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Antifungal Activity of Boric Acid, Triclosan and Zinc Oxide Against Different Clinically Relevant *Candida* Species

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Abstract

Background: The genus *Candida* includes about 200 different species, but only a few are able to produce disease in humans. The species responsible for the highest proportion of human infections is *Candida albicans*. However, in the last two decades there has been an increase in the proportion of infections caused by other *Candida* species, including *C. glabrata* (*Nakaseomyces glabrata*), *C. parapsilosis*, *C. tropicalis, C. krusei* (*Pichia kudriavzevi*) and more recently *C. auris*. Decolonization of patients has been used as an infection control strategy for bacterial infections, but information about decolonization products used in clinical practice for *Candida* and other fungal pathogens is limited. Compounds with antimicrobial activity, such as triclosan (TR), boric acid (BA) and zinc oxide (ZO), are mainly used in personal care products. These products can be used for long periods of time without an abrasive skin effect and are a possible alternative for patient decolonization in health care settings.

Objective: The aim of this study was to evaluate the antifungal activity of boric acid (BA), triclosan (TR) and zinc oxide (ZO), individually and combined, against clinically relevant *Candida* species.

Materials and methods: Compounds to be screened for antifungal activity were evaluated at different concentrations, alone, and combined, using a well diffusion assay. The statistical evaluation was performed using analysis of variance (ANOVA) and a post hoc analysis using the multiple comparisons method.

Results: Individually, BA and TR showed antifungal activity against all *Candida* species evaluated but ZO did not show any antifungal activity. Mixtures of BA [5%] - TR [0.2%]; BA [5%] - TR [0.3%]; BA [5%] - TR [0.2%] - ZO [8.6%]; and BA [5%] - TR [0.2%] - ZO [25%] yielded the highest antifungal activity. An increased antifungal effect was observed in some mixtures when compared with individual compounds.

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Conclusions: We demonstrated antifungal activity of BA and TR against multiple *Candida* species, including against a clade of the emerging healthcare-associated pathogen *C. auris*. Additionally, this study shows enhancement of the antifungal effect and no antagonism among the mixtures of these compounds. Further research is needed to determine whether these compounds can reduce the burden of *Candida* on skin.

Keywords

Candida ; Boric Acid; Zinc oxide; Triclosan; decolonization

INTRODUTION

The genus *Candida* includes about 200 different species, but only a few are opportunistic pathogens in humans. *Candida* species are found as part of the normal human microbiota of the skin and the oral, gastrointestinal, and genitourinary tracts. Infections have a wide array of clinical presentations from superficial infections of the mucosa to invasive infections causing severe disease (1). Severe infections are associated with critically ill patients, including patients with long-term hospitalization, immunocompromise, recent surgery, especially multiple abdominal surgeries, exposure to multiple antimicrobial agents during hospitalization, and those with invasive devices such as a central venous catheter (2).

Invasive infections caused by *Candida* are especially relevant in critically ill patients hospitalized in intensive care units (ICU) (3). In European ICUs the incidence is estimated at 7.07 episodes per 1000 ICU admissions, with a mortality of 42% (4). Invasive candidiasis is recognized as the third leading cause of infection in ICUs worldwide, and the emergence of novel species with multidrug resistance, like *Candida auris*, has become a serious public health threat (5–8). *Candida albicans* is the most common species associated with invasive fungal infections, but the proportion of non-*albicans Candida* infections has been steadily rising, especially the species *C. glabrata* (*Nakaseomyces glabrata*), *C. parapsilosis*, *C. tropicalis, C. krusei* (*Pichia kudriavzevi*) and more recently *C. auris* (9,10). The majority of *Candida* infections are the result of inoculation with organisms carried in the gut or on the skin of the patient. Less frequently, the infection occurs by exogenous transmission, including the hands of healthcare workers, previously reported for *C. parasilosis*, or environmental contamination as we are currently seeing with *C. auris* infections. Exogenous contamination has been linked to *Candida* outbreaks in hospitals (11).

