



## Synergistic In Vitro Interaction of Isavuconazole and Isoquercitrin against *Candida glabrata*

Petra Schwarz, Ilya Nikolskiy, Eric Dannaoui, Frank Sommer, Gert Bange,  
Patrick Schwarz

### ► To cite this version:

Petra Schwarz, Ilya Nikolskiy, Eric Dannaoui, Frank Sommer, Gert Bange, et al.. Synergistic In Vitro Interaction of Isavuconazole and Isoquercitrin against *Candida glabrata*. *Journal of Fungi*, 2022, 8 (5), pp.525. 10.3390/jof8050525 . hal-03944231

**HAL Id: hal-03944231**

**<https://hal.science/hal-03944231>**

Submitted on 7 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Article

# Synergistic In Vitro Interaction of Isavuconazole and Isoquercitrin against *Candida glabrata*

Petra V. Schwarz <sup>1</sup>, Ilya Nikolskiy <sup>1,2</sup>, Eric Dannaoui <sup>3,4,5</sup> , Frank Sommer <sup>6</sup>, Gert Bange <sup>2,7</sup> and Patrick Schwarz <sup>1,8,\*</sup> 

- <sup>1</sup> Center for Invasive Mycoses and Antifungals, Faculty of Medicine, Philipps University Marburg, D-35043 Marburg, Germany; muelle60@students.uni-marburg.de (P.V.S.); ilian@students.uni-marburg.de (I.N.)
  - <sup>2</sup> Center for Synthetic Microbiology (SYNMIKRO), Department of Chemistry, Philipps University Marburg, D-35043 Marburg, Germany; gert.bange@synmikro.uni-marburg.de
  - <sup>3</sup> Unité de Parasitologie-Mycologie, Hôpital Européen Georges-Pompidou, F-75015 Paris, France; eric.dannaoui@aphp.fr
  - <sup>4</sup> Dynamyc Research Group (EA 7380), Faculté de Médecine de Créteil, Université Paris-Est-Créteil-Val-de-Marne, F-94010 Créteil, France
  - <sup>5</sup> Faculté de Médecine, Université de Paris, F-75006 Paris, France
  - <sup>6</sup> Department of Microbiology, University Hospital Marburg, D-35032 Marburg, Germany; frank.sommer@med.uni-marburg.de
  - <sup>7</sup> “Molecular Physiology of Microbes” Group, Max Planck Institute for Terrestrial Microbiology, D-35043 Marburg, Germany
  - <sup>8</sup> Department of Internal Medicine, Respiratory and Critical Care Medicine, University Hospital Marburg, D-35033 Marburg, Germany
- \* Correspondence: patrick.schwarz@med.uni-marburg.de; Tel.: +49-6421-5862464



**Citation:** Schwarz, P.V.; Nikolskiy, I.; Dannaoui, E.; Sommer, F.; Bange, G.; Schwarz, P. Synergistic In Vitro Interaction of Isavuconazole and Isoquercitrin against *Candida glabrata*. *J. Fungi* **2022**, *8*, 525. <https://doi.org/10.3390/jof8050525>

Academic Editor: David S. Perlin

Received: 3 May 2022

Accepted: 19 May 2022

Published: 20 May 2022

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Abstract:** In vitro interactions of broad-spectrum azole isavuconazole with flavonoid isoquercitrin were evaluated by a broth microdilution checkerboard technique based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) reference methodology for antifungal susceptibility testing against 60 *Candida* strains belonging to the species *Candida albicans* ( $n = 10$ ), *Candida glabrata* ( $n = 30$ ), *Candida kefyr* ( $n = 6$ ), *Candida krusei* ( $n = 5$ ), *Candida parapsilosis* ( $n = 4$ ), and *Candida tropicalis* ( $n = 5$ ). The results were analyzed with the fractional inhibitory concentration index and by response surface analysis based on the Bliss model. Synergy was found for all *C. glabrata* strains, when the results were interpreted by the fractional inhibitory concentration index, and for 60% of the strains when response surface analysis was used. Interaction for all other species was indifferent for all strains tested, whatever interpretation model used. Importantly, antagonistic interaction was never observed.

**Keywords:** antifungal combination; *Candida*; isoquercitrin; EUCAST; in vitro; isavuconazole

## 1. Introduction

Invasive candidiasis is a life-threatening disease associated with a high mortality rate of approximately 40% [1]. Ninety-five percent of invasive *Candida* infections are caused by only five different *Candida* species: *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* [2]. The majority of infections worldwide are still caused by *C. albicans* [3], but non-*albicans* *Candida* species can represent up to 60% in the United States, or in parts of Europe [4,5]. The second most common species found in the United States and Central Europe is *C. glabrata* [6]. First-line therapy for invasive candidiasis are echinocandins. Azoles for primary therapy are not recommended anymore, but can be used as step-down therapy in case the infecting organism is susceptible [7]. *C. parapsilosis* has an intrinsic, decreased susceptibility to caspofungin [8]; *C. krusei* even has an intrinsic fluconazole resistance [9]. Echinocandin resistance in *C. glabrata* and other

*Candida* species can be acquired by mutations in the glucan synthase encoding genes, *FKS1* and *FKS2* [10,11]. Echinocandin resistance is emerging, particularly in *C. glabrata*, with variable rates of 10–13% in the United States [12,13], 2.5% in Canada [14], 2.7% in Denmark [15], and 1.1–10% in Ibero-America [16], but can be as high as 48%, as reported in Germany. Of the 176 *C. glabrata* isolates submitted to the National Reference Center for Invasive Fungal Infections from 2015 to 2019, 84 were anidulafungin resistant, based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility testing methodology. Seventy-one of these strains harbored *FKS* gene mutations. Over one-third of the echinocandin-resistant strains additionally displayed concomitant fluconazole resistance [17]. The high mortality rate, the lack of efficacy in monotherapy for some difficult-to-treat infections [18], and the emergence of antifungal resistance, especially among *C. glabrata*, underscore the need for alternative approaches. A promising strategy is the use of antifungals in combination. The main advantages of antifungal combinations are the possibility to increase efficacy [19], to reduce toxicity, to improve the pharmacokinetics of the molecules [20], and to overcome resistance [21]. However, using the common antifungals, no promising combination for the treatment of invasive candidiasis has been found [22]. As shown for *Candida* [23–25], *Aspergillus* [26,27], and Mucorales [28], combinations of non-antifungals with antifungals can also exhibit synergy. Isavuconazole is a broad-spectrum azole drug with potent in vitro activity against *Candida* blood-stream isolates, particularly against *C. albicans* and *C. glabrata* [29]. However, despite proven efficacy for the treatment of aspergillosis [30], isavuconazole could not prove non-inferiority compared with caspofungin as a primary treatment of invasive candidiasis [31]. Moreover, as the mechanisms of resistance for fluconazole and isavuconazole are similar [32], it has to be anticipated that isavuconazole resistance could also be a problem for the treatment of invasive candidiasis, but due to its limited use for treating invasive candidiasis, the rate of resistance is unknown. Isoquercitrin is a flavonoid which has shown fungicidal activity against *C. albicans* [33], which makes it an interesting partner to test in vitro combinations. Therefore, the purpose of this study was to evaluate if isoquercitrin can enhance the in vitro activity of isavuconazole against common *Candida* species, including *C. glabrata*, assessed by a checkerboard technique based on the EUCAST methodology for antifungal susceptibility testing.

