal activity, which is comparable with FLU and ITR against yeasts isolated from blood cultures. The propolis MIC in ITR and FLU dose-dependent sensitive and resistance strains such as *C. glabrata* was determined to be lower than the FLU MIC. However, further studies are required with different yeast strains for the use of propolis and in vivo

studies are also needed to assess whether this antifungal activity can be used for clinical application or not.

CONFLICT OF INTEREST

The authors declare that there are no financial conflicts of interest.

REFERENCES

1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20(1):133–163.
2. Abi-Said D, Anaissie E, Uzun O, Raad I, Pinzcowski H, Vartivarian S. The epidemiology of hematogenous candidiasis caused by different *Candida* species. Clin Infect Dis 1997;24:1122–1128.
3. Rex JH, Pfaller MA, Barry AL, Nelson PW, Webb CD. Antifungal susceptibility testing of isolates from a randomized, multicenter trial of fluconazole versus amphotericin B for the treatment of nonneutropenic patients with candidemia. Antimicrob Agents Chemother 1995;39:40–44.
4. Wingard J. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin Infect Dis 1995;20:115–125.
5. Pfaller MA, Jones RN, Doern GV, et al. Bloodstream infections due to *Candida* species: SENTRY antimicrobial surveillance program in North America and Latin America, 1997–1998. Antimicrob Agents Chemother 2000;44:747–751.
6. Pfaller MA, Messer SA, Hollis RJ, et al. Trends in species distribution and susceptibilty to fluconazole among blood stream isolates of *Candida* species in the United States. Diagn Microbiol Infect Dis 1999;33:217–222.
7. Bodey GP. Azole antifungal agents. Clin Infect Dis 1992;14:161–169.
8. Popova M, Silici S, Kaftanoglu O, Bankova V. Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine 2005;12:221–228.
9. Silici S, Kutluca S. Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J Ethnopharmacol 2005;99:69–73.
10. Uzel A, Sorkun K, Oncag O, Cogulu D, Gencay O, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res 2005;160:189–195.
11. Ozcan M, Uver A, Ceylan DA, Yetişir R. Inhibitory effect of polen and propolis extract. Nahrung 2004;48:188–194.
12. Koc AN, Silici S, Mutlu Sarıgüzel F, Sagdıc O. Antifungal activity of propolis in four different fruit juices. Food Technol Biotechnol 2007;45:57–61.
13. Koc AN, Silici S, Ayangil D, Ferahbas A, Cankaya S. Comparison of in vitro activities of antifungal drugs and ethanolic extract of propolis against *Trichophyton rubrum* and *T. mentagrophytes* by using a microdilution assay. Mycoses 2005;48:205–210.
14. Silici S, Koc AN, Ayangil D, Cankaya S. Antifungal activities of propolis collected by different races of honeybees against yeasts isolated from patients with superficial mycoses. J Pharmacol Sci 2005;99:39–44.
15. Koç AN, Silici S, Erçal BD, Kasap F, Hörmet Öz HT, Mavuş Buldu H. Antifungal activity of Turkish honey against *Candida* spp. and *Trichosporon* spp.: An *in vitro* evaluation. Med Mycol 2008;47:707–712.
16. Koç AN, Silici S, Kasap F, Hörmet-Öz HT, Mavuş Buldu H, Erçal BD. Antifungal activity of the honeybee products against *Candida* spp. and *Trichosporon* spp. J Med Food 2009;14:128–134.
17. Silici S, Koc AN. Comparative study of in vitro methods to analyse the antifungal activity of propolis against yeasts isolated from patients with superficial mycoses. Lett Appl Microbiol 2006;43:318–324.
18. Silici S, Koç AN, Mutlu Sarıgüzel F, Sagdıc O. Mould inhibition in different fruit juices by propolis. Arch Lebensmittelhygiene 2005;56:87–90.
19. Kolankaya D, Selmanoglu G, Sorkun K, Salih B. Protective effects of Turkish propolis on alcohol-induced serum lipid changes and liver injury in male rates. Food Chem 2002;78:213–217.
20. Orhan H, Marol S, Hepsen IF, Sahin G. Effects of some probable antioxidants on selenite-induced cataract formation and oxidative stress-related parameters in rats. Toxicology 1999;139:219–232.
21. Ozkul Y, Silici S, Eroglu E. The anticarcinogenic effect of propolis in human lymphocytes culture. Phytomedicine 2005;12:742–747.
22. Miyataka H, Nishiki M, Matsumoto H, Fujimoto T, Matsuka M, Satoh T. Evaluation of propolis. I. Evaluation of Brazilian and Chinese propolis by enzymatic and physico-chemical methods. Biol Pharm Bull 1997;20:496–501.
23. Dimov V, Ivanovska N, Manolova N, Bankova V, Nikolov N, Popov S. Immunomodulatory action of propolis. Influence on anti-infections protection and macrophage function. Apidologie 1991;22:155–162.
24. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Third Edition. CLSI Document M27-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
25. White TC, Marr KA, Bowden RA. Clinical cellular and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 1998;11:382–402.
26. Cameron ML, Schell WA, Bruch S, et al. Correlation of in vitro fluconazole resistance of *Candida* isolates in relation to therapy and symptoms of individuals seropositive for human immunodeficiency virus type I. Antimicrob Agents Chemother 1993;37:2449–2453.
27. Espinel-Ingroff A, Kerkering TM, Goldson PR, Shadomy S. Comparison study of broth macrodilution and broth microdilution antifungal susceptibility testing of yeast isolates. Diagn Microbiol Infect Dis 1991;19:9–13.
28. Elder JV, Joosten L, Verhaeghe A, Surmont I. Fluconazole and amphotericin B antifungal susceptibility testing by National Committee for Clinical Laboratory Standards broth macrodilution method compared with E test and semiautomated broth microdilution test. J Clin Microbiol 1996;32:2099–2102.
29. Ghisalbert EL. Propolis: A review. Bee World 1979;60:59–84.
30. Ota C, Unterkircher C, Fantinato V, Shimuzu MT. Antifungal activity of propolis on different species of *Candida*. Mycoses 2001;44:375–378.