As part of infection control practices, decolonization of patients is an important control measure for resistant multidrug pathogens (12). The Infectious Diseases Society of America (IDSA), has published guidelines for decolonization of resistant microorganisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA). These guidelines recommend daily chlorhexidine washes for 5 to 14 days or diluted bleach baths for 15 minutes twice a week for 3 months (13). However, evidence of the efficacy of chlorhexidine for the decolonization of skin containing colonizing *Candida* species is lacking. With the emergence of *C. auris* more studies have evaluated compounds with the capacity to decolonize skin (6, 14). Additionally, it is important to note that the prolonged use of chlorhexidine can generate an abrasive effect on the skin, which can enable the development invasive infections (15).

As an alternative, compounds with antimicrobial activity, which are mainly used in personal hygiene products, such as foot powder and deodorants, can be used continuously without an abrasive effect on the skin, and are recommended for daily and prolonged use. Within this group of active compounds, triclosan (TR), boric acid (BA) and zinc oxide (ZO) are some of the most commonly used (16). These compounds have different antimicrobial mechanisms. Studies evaluating triclosan against bacteria showed that antibacterial activity occurs by the blocking of lipid synthesis, through the inhibition of an enoyl-acyl carrier protein dependent on NADH or FabI (17) but not much is known about its mechanism of action against fungi. Recently it was found that exposure of *Schizosaccharomyces pombe* to triclosan produces more oxidative stress and less protection by efflux pumps (18). The mechanism of action of boric acid is inhibition of oxidative metabolism causing a decrease in production of cellular ergosterol and interfering in hyphae transformation by inhibiting apical growth through cytoskeletal disruption (19). Zinc oxide acts by generating reactive oxygen species (ROS), producing damage to the cell and the cellular components through oxidative stress (20, 21). The individual activity of BA, TR and ZO has been described, but the increasing or potentiating effect of the antimicrobial activity by combination of these compounds is unknown.

The aim of this study was to evaluate the antifungal activity of BA, TR, and ZO individually and in mixtures against clinically relevant *Candida* species.

MATERIALS AND METHODS

Compounds.

We evaluated boric acid (MERCK, reference: 1.00165.1000), triclosan (CHEMICALS LM, reference: 3518VN) and zinc oxide (Protokimica, reference: 10225). Compounds were prepared in different concentrations, individually and combined (Supplementary Table 1). Fifty grams of each of the compounds were prepared by the geometric dilution method of mixing (22), using industrial talc as excipient (Protokimica, reference: GT-F-41) to obtain the mixtures. Maximum concentrations were chosen based on the maximum concentrations allowed by the European Commission Data Base for Information on Cosmetics and Ingredients (COSING) (23). Minimum concentrations were selected based on the concentration used in personal care products sold over the counter (OTC).

Controls:

We used as a negative control industrial talc (Protokimica, reference: GT-F-41), and as a positive controls antifungal discs with 0.5 μg/μl of amphotericin B (Bristol-Myers Squibb, reference: 16049TB24) and 1.25 μg/μl of Fluconazole (Diflucan, Pfizer, reference: A429304).

Candida species isolates:

isolates used to evaluate antifungal activity included: *C. albicans* (ATCC 90028), *C. tropicalis* (ATCC 750), *C. parapsilosis* (ATCC 22019), *C. glabrata* (ATCC 64677), currently name as *Nakaseomyces glabrata*, and *C. krusei* (ATCC 6258), currently name as *Pichia kudriavzevi*. Additionally, two *Candida auris* isolates from Colombian patients

were provided by the Microbiology Group of the National Institute of Health (INS), Bogotá, Colombia (H0059-I-13-74 and H0059-I-87; South American, IV clade). Antifungal susceptibility of *C. auris* strains was performed by INS using reference Clinical and Laboratory Standards Institute (CLSI) M27 methods.

Antifungal activity evaluation:

Susceptibility testing was performed using a modification of the antifungal disc diffusion susceptibility test described in the CLSI standard M44-A2 (24). This modification replaced antifungal discs by injection of the active ingredients into agar wells (25). A suspension of the microorganisms was prepared from a pure subculture on Sabouraud agar (24–48 hours of growth at 37 °C). Colonies were suspended in sterile 0.85% sodium chloride solution (Corpaul, reference: 75808217), and adjusted to a 0.5 turbidity of McFarland, according to the CLSI protocol (24).