## 2. Materials and Methods

### 2.1. Strains

This study included a total of 60 clinical *Candida* strains belonging to six different species. Included were 10 *C. albicans*, 30 *C. glabrata*, 6 *Candida kefyr*, 5 *C. krusei*, 4 *C. parapsilosis*, and 5 *C. tropicalis* strains. Three strains belonged to international collections; two of these strains belonged to the American Type Culture Collection and the other to the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; <https://www.dsmz.de> (accessed on 1 May 2022)). The other 57 strains were obtained from the Department of Microbiology of the University Hospital Marburg. The strains not belonging to international collections were all identified to the species level by the sequencing of the complete Internal Transcribed Spacer (ITS)1–5.8S-ITS2 region, as described elsewhere [34]. All sequences were deposited at GenBank under the accession numbers OL351325 to OL351353, OL351355, and OL351356 [24], under OM859334 to OM859338 [23], and under ON391951 to ON391970. Each batch of microplates was tested by the quality control strains *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019, as recommended by EUCAST.

### 2.2. Drugs

Isavuconazole powder was obtained from Pfizer (Berlin, Germany). The stock solution of isavuconazole was prepared in dimethyl sulfoxide (DMSO) at a concentration of 3200 µg/mL. Isoquercitrin was purchased as powder from Merck (Darmstadt, Germany), and was solved in DMSO at a concentration of 12,800 µg/mL. Both stock solutions were kept at −25 °C until use.

### 2.3. Medium Preparation

All experiments were carried out in Roswell Park Memorial Institute 1640 (RPMI) medium (with L-glutamine and pH indicator, but without bicarbonate) (Merck). The medium was prepared in double strength and contained 2% (*w/v*) of D-Glucose and the buffer 3-(*N*-morpholino) propanesulfonic acid (Merck) at a final concentration of 0.165 mol/L. After adjustment of the pH to 7.0 with 2 molar NaOH, the medium was sterilized by vacuum filtration through a 0.22 µm pore-sized filter (Merck).

### 2.4. Microplate Preparation

All experiments of this study were carried out in Nunclon™ delta surface 96-well microtiter plates for adherent cells (Thermo Fisher Scientific, Darmstadt, Germany). A two-dimensional checkerboard was used to evaluate the combination of isavuconazole with isoquercitrin [35]. Therefore, an antifungal susceptibility testing protocol, modified for broth microdilution combination studies, based on the EUCAST guidelines was used [36]. Each drug was two-fold serial diluted in the double strength RPMI medium. As the tested *Candida* species exhibited different susceptibility profiles to isavuconazole, two kinds of microplates were prepared. To test *C. albicans*, *C. kefyr*, *C. parapsilosis*, and *C. tropicalis* strains, the final concentrations for isavuconazole on the microplates ranged from 0.00006 to 0.03 µg/mL, while 0.001 to 0.5 µg/mL was used to test *C. glabrata* and *C. krusei* strains. Isoquercitrin ranged from 1 to 64 µg/mL on all prepared microplates. The last column of the microplates contained only the RPMI medium without drugs and was used as the growth control (positive control). Before the addition of the inoculum, each well of the microplates contained 1% (*v/v*) DMSO.

### 2.5. Inoculum Preparation and Inoculation of Microplates

*Candida* strains were cultured from stocks frozen in 40% (*v/v*) glycerol at 35 °C and 95% humidity on Sabouraud dextrose agar slants, supplemented with chloramphenicol and gentamicin (Bio-Rad Laboratories, Feldkirchen, Germany). Twenty-four hours before the experiments, the strains were subcultured again under the same conditions. After subculture, yeast cells were transferred to sterile tubes, containing pure sterile water, by using inoculation loops. The final inoculum was adjusted to  $2 \times 10^5$  colony-forming units (CFU)/mL after counting the cells in a hemocytometer. Directly after adjustment, Eppendorf Xplorer plus (Eppendorf, Hamburg, Germany) electric multichannel pipettes were used to distribute 100 µL of the inoculum into each well of the microplates. To ensure the inoculum size, the final inoculum was further diluted to 1:10 and 50 µL were spread once on the Sabouraud dextrose agar plates with a sterile Drigalski spatula. The CFU were counted 24 hours after incubation. The microplates were incubated for 24 h at 35 °C and 95% humidity and the optical densities were read spectrophotometrically at a wavelength of 530 nm, using a MultiScan FC spectrometer (Thermo Fisher Scientific). Before spectrophotometric reading, all microplates were shaken for 2 min at 1100 rpm with the PMS-1000 Microplate Shaker (Grant Instruments, Shepreth, UK) to dissolve the yeast colonies in the wells. A blank plate, in which each well was inoculated with 100 µL of sterile distilled water, was incubated under the same conditions as mentioned above (negative control). The blank plate was read spectrophotometrically at 530 nm and the optical density values were subtracted from the values of the microplates inoculated with the *Candida* strains. The resulting optical density values were transformed into the percentage of growth compared with the growth control, and used for the calculation of the results. Combination experiments were run twice.

### 2.6. Interpretation of the Results by Fractional Inhibition Concentration Index

Fifty percent of inhibition was chosen as an endpoint for the determination of the MICs alone of both drugs and in combination. High off-scale MICs were converted to the next log<sub>2</sub> dilution. The fractional inhibition concentration index (FICI) was calculated the following way:  $FICI = (MIC_{\text{isavuconazole in combination}} / MIC_{\text{isavuconazole alone}}) +$

( $MIC_{\text{isoquercitrin in combination}} / MIC_{\text{isoquercitrin alone}}$ ). For FICI values  $\leq 0.5$ , synergy was concluded; indifference was concluded if the values were  $>0.5$  to 4, and antagonism was concluded if the values were  $>4$  [37].

### 2.7. Interpretation of the Results by Response Surface Analysis

Response surface analysis is an MIC and inhibition endpoint independent method to analyze checkerboard data. It allows the determination and visualization of the drug interactions on the complete surface of the microplate. FICI analyzes are limited to the evaluation of the interaction only for the MICs in combination. To analyze the checkerboard data, special programs are used; in the case of this study, all calculations were done by the Combenefit software [38]. First, the program calculates dose–response curves for the drugs alone, which are based on the growth rates in the wells of the single molecules. From the dose–response curves of the two drugs alone, according to the chosen theoretical model (this study uses the Bliss independence model), a dose–response surface with an indifferent interaction is calculated. The Bliss independence model is based on the hypothesis that drugs act independently from each other. Second, to evaluate the interaction of the combination, the program compares the experimentally obtained dose–response surface with the calculated indifferent dose–response surface. Synergy is observed if the experimentally obtained dose–response surface lies below the calculated dose–response surface. This corresponds to less growth on the microplate, compared with an indifferent interaction. If more growth is obtained on the plate compared with an indifferent interaction, which means that the experimentally obtained dose–response surface lies above the calculated indifferent dose–response surface, antagonism is concluded. Third, the program calculates the SUM-SYN-ANT metric to quantitatively assess the interaction of the drugs. The SUM-SYN-ANT metric is the sum of all the dose–response surface values lying below the calculated indifferent dose–response surface (SYN-SUM), minus the sum of all values lying above (ANT-SUM). Broth microdilution techniques have an intrinsic variability which makes the definition of a threshold necessary. This threshold defines the values for which the interaction of the combination is assumed to be indifferent. Threshold determination can be done experimentally by combining the active molecules with themselves. In the case of this study, the active molecule isavuconazole was tested with itself on the two-dimensional checkerboard with two-fold serial dilutions using the preparation, incubation, and analyzation protocol described above. The quality control strain *C. krusei* ATCC 6258 was used for threshold determination. The strain was tested in triplicate with the highest isavuconazole of 0.12 µg/mL in both axes. Based on these results, synergy was assumed when the SUM-SYN-ANT was  $\geq 56.0\%$ , and antagonism when  $\leq -56.0\%$ . Between  $-56.0$  and  $56.0\%$ , indifference was concluded. For the determination of the SUM-SYN-ANT metric of the tested strains, the data of both runs were combined.

## 3. Results

In the first part of this study the combination of isavuconazole with isoquercitrin was screened against a panel of 35 *Candida* strains belonging to common *Candida* species using the EUCAST broth microdilution technique modified for combination studies. The data of these experiments were analyzed by FICI and response surface analysis, and the results are presented in Table 1. Based on these results, 25 additional *C. glabrata* strains were tested and analyzed the same way. The results of these analyses are presented in Table 2. A summary of all results is presented in Table 3. Figure 1 shows the synergy distributions for the combination of isavuconazole with isoquercitrin against all *C. glabrata* strains tested.

**Table 1.** Interaction of isavuconazole with isoquercitrin against common *Candida* species evaluated by checkerboard and interpretation by fractional inhibitory concentration index and response surface analysis.

| Species                | Collection Number | Checkerboard MICs (µg/mL) |      |            | FICI   | INTPN | Response Surface Analysis |       |
|------------------------|-------------------|---------------------------|------|------------|--------|-------|---------------------------|-------|
|                        |                   | ISA                       | ISOQ | ISA/ISOQ   |        |       | ΣSYN-ANT (ΣSYN; ΣANT)     | INTPN |
| <i>C. albicans</i>     | V2105126          | 0.002                     | 128  | 0.001/64   | 1      | IND   | 17.47 (19.03; −1.56)      | IND   |
| <i>C. albicans</i>     | N2101578          | 0.004                     | 128  | 0.004/1    | 1.0078 | IND   | −7.17 (8.40; −15.57)      | IND   |
| <i>C. albicans</i>     | V2105568          | 0.001                     | 64   | 0.00006/32 | 0.5625 | IND   | 27.02 (27.98; −0.96)      | IND   |
| <i>C. albicans</i>     | N2101577          | 0.002                     | 128  | 0.002/1    | 1.0078 | IND   | 34.95 (35.70; −0.75)      | IND   |
| <i>C. albicans</i>     | V2105825iso3      | 0.001                     | 32   | 0.0005/16  | 1      | IND   | 22.97 (23.54; −0.57)      | IND   |
| <i>C. albicans</i>     | ATCC 14053        | 0.002                     | 32   | 0.001/1    | 0.5313 | IND   | 16.83 (18.45; −1.62)      | IND   |
| <i>C. albicans</i>     | V2105529          | 0.001                     | 32   | 0.0005/8   | 0.75   | IND   | 28.19 (28.66; −0.47)      | IND   |
| <i>C. albicans</i>     | V2106139          | 0.002                     | 64   | 0.00006/32 | 0.5313 | IND   | 0.85 (10.26; −9.41)       | IND   |
| <i>C. albicans</i>     | V2106041          | 0.001                     | 128  | 0.001/1    | 1.0078 | IND   | 11.79 (12.27; −0.48)      | IND   |
| <i>C. albicans</i>     | V2106305          | 0.004                     | 128  | 0.002/1    | 0.5078 | IND   | 12.60 (13.34; −0.74)      | IND   |
| <i>C. glabrata</i>     | V2105272          | 0.5                       | 64   | 0.06/4     | 0.1875 | SYN   | 93.42 (93.60; −0.18)      | SYN   |
| <i>C. glabrata</i>     | V2105282          | 0.5                       | 64   | 0.016/8    | 0.1563 | SYN   | 84.19 (84.83; −0.64)      | SYN   |
| <i>C. glabrata</i>     | N2101711          | 0.125                     | 32   | 0.03/2     | 0.3125 | SYN   | 55.66 (56.95; −1.29)      | IND   |
| <i>C. glabrata</i>     | V2105636          | 0.125                     | 32   | 0.016/4    | 0.25   | SYN   | 70.11 (70.56; −0.45)      | SYN   |
| <i>C. glabrata</i>     | DSM 70614         | 0.125                     | 16   | 0.03/2     | 0.375  | SYN   | 46.95 (48.26; −1.31)      | IND   |
| <i>C. krusei</i>       | V2105825iso4      | 0.06                      | 128  | 0.06/1     | 1.0078 | IND   | 22.13 (24.96; −2.83)      | IND   |
| <i>C. krusei</i>       | V2105866          | 0.06                      | 128  | 0.06/1     | 1.0078 | IND   | −38.29 (1.94; −40.23)     | IND   |
| <i>C. krusei</i>       | V2106177          | 0.06                      | 128  | 0.03/1     | 0.5078 | IND   | 9.70 (19.83; −10.13)      | IND   |
| <i>C. krusei</i>       | V2105920          | 0.06                      | 128  | 0.03/1     | 0.5078 | IND   | −4.50 (10.81; −15.31)     | IND   |
| <i>C. krusei</i>       | ATCC 6258         | 0.06                      | 128  | 0.03/2     | 0.5156 | IND   | −30.31 (5.67; −35.98)     | IND   |
| <i>C. parapsilosis</i> | V2105056          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | −1.10 (11.54; −12.64)     | IND   |
| <i>C. parapsilosis</i> | V2105223          | 0.008                     | 128  | 0.004/16   | 0.625  | IND   | −40.04 (9.22; −49.26)     | IND   |
| <i>C. parapsilosis</i> | B2107379          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | 6.03 (13.39; −7.36)       | IND   |
| <i>C. parapsilosis</i> | ATCC 22019        | 0.016                     | 128  | 0.016/1    | 1.0078 | IND   | −8.18 (5.27; −13.45)      | IND   |
| <i>C. tropicalis</i>   | V2105128          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | −5.10 (4.14; −9.24)       | IND   |
| <i>C. tropicalis</i>   | V2105245          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | −9.39 (2.91; −12.30)      | IND   |
| <i>C. tropicalis</i>   | V2105598          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | 15.03 (16.16; −1.13)      | IND   |
| <i>C. tropicalis</i>   | B1907975          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | −2.05 (6.51; −8.56)       | IND   |
| <i>C. tropicalis</i>   | V2106298          | 0.008                     | 128  | 0.008/1    | 1.0078 | IND   | −41.98 (1.63; −43.61)     | IND   |
| <i>C. kefyr</i>        | V2106126          | 0.002                     | 128  | 0.001/32   | 0.75   | IND   | 7.71 (12.12; −4.41)       | IND   |
| <i>C. kefyr</i>        | N2101899          | 0.0005                    | 32   | 0.0002/8   | 0.75   | IND   | 9.04 (17.00; −7.96)       | IND   |
| <i>C. kefyr</i>        | N2102541          | 0.002                     | 128  | 0.001/8    | 0.5625 | IND   | 8.52 (18.35; −9.83)       | IND   |
| <i>C. kefyr</i>        | V2107293          | 0.004                     | 128  | 0.004/16   | 1.125  | IND   | −4.30 (7.64; −11.94)      | IND   |
| <i>C. kefyr</i>        | V2107534          | 0.0005                    | 128  | 0.0005/2   | 1.0156 | IND   | 10.99 (16.08; −5.09)      | IND   |
| <i>C. kefyr</i>        | V2108462          | 0.002                     | 128  | 0.002/2    | 1.0156 | IND   | 10.42 (24.18; −13.76)     | IND   |

FICI, fractional inhibitory concentration index; INTPN, interpretation; SYN, synergy; IND, no interaction; ISA, isavuconazole; ISOQ, isoquercitrin; ATCC, American Type Culture Collection; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen.