Evaluation of antifungal activity was performed using Petri dishes with Mueller Hinton agar, supplemented with glucose. The inoculum was first spread on the agar. A 9 mm diameter punch was used to make a hole in the agar. Subsequently the wells were filled with 200 mg of the active compounds or negative control. Antifungal discs on the surface of the agar were also used as controls.

Petri dishes were incubated at 37 °C in aerobic conditions. Plates were read by measuring of the zone of inhibition diameter. Reading was performed at 24 hours for *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C krusei*, and at 48 hours for *C. glabrata* and *C. auris*.

Experimental design and statistical analysis:

Antifungal activity assays were performed at three different times, each time in duplicate. Assay reproducibility was evaluated by calculation of the coefficient of variation. We calculated mean zone of inhibition diameter ($\bar{x}$) and standard deviations (SD) using ANOVA, following by a post hoc analysis using the Bonferroni multiple comparisons method. Statistical analysis was performed using GraphPad Prism version 4.

Ethics statement:

No ethical approval was required as the research in this article related to micro-organisms.

RESULTS

Negative controls had no antifungal activity against any *Candida* isolates tested with a 9 mm zone of inhibition diameters (Figure 1, Table 1).

Fluconazole showed antifungal activity against most of the *Candida* isolates tested, with a mean zone of inhibition diameter from 15.2 mm to 30.7 mm, depending on the species. All isolates had prominent zones of inhibition when tested against amphotericin B, with a mean halo greater that 17.2 mm. The exception was *C. auris* H0059-I-87 which appeared resistant to amphotericin B, with 9.7 ± 0.5 mm of inhibition (Table 1, Figure 1).

All experiments were high reproducible (intra- and inter-assay coefficient of variation was <20%). Due to the consistent antifungal activity of amphotericin B against nearly all *Candida* species tested during this study, we used this control as a parameter to evaluate the antifungal capacity of the evaluated compounds (26). Compounds with a zone of inhibition diameter higher than the average of the amphotericin B control, calculated for each species, were considered to have high antifungal activity, and compounds with zone of inhibition diameters below the average of the amphotericin B control for each species were considered to have low antifungal activity.

Figure 2 presents examples of the well diffusion method. Zone diameters for the tested compounds and combinations ranged from 9 mm to 37.7 mm (Table 1 and Figure 1). The antifungal effect varied by active ingredient -individual or in mixtures-, the concentration of the compounds, and the *Candida* species tested. Data from individual compounds showed that BA and TR produced inhibition zones for all concentrations and all species of *Candida* analyzed. Nevertheless, BA at 1.9% did not produced an inhibition zone for *C. glabrata* and *C. auris* H0059-I-13-74. Zinc oxide produced no inhibition zones for any *Candida* isolates at either 8.6% or 25% (Figure 1, Table 1).

Analyzing the individual antifungal activity of BA and TR in comparison with amphotericin B, we found that BA [5.0%] produced the largest zones of inhibition for most of the *Candida* species evaluated. The exception was with *C. auris* H0059-I-13-74 where BA [5.0%] produced a slightly smaller inhibition zone than the amphotericin B control with zone of inhibition diameters of 18 ± 1.7 mm vs 19.8 ± 1.2 mm, respectively. Boric Acid [1.9%]; TR [0.2%]; and TR [0.3%] inhibition zones were smaller than amphotericin B for almost all strains except for *C. krusei* and *C. auris* H0059-I-87 (Figure 1 and Table 1).

Once the active ingredients were evaluated in combination, some mixtures presented a statistically significant enhanced antifungal effect compared with individual active compounds. It is important to note that no enhancement was found in *C. auris* H0059-I-87 nor in *C. krusei* 6258, where the BA [5.0%] produced the largest zones, 29.3±1 and 36.5±1.6 mm, respectively. In both cases, and no mixture enhanced the effect in any *Candida* species.

When mixtures were compared with the amphotericin B positive control, high antifungal activity was observed in all *Candida* species using two compound mixtures, BA [5%] -TR [0.2%] and BA [5%] -TR [0.3%] with mean inhibition zones from 20.2 mm to 37.7 mm. Adding ZO to these two mixtures, to produce a triple combination, neither enhanced nor inhibited the antifungal activity.