**Table 2.** Interaction of isavuconazole with isoquercitrin against *C. glabrata* evaluated by checkerboard and interpretation by fractional inhibitory concentration index and response surface analysis.

| Species            | Collection Number | Checkerboard MICs (µg/mL) |      |          | Response Surface Analysis |       |                       |       |
|--------------------|-------------------|---------------------------|------|----------|---------------------------|-------|-----------------------|-------|
|                    |                   | ISA                       | ISOQ | ISA/ISOQ | FICI                      | INTPN | ΣSYN-ANT (ΣSYN; ΣANT) | INTPN |
| <i>C. glabrata</i> | U2105834          | 0.25                      | 32   | 0.03/4   | 0.25                      | SYN   | 81.95 (83.97; −2.02)  | SYN   |
| <i>C. glabrata</i> | V2105576          | 0.25                      | 32   | 0.03/4   | 0.25                      | SYN   | 53.99 (56.11; −2.12)  | IND   |
| <i>C. glabrata</i> | N2102530          | 0.5                       | 32   | 0.06/2   | 0.1875                    | SYN   | 95.80 (96.38; −0.58)  | SYN   |
| <i>C. glabrata</i> | U2106503          | 0.5                       | 32   | 0.06/2   | 0.1875                    | SYN   | 82.48 (82.62; −0.14)  | SYN   |
| <i>C. glabrata</i> | U2106602          | 0.5                       | 16   | 0.125/2  | 0.375                     | SYN   | 25.99 (37.29; −11.30) | IND   |
| <i>C. glabrata</i> | U2106664          | 0.125                     | 32   | 0.016/4  | 0.25                      | SYN   | 47.50 (48.14; −0.64)  | IND   |
| <i>C. glabrata</i> | U2106745          | 0.25                      | 32   | 0.03/4   | 0.25                      | SYN   | 78.88 (81.92; −3.04)  | SYN   |
| <i>C. glabrata</i> | U2107113          | 0.25                      | 64   | 0.03/2   | 0.1563                    | SYN   | 76.94 (77.70; −0.76)  | SYN   |
| <i>C. glabrata</i> | U2107210          | 0.125                     | 64   | 0.03/4   | 0.3125                    | SYN   | 51.26 (54.21; −2.95)  | IND   |
| <i>C. glabrata</i> | U2107214          | 0.25                      | 64   | 0.06/8   | 0.375                     | SYN   | 56.24 (61.55; −5.31)  | SYN   |
| <i>C. glabrata</i> | V2107409          | 0.25                      | 64   | 0.03/8   | 0.25                      | SYN   | 55.26 (56.33; −1.07)  | IND   |
| <i>C. glabrata</i> | N2102703          | 0.5                       | 64   | 0.06/4   | 0.1875                    | SYN   | 93.46 (94.17; −0.71)  | SYN   |
| <i>C. glabrata</i> | N2102712          | 1                         | 64   | 0.06/4   | 0.125                     | SYN   | 87.70 (87.85; −0.15)  | SYN   |
| <i>C. glabrata</i> | N2102714          | 0.25                      | 64   | 0.03/4   | 0.1875                    | SYN   | 77.59 (77.66; −0.07)  | SYN   |
| <i>C. glabrata</i> | U2107517          | 0.5                       | 64   | 0.06/8   | 0.25                      | SYN   | 51.55 (53.74; −2.19)  | IND   |
| <i>C. glabrata</i> | U2107630          | 0.25                      | 32   | 0.06/4   | 0.375                     | SYN   | 35.48 (39.20; −3.72)  | IND   |
| <i>C. glabrata</i> | U2107836          | 0.25                      | 64   | 0.03/8   | 0.25                      | SYN   | 39.54 (40.73; −1.19)  | IND   |
| <i>C. glabrata</i> | V2108007          | 0.25                      | 64   | 0.03/8   | 0.25                      | SYN   | 57.34 (58.43; −1.09)  | SYN   |
| <i>C. glabrata</i> | V2108459          | 0.25                      | 64   | 0.06/2   | 0.2813                    | SYN   | 37.39 (39.26; −1.87)  | IND   |
| <i>C. glabrata</i> | B2109750          | 0.25                      | 64   | 0.03/8   | 0.25                      | SYN   | 70.15 (70.26; −0.11)  | SYN   |
| <i>C. glabrata</i> | A2100553          | 0.25                      | 64   | 0.03/8   | 0.25                      | SYN   | 60.71 (61.60; −0.89)  | SYN   |
| <i>C. glabrata</i> | U2107634          | 0.5                       | 64   | 0.06/4   | 0.1875                    | SYN   | 72.52 (73.38; −0.86)  | SYN   |
| <i>C. glabrata</i> | U2107796          | 0.25                      | 16   | 0.06/4   | 0.5                       | SYN   | 55.06 (55.47; −0.41)  | IND   |
| <i>C. glabrata</i> | U2108032          | 0.125                     | 16   | 0.016/4  | 0.375                     | SYN   | 65.49 (66.03; −0.54)  | SYN   |
| <i>C. glabrata</i> | U2107634          | 0.5                       | 32   | 0.06/2   | 0.1875                    | SYN   | 64.03 (65.77; −1.74)  | SYN   |

FICI, fractional inhibitory concentration index; INTPN, interpretation; SYN, synergy; IND, no interaction; ISA, isavuconazole; ISOQ, isoquercitrin.

**Table 3.** Summary of the in vitro interactions of isavuconazole with isoquercitrin against common *Candida* species evaluated by checkerboard and interpretation by fractional concentration index and response surface analysis.

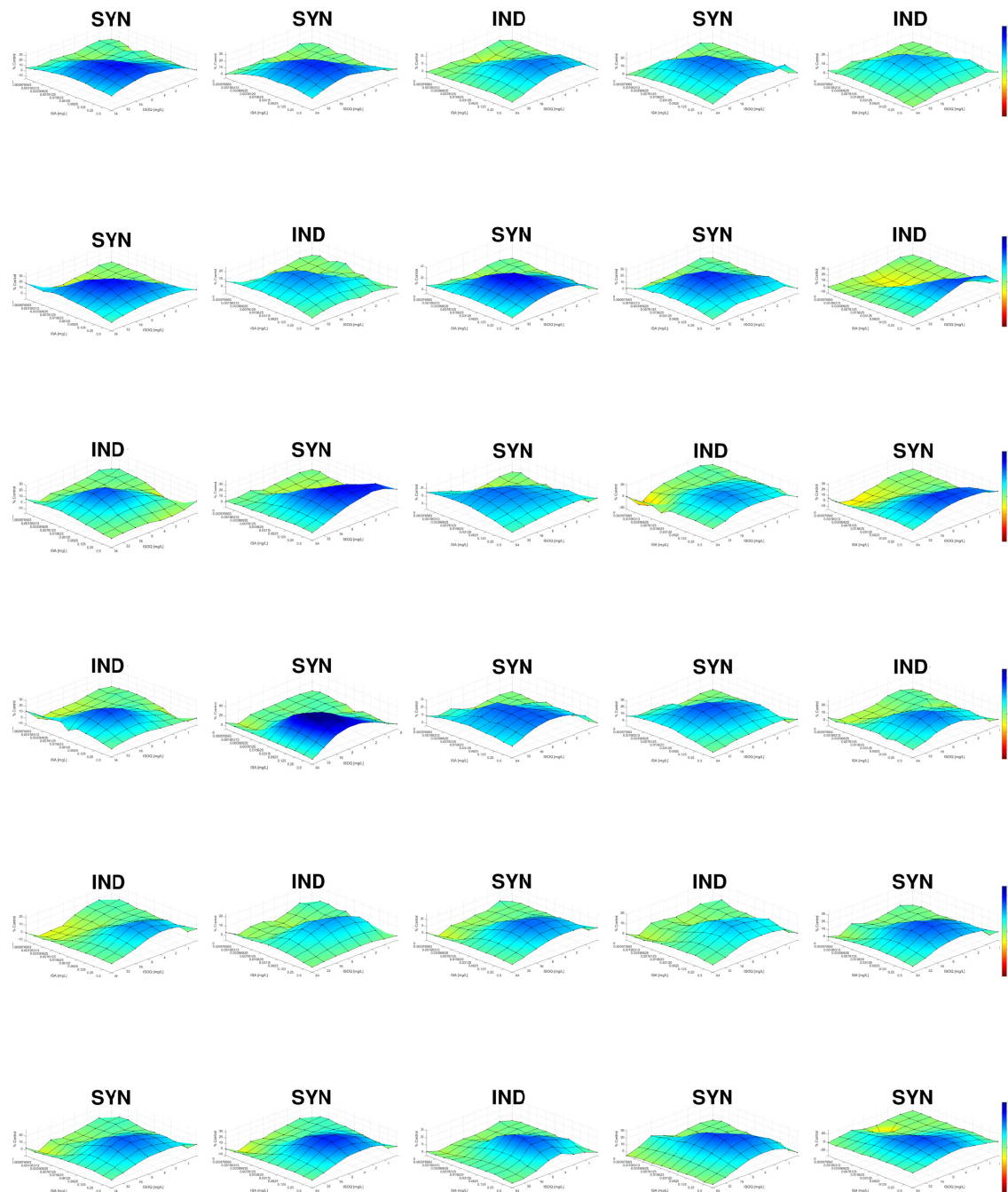
| Species (Strains), Interpretation Model | % of Strains with the Following Interaction |              |            |
|---|---|--------------|------------|
|   | Synergy                                     | Indifference | Antagonism |
| <i>C. albicans</i> (10), FICI           | 0   | 100          | 0          |
| <i>C. albicans</i> (10), RSA            | 0   | 100          | 0          |
| <i>C. glabrata</i> (30), FICI           | 100   | 00           | 0          |
| <i>C. glabrata</i> (30), RSA            | 60  | 40           | 0          |
| <i>C. krusei</i> (5) FICI               | 0   | 100          | 0          |
| <i>C. krusei</i> (5), RSA               | 0   | 100          | 0          |
| <i>C. parapsilosis</i> (4), FICI        | 0   | 100          | 0          |
| <i>C. parapsilosis</i> (4), RSA         | 0   | 100          | 0          |
| <i>C. tropicalis</i> (5), FICI          | 0   | 100          | 0          |



Table 3. Cont.

| Species (Strains),<br>Interpretation Model | % of Strains with the Following Interaction |              |            |
|--|---|--------------|------------|
|  | Synergy                                     | Indifference | Antagonism |
| <i>C. tropicalis</i> (5), RSA              | 0   | 100          | 0          |
| <i>C. kefyr</i> (6), FICI                  | 0   | 100          | 0          |
| <i>C. kefyr</i> (6), RSA                   | 0   | 100          | 0          |

FICI, fractional inhibitory concentration index; RSA, response surface analysis.



**Figure 1.** Synergy distribution for the combination of isavuconazole with isoquercitrin against all *C. glabrata* strains tested. Always from the left to the right, first row: V2105272, V2105282, N2101711, V2105636, DSM 70614; second row: U2105834, V2105576, N2102530, U2106503, U2106602; third row:



U2106664, U2106745, U2107113, U2107210, U2107214; fourth row: V2107409, N2102703, N2102712, N2102714, U2107517; fifth row: U2107630, U2107836, V2108007, V2108459, B2109750; last row: A2100553, U2107634, U2107796, U2108032, U2107634. The mode of interaction was defined based on the SUM-SYN-ANT metric. IND, indifference; SYN, synergy.

The MICs for isavuconazole were within the range of the EUCAST quality control range for this antifungal and are presented in Table 1 (first batch of microplates). The MICs for the quality controls of the second batch of microplates were exactly the same (data not shown). For isoquercitrin, no quality control ranges exist, but the MICs for isoquercitrin of the two quality controls were the same in both batches of microplates.

The 60 *Candida* strains exhibited MICs for isavuconazole ranging from 0.0005 to 1 µg/mL (Tables 1 and 2) with an MIC<sub>50</sub>, MIC<sub>90</sub>, and geometric mean MIC of 0.06, 0.5, and 0.037 µg/mL, respectively. Isavuconazole MICs ranged from 0.001 to 0.004, 0.008 to 0.016, and 0.0005 to 0.004 µg/mL for *C. albicans*, *C. parapsilosis*, and *C. kefyr*, respectively, or were 0.06 and 0.008 µg/mL for *C. krusei* and *C. tropicalis*, respectively. For the strains of *C. glabrata*, MICs for isavuconazole ranged from 0.125 to 1 µg/mL with an MIC<sub>50</sub>, MIC<sub>90</sub>, and geometric mean MIC of 0.25, 0.5, and 0.28 µg/mL, respectively. When tested alone, isoquercitrin exhibited MICs ranging from 16 to 128 µg/mL for the different species (128 µg/mL being the high off-scale MIC) with an MIC<sub>50</sub>, MIC<sub>90</sub>, and a geometric mean MIC of 64, 128, and 65.5 µg/mL, respectively. Best activity of isoquercitrin was seen against *C. glabrata* with MICs ranging from 16 to 64 µg/mL and a geometric mean MIC of 42.22 µg/mL. Isoquercitrin showed no activity against *C. krusei*, *C. parapsilosis*, and *C. tropicalis*; the MICs of all strains were >64 µg/mL. Almost no activity was seen against *C. kefyr*, with a geometric mean MIC of 101.6 µg/mL. Apart from *C. glabrata*, isoquercitrin exhibited only against *C. albicans* a certain degree of activity with a geometric mean MIC of 73.52 µg/mL. Between the experiments, isavuconazole and isoquercitrin MICs were within  $\pm 1 \log_2$  dilutions in 98.33% of the cases for all *Candida* strains tested (data not shown). Interaction was synergistic for 100% of the tested *C. glabrata* strains ( $n = 30$ ), with FICIs ranging from 0.125 to 0.5 with a geometric mean FICI of 0.25. Interaction against all other tested species was indifferent.

Although synergy was less frequently obtained than by FICI analysis, the interaction of the combination evaluated by response surface analysis was still synergistic for the majority (60%) of the *C. glabrata* strains tested. The SUM-SYN-ANT metric for the synergistic strains ranged from 56.24 to 95.8, with a mean of 76.06. The mean of the SUM-SYN-ANT metric of all strains of *C. glabrata* tested was 64.15. As obtained by FICI analysis, the interaction of the combination evaluated by response surface analysis against all other strains of the tested species was indifferent.

#### 4. Discussion

Polyphenols are natural organic compounds comprising of multiple phenol units, found in fruits, vegetables, cereals, and beverages such as red wine or tea [39]. They are secondary metabolites of plants, and involved in the host defense against ultraviolet radiation or pathogens [40]. Epidemiological studies suggested that long-term use of plant polyphenol-rich diets could protect against the development of cancers, cardiovascular diseases, diabetes, aging, asthma, neurodegenerative diseases, and infections [41]. Flavonoids are a class of polyphenols which have a 15-carbon skeleton comprising of two phenyl rings connected over a heterocyclic ring containing embedded oxygen, have the ability to inhibit spore germination in plant pathogenic fungi, and have, therefore, been proposed for use against fungal pathogens of man [42]. Flavonoids have shown antifungal activity against dermatophytes [43,44], human opportunistic filamentous fungi [43,45–48], and yeasts, including *Candida* species [43,49–52]. Isoquercitrin is a flavonoid which can be isolated from areal parts of *Aster yomena*, a perennial herb which grows in the southern part of Korea, and is used as a traditional medication to treat inflammation, colds, and bronchial asthma [53]. Apart from its anti-allergic [54], antibiotic [55], anti-hyperlipidemic [56], anti-inflammatory [57], and antioxidant properties [58], it causes fungicidal membrane

disturbance [33] and reactive oxygen species (ROS)-mediated apoptosis in *C. albicans* [59], which make the drug an interesting partner to test combinations with antifungals.