The best results for *C. auris* isolates were produced using the combination BA [5%] -TR [0.2%] for *C. auris* H0059-I-13-74 with 28.3 ± 2.1 mm, and BA [5%] alone for *C. auris* H0059-I-87 with 29.3 ± 1.0 mm. The mixtures of BA [5.0%] - TR [0.3%] and BA [5.0%] - TR [0.2%] produced large inhibition zones for both clinical isolates of *C. auris*. Additionally, *C. auris* H0059-I-13-74 was resistant to fluconazole by broth microdilution and all the mixtures containing BA [5.0 %] produced larger zones than fluconazole but comparable zones of inhibition to those produced for amphotericin B. In *C glabrata*, BA

[5.0%] -TR [0.3%] and BA [5.0%] -TR [0.2%] also produced larger zones of inhibition than amphotericin B (Figure 1, Table 1).

When the combinations were compared with active ingredients alone, some mixtures presented enhanced antifungal effect. An example of this was BA [5.0%] – TR [0.3%] – ZO [25%] in *C. tropicalis* where the zone of inhibition of 28.2 ± 3.5 mm was larger than those of BA [0.5%] or TR [0.3%] alone that were 21±3.1 mm and 15.1±1.2 mm respectively. This potentiated effect was notable with BA [5.0%] - TR [0.2%] and BA [5.0 %] - TR [0.3%], where both combinations had enhanced activity against *C. glabrata* and *C. auris* H0059-I-13-74 compared with BA [5.0%], TR [0.2%] or TR [0.3%] alone (Table 1). It is important to note that no enhancement was found for combinations against *C. auris* H0059-I-87 nor in *C. krusei* 6258, and there was no combination that improved the antimicrobial activity in all the *Candida* species (Table 1).

Reduction of the antifungal effect was observed when BA and ZO were tested in combination. The major difference was observed in *C. auris* H0059-I-87 when ZO [8.6%] was in combination with BA [5%], 15.8± 1 mm inhibition zone, in contrast with 29.3± 3.5 mm inhibition only using BA [5%]. Smaller differences were also observed in *C. albicans*, *C. krusei*, *C. tropicalis* and *C. glabrata* when BA and ZO were tested in combination.

DISCUSSION

Previous studies have shown the antifungal activity of BA (26, 19), TR (28), and ZO (29, 30). Evaluating the individual antifungal activity alone and in combination with antifungal medications has shown both synergy and antagonism (31,32), but no studies have evaluated the effects of the compounds themselves in combination. The present study analyzed the antifungal activity of these compounds, alone and combined, against clinically relevant *Candida* species.

In this study, most *Candida* strains were susceptible to amphotericin B, and given this low resistance in general, this parameter was used to assign high or low antifungal activity of the evaluated compounds or combinations. Antifungal activity of BA and TR was observed at all concentrations and in all species of *Candida* analyzed in this work, except with the BA [1.9%] in *C glabrata* and *C. auris* H0059-I-13-74. Compared with amphotericin B inhibition zone diameters, high antifungal activity was obtained with BA [5%] in five of the six species and low antifungal activity was found in BA [1.9%], TR [0.2%] and TR [0.3%] in almost all of *Candida* isolates.

The antifungal activity of BA in the treatment of recurrent vaginal candidiasis has been recognized for a long time. It has been used effectively at concentrations of 5%, which agrees with the results obtained in our study (33, 34, 35). There are reports that evaluate the *in vitro* antimicrobial activity of BA against clinical isolates of *Candida* associated with vaginal infections, but none using an antifungal diffusion susceptibility assay. Using broth microdilution, Romano et al. obtained MIC values for BA ranging from 0.094% to 0.187% against various *Candida* isolates (27); De Seta et al, using an agar dilution method, found that all isolates of *Candida* they tested failed to form visible colonies on BA at 50,000 mg/L,

a concentration comparable to ours. In the same study an MIC value of 3,125 mg/L by broth microdilution was obtained for 96 of the tested strains showing the difference between the testing method used (19).

There are a number of reports that evaluated the antimicrobial and antifungal activity of TR, but not many evaluated *Candida* species and those that did used broth microdilution. In one study, the MIC values reported for different *Candida* species ranged from 0.8 to 25 mg/L (28). There have been found any studies which evaluated TR activity using a well or disc diffusion assay.

No antifungal activity was observed for any of the ZO concentrations used against the isolates evaluated in this study. *In vitro* antimicrobial activity of ZO has been described using the agar well diffusion of method, for instance, in oral pathogenic bacteria such as *S. aureus*, *E. coli*, *P. aeruginosa* and *E. faecalis* (37). Recently, new Zinc (Zn) formulations in nanoparticules with significant antibacterial and antifungal activity in a broad spectrum of microorganisms, such as *S. aureus* and some *Aspergillus* species, have been described (38, 39). The *In vitro* antifungal activity of nano-ZO was evaluated by broth microdilution against a standard strain of *C. albicans*. MIC and MFC of nano-ZO was recorded as 400 μg/mL. Nevertheless, the authors concluded that, ZO nanoparticles have anti *C. albicans* properties and use for treatment of infections caused by this fungus should be investigated (40).

The analysis of the dual and triple mixtures demonstrated an increased antifungal effect compared with the results obtained for the individual compounds. The analysis of all combinations and strains of *Candida* evaluated in this work showed that the four formulations with high inhibitory capacity, superior even to amphotericin B, all contained BA [5%] and had activity against all strains. These mixtures were BA [5.0%] - TR [0.2%], BA [5.0 %] - TR [0.3%], BA [5.0%] - TR [0.2%] - ZO [8.6%] and BA [5.0%] - TR [0.2%] - ZO [25%].

Although there are reports of the activity of BA [5.0%] as well as TR [0.2%] and TR [0.3%] against different species of *Candida* (19, 28), there are no reports of their combined activity. Several combinations of these compounds produced enhancement of the antifungal activity against *Candida* species when compared with the active ingredient alone, showing increased antifungal effect between BA and TR and between TR and ZO. It is important to note that no combination showed antagonism. The overall activity of ZO was weak to absent but it is worth noting that in *C. albicans*, *C. tropicalis* and *C. auris* H0059-I-13-74 some of the combinations containing OZ and TR showed increased antifungal effect (Table 1). Reduction of BA antimicrobial activity was observed in some mixtures with ZO, Shete et al, described the conversion of ZO to zinc borate in presence of BA (41). This chemical conversion probably occurred in our mixtures, and it could be the cause of the reduction in antifungal activity.

The well diffusion method used in this this study was based on the CLSI disc diffusion method (M44-A2), it is important to highlight that this is a simple, cheap, and robust methodology that is widely used, especially in resource-limited settings. This method also

allows the use of many different compounds, especially those that are insoluble in the broth media used for susceptibility testing, like TR, BA and ZO (42). One of the disadvantages of the well diffusion technique is that it does not allow to determination of synergy and the FIC coefficient (43). In this study, an increased antifungal effect was observed with some triclosan and boric acid mixtures, but the presence of a synergic effect could not be determined by agar diffusion. Future studies aimed at obtaining the FIC coefficient, based the minimum inhibitory concentration are need. Most of isolates used in this study come from the ATCC collection, these isolates may not represent the susceptibility of clinical isolates of *Candida* species. Further studies are necessary for evaluating the antimicrobial activity of TR, BA and ZO in clinical isolates (6).