The MICs of isavuconazole for the *Candida* species tested in this study were in the same range as described previously [60–62]. MICs of isoquercitrin of some of our *C. albicans* strains were in the same range as reported by others who used EUCAST methodology for MIC determination [63]; however, the majority of our strains had higher MICs. Another study which evaluated the activity of different polyphenols isolated from *Pterogyne nitens* found, in accordance with this study, high MICs of isoquercitrin against *C. albicans*, *C. krusei*, and *C. parapsilosis*, but CLSI (Clinical and Laboratory Standards Institute) methodology was used for susceptibility testing.

MICs of isoquercitrin in combination for *C. glabrata* ranged from 2 to 8 µg/mL with a geometric mean MIC of 4 µg/mL. To our knowledge, peak serum levels of isoquercitrin in patients have not been studied so far. In rats, peak plasma levels of about 15 µg/mL were reached after a single intravenous administration of 5 mg/kg of body weight. With doses of 20 mg/kg of body weight, peak plasma levels of even 60 µg/mL have been reached [64]. These levels would have largely been enough against the *C. glabrata* strains tested in this study. However, even if achievable serum levels in patients would be lower, the synergy of the combination could not directly be out of the question, as it has been shown in patients that even lower serum levels than those of the MICs can lead to in vivo synergy [65].

Only one study evaluated the interaction of isoquercitrin with azoles against *Candida*. The combination of isoquercitrin with fluconazole was evaluated by checkerboard against one strain of *C. albicans* and found to be synergistic. The authors further showed that the addition of fluconazole to isoquercitrin increases the effect of isoquercitrin in lowering the activity of the superoxide dismutase and increasing metacaspase activation and DNA condensation, leading to ROS accumulation, oxidative stress, and induction of apoptosis. In the same study, the combination of isoquercitrin with amphotericin or flucytosine was tested against one strain of *C. albicans*, yielding synergy and indifference, respectively [66]. In our study, the combination of isoquercitrin and isavuconazole against 10 *C. albicans* strains evaluated by checkerboard and interpreted by FICI and by response surface analysis exhibited indifference. The different interaction could be specific to the strain used or associated with the different azole.

Against other *Candida* species, the combination of isoquercitrin has never been tested before. We found indifference of the combination of isoquercitrin with isavuconazole for all *C. krusei*, *C. kefyr*, *C. parapsilosis*, and *C. tropicalis* strains tested. In contrast to these results, combination was synergistic against all 30 *C. glabrata* strains tested, when the results of the checkerboard were interpreted by FICI. When the interpretation of the results was carried out by response surface analysis, synergy was still obtained for 60% of the *C. glabrata* strains tested. The discrepancy between the FICI and the response surface analysis results can be explained by the stringent threshold (56.0) used in this study compared with previous works, where lower thresholds have been used [24,25]. Nevertheless, despite the indifference of 40% of the *C. glabrata* results by response surface analysis, the mean of the SUM-SYN-ANT metric of all strains was 64.15, and therefore higher than the threshold.

One of the limitations of this study is that molecular determination of azole resistance or *FKS* gene mutation has not been performed for the *C. glabrata* strains used in this study. This limitation is aggravated by the lack of breakpoint and epidemiological cut-off value definition by EUCAST for isavuconazole against *C. glabrata*. It has been shown that *C. glabrata* strains with proven molecular azole resistance exhibit MICs to isavuconazole of 1 to  $\geq 8$  µg/mL [67,68]. In this study, one strain exhibited an MIC of 1 µg/mL. However, it cannot be concluded that this strain owns an azole-resistance mutation because the MICs in the mentioned studies were determined by CLSI methodology and, also, because wild-type strains can exhibit MICs of 1 and even 2 µg/mL to isavuconazole [67]. *C. glabrata* strains resistant to echinocandins are often cross-resistant to azoles. Even if the strain in this study with the MIC of 1 µg/mL would be isavuconazole resistant, it cannot be concluded that the strains are mandatory also echinocandin resistant [69]. Therefore, based on the results of

this study, it cannot be concluded that the combination of isavuconazole and isoquercitrin can overcome proven azole or echinocandin resistance.

To summarize, we demonstrated that the combination of isavuconazole with isoquercitrin interacts synergistically against *C. glabrata*. Interaction against *C. albicans*, *C. krusei*, *C. kefyr*, *C. parapsilosis*, and *C. tropicalis* exhibited only indifference, but importantly antagonistic interaction was never observed. These results warrant further animal experiments.

**Author Contributions:** P.S. and P.V.S. wrote the first draft of the manuscript. P.S. and I.N. carried out the experiments. P.V.S., P.S. and I.N. performed the analysis of the results. E.D., P.S., G.B. and F.S. contributed to the revisions. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by internal funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The datasets of the sequenced *Candida* strains used in this study can be found at GenBank (<https://www.ncbi.nlm.nih.gov/genbank/> (accessed on 1 May 2022)) under the accession numbers OL351325 to OL351353, OL351355, and OL351356, under OM859334 to OM859338, and under ON391951 to ON391970.

**Conflicts of Interest:** Patrick Schwarz has received research grants from Basilea Pharmaceutica, Gilead, and Pfizer, received travel grants from Gilead and Pfizer, and speaking fees from Pfizer. During the past 5 years, Eric Dannaoui has received research grants from MSD and Gilead, travel grants from Gilead, MSD, Pfizer, and Astellas, and speaker's fees from Gilead, MSD, and Astellas. The other authors have none to declare.