The results of this study provide a framework for additional areas of research towards patient *Candida* decolonization in the hospital setting. As we said before, future investigation is necessary to evaluate the antimicrobial activity of triclosan, boric acid and zinc oxide, and combinations *in vivo*. This study complements our knowledge of the antimicrobial activity of these compounds and sets a standard for defining the minimum ranges of activity of these active ingredients against various *Candida* species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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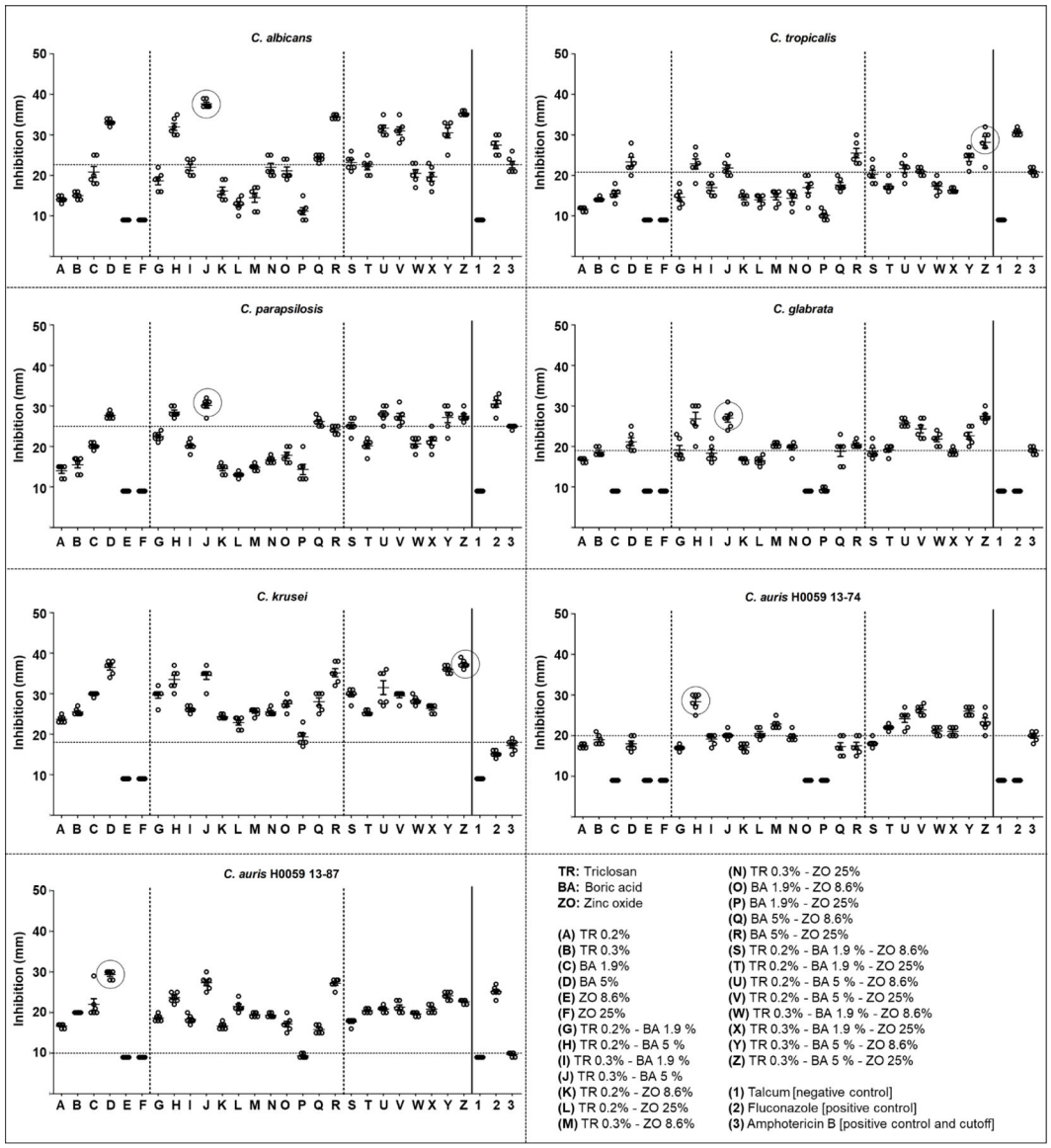

Figure 1.
Antifungal activity of compounds against *Candida* species.

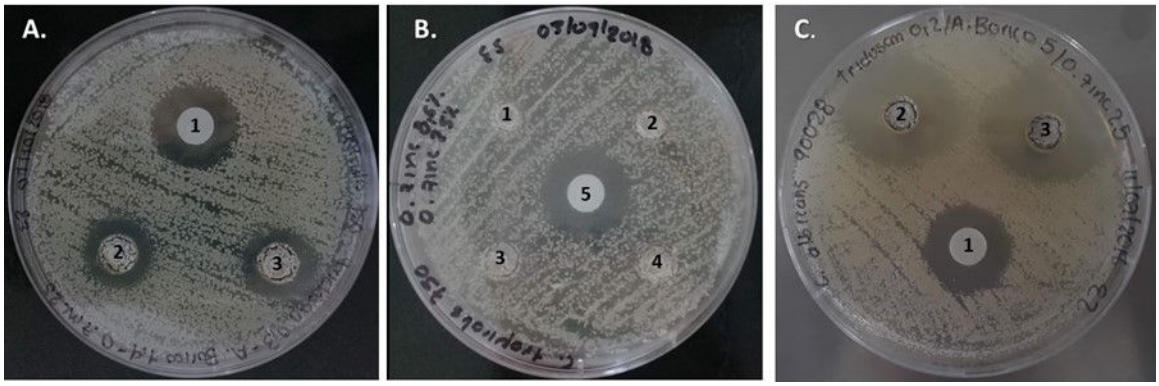

Figure 2.

Some examples of well diffusion testing. (A) Candidatropicalis: 1) amphotericin B, 2 and 3) BA [1.9%]—[ZO 25%]. (B) C. tropicalis: 1 and 2) [ZO 8,6%], 3 and 4) [Z0 25%], 5) amphotericin B. (C) C. albicans: 1) amphotericin B, 2 and 3) BA [5%]—[TR 0.2%]—[ZO 25%]

Table. 1:

Antifungal activity of TR, BA, and ZO and combinations against different species of *Candida* as defined by the diameter of the zone of inhibition, in mm.

Active Ingredients	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*	*C. krusei*	*C. auris H0059-I-13-74*	*C. auris H0059-I-87*	
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Amphotericin B (cut-off)	**22.7 ± 2.1**	**20.8 ± 0.3**	**24.8 ± 0.4**	**19 ± 0.9**	**17.2 ± 1.5**	**19.8 ± 1.2**	**9.7 ± 0.5**	
Fluconazole (positive control)	27.5 ± 2.3	30.7 ± 0.8	30.5 ± 2.1	9 ± 0	15.2 ± 0.8	9 ± 0	*25.2 ± 1.3	
Talc (negative control)	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	
BA 1.9%	20.3 ± 3.8	*13.6 ± 2.3	20.2 ± 0.8	9 ± 0	29.8 ± 0.4	9 ± 0	22 ± 3.5	
BA 5.0%	33.2 ± 0.8	21 ± 3.1	27.7 ± 0.8	21.2 ± 2.4	36.5 ± 1.6	18 ± 1.7	29.3 ± 1	
ZO 8.6%	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	
ZO 25%	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	9 ± 0	
TR 0.2%	14.1 ± 0.8	12.9 ± 1.5	14 ± 1.5	16.7 ± 0.5	23.7 ± 0.8	17.5 ± 0.5	16.7 ± 0.5	
TR 0.3%	15 ± 0.9	15.1 ± 1.2	15.5 ± 2	18.7 ± 1	25.5 ± 0.8	19 ± 1.3	20 ± 0	
BA 1.9% - ZO 8.6%	21.2 ± 2.2	16.7 ± 3.8	17.8 ± 1.8	9 ± 0	27.5 ± 1.6	9 ± 0	17.2 ± 1.7	
BA 1.9% - ZO 25%	11.2 ± 2.2	*10.2 ± 1.2	14.3 ± 3.1	9.3 ± 0	19.3 ± 2.2	9 ± 0	9.3 ± 0.5	
BA 5.0% - ZO 8.6%	24.3 ± 0.8	17.7 ± 1.5	26.2 ± 1.2	18.8 ± 3.2	28 ± 2.3	17.3 ± 2.3	15.8 ± 1	
BA 5.0% - ZO 25%	34.5 ± 0.5	25.5 ± 2.9	24.2 ± 1.2	20.5 ± 0.8	35.2 ± 2.5	18 ± 1.7	27.2 ± 1.2	
BA 1.9 % - TR 0.2%	18.7 ± 2.3	14.7 ± 2.2	22.5 ± 1	19.2 ± 2.6	29.7 ± 2	17 ± 0.6	18.7 ± 0.8	
BA 1.9 % - TR 0.3%	22 ± 1.9	18 ± 1.6	20.2 ± 1.3	18.3 ± 2.3	26.2 ± 0.8	19.2 ± 1.3	18.3 ± 1	
BA 5.0 % - TR 0.2%	32 ± 2.1	22.8 ± 3.1	28.5 ± 1.2	26.8 ± 4	33.5 ± 2.6	28.3 ± 2.1	23.7 ± 1.2	¥
BA 5.0 % - TR 0.3%	37.7 ± 1	21.8 ± 1.9	30.2 ± 1.7	27 ± 2.4	34.5 ± 2.3	20.2 ± 1	27.3 ± 1.8	¥
TR 0.2% - ZO 8.6%	16.2 ± 2.3	14.5 ± 1.2	14.5 ± 1.2	16.7 ± 0.5	24.3 ± 0.5	17.2 ± 1	16.7 ± 0.8	
TR 0.2% - ZO 25%	13 ± 1.7	14 ± 1.3	13 ± 0.6	16.7 ± 1	22.8 ± 1.5	20.5 ± 1.2	21.5 ± 1.4	
TR 0.3% - ZO 8.6%	15 ± 2.8	14.7 ± 1.8	14.8 ± 0.8	20.5 ± 0.5	25.5 ± 0.8	22.8 ± 1.2	19.5 ± 0.5	
TR 0.3% - ZO 25%	22 ± 2.4	14.3 ± 2	16.7 ± 0.8	19.7 ± 1.4	25.5 ± 0.8	19.8 ± 1.2	19.3 ± 0.5	
BA 1.9% - TR 0.2% - ZO 8.6%	23.2 ± 1.8	20.3 ± 2.3	25.2 ± 1.8	18.8 ± 1.8	29.8 ± 0.5	18.2 ± 1	17.7 ± 0.8	
BA 1.9% - T 0.2% - ZO 25%	22.2 ± 1.9	17.3 ± 1.4	20.2 ± 1.7	19.2 ± 1.2	25.3 ± 0.5	22 ± 0.6	20.5 ± 0.5	
BA 5.0% - T 0.2% - ZO 8.6%	31.7 ± 1.9	21.7 ± 2.4	28 ± 1.9	25.8 ± 1	31.5 ± 4.2	24.2 ± 2.2	20.8 ± 0.8	¥