## References

1. Kullberg, B.J.; Arendrup, M.C. Invasive candidiasis. *N. Engl. J. Med.* **2015**, *373*, 1445–1456. [CrossRef] [PubMed]
2. Yapar, N. Epidemiology and risk factors for invasive candidiasis. *Ther. Clin. Risk Manag.* **2014**, *10*, 95–105. [CrossRef] [PubMed]
3. Pfaller, M.A.; Diekema, D.J.; Gibbs, D.L.; Newell, V.A.; Ellis, D.; Tullio, V.; Rodloff, A.; Fu, W.; Ling, T.A.; Global Antifungal Surveillance Group. Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2007: A 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J. Clin. Microbiol.* **2010**, *48*, 1366–1377. [CrossRef] [PubMed]
4. Montagna, M.T.; Caggiano, G.; Lovero, G.; De Giglio, O.; Coretti, C.; Cuna, T.; Iatta, R.; Giglio, M.; Dalfino, L.; Bruno, F.; et al. Epidemiology of invasive fungal infections in the intensive care unit: Results of a multicenter Italian survey (AURORA Project). *Infection* **2013**, *41*, 645–653. [CrossRef]
5. Vannini, M.; Emery, S.; Lieutier-Colas, F.; Legueult, K.; Mondain, V.; Retur, N.; Gastaud, L.; Pomares, C.; Hasseine, L. Epidemiology of candidemia in NICE area, France: A five-year study of antifungal susceptibility and mortality. *J. Mycol. Med.* **2022**, *32*, 101210. [CrossRef]
6. Pappas, P.G.; Lionakis, M.S.; Arendrup, M.C.; Ostrosky-Zeichner, L.; Kullberg, B.J. Invasive candidiasis. *Nat. Rev. Dis. Primers* **2018**, *4*, 18026. [CrossRef]
7. Cornely, O.A.; Bassetti, M.; Calandra, T.; Garbino, J.; Kullberg, B.J.; Lortholary, O.; Meersseman, W.; Akova, M.; Arendrup, M.C.; Arikan-Akdagli, S.; et al. ESCMID guideline for the diagnosis and management of *Candida* diseases 2012: Non-neutropenic adult patients. *Clin. Microbiol. Infect.* **2012**, *18* (Suppl. S7), 19–37. [CrossRef]
8. Pfaller, M.A.; Boyken, L.; Hollis, R.J.; Messer, S.A.; Tendolkar, S.; Diekema, D.J. In vitro susceptibilities of *Candida* spp. to caspofungin: Four years of global surveillance. *J. Clin. Microbiol.* **2006**, *44*, 760–763. [CrossRef]
9. Orozco, A.S.; Higginbotham, L.M.; Hitchcock, C.A.; Parkinson, T.; Falconer, D.; Ibrahim, A.S.; Ghannoum, M.A.; Filler, S.G. Mechanism of fluconazole resistance in *Candida krusei*. *Antimicrob. Agents Chemother.* **1998**, *42*, 2645–2649. [CrossRef]
10. Garcia-Effron, G.; Lee, S.; Park, S.; Cleary, J.D.; Perlin, D.S. Effect of *Candida glabrata* FKS1 and FKS2 mutations on echinocandin sensitivity and kinetics of 1,3-beta-D-glucan synthase: Implication for the existing susceptibility breakpoint. *Antimicrob. Agents Chemother.* **2009**, *53*, 3690–3699. [CrossRef]
11. Dannaoui, E.; Desnos-Ollivier, M.; Garcia-Hermoso, D.; Grenouillet, F.; Cassaing, S.; Baixench, M.T.; Bretagne, S.; Dromer, F.; Lortholary, O.; French Mycoses Study Group. *Candida* spp. with acquired echinocandin resistance, France, 2004–2010. *Emerg. Infect. Dis.* **2012**, *18*, 86–90. [CrossRef] [PubMed]
12. Alexander, B.D.; Johnson, M.D.; Pfeiffer, C.D.; Jimenez-Ortigosa, C.; Catania, J.; Booker, R.; Castanheira, M.; Messer, S.A.; Perlin, D.S.; Pfaller, M.A. Increasing echinocandin resistance in *Candida glabrata*: Clinical failure correlates with presence of FKS mutations and elevated minimum inhibitory concentrations. *Clin. Infect. Dis.* **2013**, *56*, 1724–1732. [CrossRef] [PubMed]

13. Messer, S.A.; Carvalhaes, C.G.; Castanheira, M.; Pfaller, M.A. In vitro activity of isavuconazole versus opportunistic filamentous fungal pathogens from the SENTRY antifungal surveillance program, 2017–2018. *Diagn. Microbiol. Infect. Dis.* **2020**, *97*, 115007. [CrossRef]
14. Fuller, J.; Dingle, T.C.; Bull, A.; Shokoples, S.; Laverdiere, M.; Baxter, M.R.; Adam, H.J.; Karlowsky, J.A.; Zhanel, G.G.; Canadian Antimicrobial Resistance Alliance; et al. Species distribution and antifungal susceptibility of invasive *Candida* isolates from Canadian hospitals: Results of the CANWARD 2011–16 study. *J. Antimicrob. Chemother.* **2019**, *74*, iv48–iv54. [CrossRef] [PubMed]
15. Astvad, K.M.T.; Johansen, H.K.; Roder, B.L.; Rosenvinge, F.S.; Knudsen, J.D.; Lemming, L.; Schonheyder, H.C.; Hare, R.K.; Kristensen, L.; Nielsen, L.; et al. Update from a 12-year nationwide fungemia surveillance: Increasing intrinsic and acquired resistance causes concern. *J. Clin. Microbiol.* **2018**, *56*, e01564–17. [CrossRef]
16. Martinez-Herrera, E.; Frias-De-Leon, M.G.; Hernandez-Castro, R.; Garcia-Salazar, E.; Arenas, R.; Ocharan-Hernandez, E.; Rodriguez-Cerdeira, C. Antifungal resistance in clinical isolates of *Candida glabrata* in Ibero-America. *J. Fungi* **2021**, *8*, 14. [CrossRef]
17. Aldejohann, A.M.; Herz, M.; Martin, R.; Walther, G.; Kurzai, O. Emergence of resistant *Candida glabrata* in Germany. *JAC Antimicrob. Resist.* **2021**, *3*, dlab122. [CrossRef]
18. Vitale, R.G. Role of antifungal combinations in difficult to treat *Candida* infections. *J. Fungi* **2021**, *7*, 731. [CrossRef]
19. Vitale, R.G.; Afeltra, J.; Dannaoui, E. Antifungal combinations. *Methods Mol. Med.* **2005**, *118*, 143–152. [CrossRef]
20. Perfect, J.R.; Dismukes, W.E.; Dromer, F.; Goldman, D.L.; Graybill, J.R.; Hamill, R.J.; Harrison, T.S.; Larsen, R.A.; Lortholary, O.; Nguyen, M.H.; et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin. Infect. Dis.* **2010**, *50*, 291–322. [CrossRef]
21. Kontoyiannis, D.P.; Lewis, R.E. Toward more effective antifungal therapy: The prospects of combination therapy. *Br. J. Haematol.* **2004**, *126*, 165–175. [CrossRef] [PubMed]
22. Johnson, M.D.; MacDougall, C.; Ostrosky-Zeichner, L.; Perfect, J.R.; Rex, J.H. Combination antifungal therapy. *Antimicrob. Agents Chemother.* **2004**, *48*, 693–715. [CrossRef] [PubMed]
23. Schwarz, P.; Nikolskiy, I.; Bidaud, A.L.; Sommer, F.; Bange, G.; Dannaoui, E. In vitro synergy of isavuconazole combined with colistin against common *Candida* species. *Front. Cell Infect. Microbiol.* **2022**, *12*. [CrossRef] [PubMed]
24. Schwarz, P.; Nikolskiy, I.; Bidaud, A.L.; Sommer, F.; Bange, G.; Dannaoui, E. In vitro activity of amphotericin B in combination with colistin against fungi responsible for invasive infections. *J. Fungi* **2022**, *8*, 115. [CrossRef]
25. Schwarz, P.; Bidaud, A.L.; Dannaoui, E. In vitro synergy of isavuconazole in combination with colistin against *Candida auris*. *Sci. Rep.* **2020**, *10*, 21448. [CrossRef]
26. Schwarz, P.; Djenontin, E.; Dannaoui, E. Colistin and isavuconazole interact synergistically in vitro against *Aspergillus nidulans* and *Aspergillus niger*. *Microorganisms* **2020**, *8*, 1447. [CrossRef]
27. Schwarz, P.; Dannaoui, E. In vitro interaction between isavuconazole and tacrolimus, cyclosporin A, or sirolimus against *Aspergillus* Species. *J. Fungi* **2020**, *6*, 103. [CrossRef]
28. Schwarz, P.; Schwarz, P.V.; Felske-Zech, H.; Dannaoui, E. In vitro interactions between isavuconazole and tacrolimus, cyclosporin A or sirolimus against Mucorales. *J. Antimicrob. Chemother.* **2019**, *74*, 1921–1927. [CrossRef]
29. Seifert, H.; Aurbach, U.; Stefanik, D.; Cornely, O. In vitro activities of isavuconazole and other antifungal agents against *Candida* bloodstream isolates. *Antimicrob. Agents Chemother.* **2007**, *51*, 1818–1821. [CrossRef]
30. Maertens, J.A.; Raad, I.I.; Marr, K.A.; Patterson, T.F.; Kontoyiannis, D.P.; Cornely, O.A.; Bow, E.J.; Rahav, G.; Neofytos, D.; Aoun, M.; et al. Isavuconazole versus voriconazole for primary treatment of invasive mould disease caused by *Aspergillus* and other filamentous fungi (SECURE): A phase 3, randomised-controlled, non-inferiority trial. *Lancet* **2016**, *387*, 760–769. [CrossRef]
31. Kullberg, B.J.; Viscoli, C.; Pappas, P.G.; Vazquez, J.; Ostrosky-Zeichner, L.; Rotstein, C.; Sobel, J.D.; Herbrecht, R.; Rahav, G.; Jaruratanasirikul, S.; et al. Isavuconazole versus caspofungin in the treatment of candidemia and other invasive *Candida* infections: The ACTIVE trial. *Clin. Infect. Dis.* **2019**, *68*, 1981–1989. [CrossRef] [PubMed]
32. Ellsworth, M.; Ostrosky-Zeichner, L. Isavuconazole: Mechanism of action, clinical efficacy, and resistance. *J. Fungi* **2020**, *6*, 324. [CrossRef] [PubMed]
33. Yun, J.; Lee, H.; Ko, H.J.; Woo, E.R.; Lee, D.G. Fungicidal effect of isoquercitrin via inducing membrane disturbance. *Biochim. Biophys. Acta* **2015**, *1848*, 695–701. [CrossRef] [PubMed]
34. Schwarz, P.; Bretagne, S.; Gantier, J.C.; Garcia-Hermoso, D.; Lortholary, O.; Dromer, F.; Dannaoui, E. Molecular identification of zygomycetes from culture and experimentally infected tissues. *J. Clin. Microbiol.* **2006**, *44*, 340–349. [CrossRef] [PubMed]
35. Bidaud, A.L.; Schwarz, P.; Herbreteau, G.; Dannaoui, E. Techniques for the assessment of in vitro and in vivo antifungal combinations. *J. Fungi* **2021**, *7*, 113. [CrossRef] [PubMed]
36. Arendrup, M.C.; Meletiadis, J.; Mouton, J.W.; Lagrou, K.; Hamal, P.; Guinea, J. Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Yeasts (EUCAST Definitive Document E.Def 7.3.2). 2020. Available online: [https://www.eucast.org/astoffungi/methodsinantifungalsusceptibilitytesting/susceptibility\\_testing\\_of\\_yeasts/](https://www.eucast.org/astoffungi/methodsinantifungalsusceptibilitytesting/susceptibility_testing_of_yeasts/) (accessed on 1 May 2022).
37. Odds, F.C. Synergy, antagonism, and what the checkerboard puts between them. *J. Antimicrob. Chemother.* **2003**, *52*, 1. [CrossRef]
38. Di Veroli, G.Y.; Fornari, C.; Wang, D.; Mollard, S.; Bramhall, J.L.; Richards, F.M.; Jodrell, D.I. Combeneft: An interactive platform for the analysis and visualization of drug combinations. *Bioinformatics* **2016**, *32*, 2866–2868. [CrossRef]