Active Ingredients	*C. albicans*	*C. tropicalis*	*C. parapsilosis*	*C. glabrata*	*C. krusei*	*C. auris H0059-I-13-74*	*C. auris H0059-I-87*	
	$\overline{x} \pm SD$	$\overline{x} \pm SD$	$\overline{x} \pm SD$	$\overline{x} \pm SD$	$\overline{x} \pm SD$	$\overline{x} \pm SD$	$\overline{x} \pm SD$	
BA 5.0% - T 0.2% - ZO 25%	31 ± 2.4	21 ± 0.9	27.3 ± 2.1	24.3 ± 2.3	29.5 ± 1.2	26.3 ± 1.2	21.3 ± 1.4	¥
BA 1.9% - T 0.3% - ZO 8.6%	20.5 ± 2.3	17.3 ± 1.8	20.7 ± 1.6	21.8 ± 1.6	28.3 ± 1	21.2 ± 1	19.7 ± 0.5	
BA 1.9% - T 0.3% - ZO 25%	19.7 ± 2.6	24.3 ± 2.1	21.3 ± 2.3	18.7 ± 0.8	26.3 ± 1	21 ± 1.1	20.8 ± 1	
BA 5.0% - T 0.3% - ZO 8.6%	30.5 ± 3	16.3 ± 0.5	27.2 ± 2.1	22.7 ± 2.1	36 ± 0.9	26 ± 1.1	24.2 ± 1	
BA 5.0% - T 0.3% - ZO 25%	35.3 ± 0.5	28.2 ± 3.5	27.5 ± 1.4	17.5 ± 1.4	37.3 ± 1	23.3 ± 2.4	22.7 ± 0.5	

(TR) Triclosan; (BA) Boric Acid; (ZO): Zinc Oxide;

*(*)*Trailing growth. Green cells: zone of inhibition greater than or equal to Amphotericin B (high antifungal activity). Red cells: zone of inhibition less than Amphotericin B (low antifungal activity);

*(¥)*High antifungal activity in all isolates.