39. Cory, H.; Passarelli, S.; Szeto, J.; Tamez, M.; Mattei, J. The role of polyphenols in human health and food systems: A mini-review. *Front. Nutr.* **2018**, *5*, 87. [\[CrossRef\]](#)
40. Beckman, C.H. Phenolic-storing cells: Keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? *Physiol. Mol. Plant Pathol.* **2000**, *57*, 101–110. [\[CrossRef\]](#)
41. Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell Longev.* **2009**, *2*, 270–278. [\[CrossRef\]](#)
42. Cushnie, T.P.; Lamb, A.J. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **2005**, *26*, 343–356. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Faustino, M.V.; Pinto, D.C.G.A.; Gonçalves, M.J.; Salgueiro, L.; Silveira, P.; Silva, A.M.S. *Calendula* L. species polyphenolic profile and in vitro antifungal activity. *J. Funct. Foods* **2018**, *45*, 254–267. [\[CrossRef\]](#)
44. Foss, S.R.; Nakamura, C.V.; Ueda-Nakamura, T.; Cortez, D.A.; Endo, E.H.; Dias Filho, B.P. Antifungal activity of pomegranate peel extract and isolated compound punicalagin against dermatophytes. *Ann. Clin. Microbiol. Antimicrob.* **2014**, *13*, 32. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Aguirre-Joya, J.A.; Pastrana-Castro, L.; Nieto-Oropeza, D.; Ventura-Sobrevilla, J.; Rojas-Molina, R.; Aguilar, C.N. The physico-chemical, antifungal and antioxidant properties of a mixed polyphenol based bioactive film. *Heliyon* **2018**, *4*, e00942. [\[CrossRef\]](#)
46. Ascacio-Valdes, J.; Burboa, E.; Aguilera-Carbo, A.F.; Aparicio, M.; Perez-Schmidt, R.; Rodriguez, R.; Aguilar, C.N. Antifungal ellagitannin isolated from *Euphorbia antisyphilitica* Zucc. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 41–46. [\[CrossRef\]](#)
47. Kanwal, Q.; Hussain, I.; Latif Siddiqui, H.; Javaid, A. Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves. *Nat. Prod. Res.* **2010**, *24*, 1907–1914. [\[CrossRef\]](#)
48. Rongai, D.; Pulcini, P.; Pesce, B.; Milano, F. Antifungal activity of pomegranate peel extract against fusarium wilt of tomato. *Eur. J. Plant Pathol.* **2017**, *147*, 229–238. [\[CrossRef\]](#)
49. Filho, A.A.O.; Oliveira, H.B.D.; de Sousa, J.P.; Meireles, D.R.P.; de Alzevedo Maia, G.L.; Filho, J.M.B.; de Siqueira Júnior, J.P.; de Oliveira Lima, E. In vitro anti-*Candida* activity and mechanism of action of the flavonoid isolated from *Praxelis clematidea* against *Candida albicans* species. *J. Appl. Pharm. Sci.* **2016**, *6*, 066–069. [\[CrossRef\]](#)
50. Meragelman, T.L.; Tucker, K.D.; McCloud, T.G.; Cardellina, J.H., 2nd; Shoemaker, R.H. Antifungal flavonoids from *Hildegardia barteri*. *J. Nat. Prod.* **2005**, *68*, 1790–1792. [\[CrossRef\]](#)
51. Nair, M.S.; Saxena, A.; Kaur, C. Characterization and antifungal activity of pomegranate peel extract and its use in polysaccharide-based edible coatings to extend the shelf-life of *Capsicum* (*Capsicum annuum* L.). *Food Bioprocess Technol.* **2018**, *11*, 1317–1327. [\[CrossRef\]](#)
52. Yamaguchi, M.U.; Garcia, F.P.; Cortez, D.A.; Ueda-Nakamura, T.; Filho, B.P.; Nakamura, C.V. Antifungal effects of Ellagitannin isolated from leaves of *Ocotea odorifera* (Lauraceae). *Antonie. Van. Leeuwenhoek* **2011**, *99*, 507–514. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Kim, A.R.; Jin, Q.; Jin, H.G.; Ko, H.J.; Woo, E.R. Phenolic compounds with IL-6 inhibitory activity from *Aster yomena*. *Arch. Pharm. Res.* **2014**, *37*, 845–851. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Makino, T.; Kanemaru, M.; Okuyama, S.; Shimizu, R.; Tanaka, H.; Mizukami, H. Anti-allergic effects of enzymatically modified isoquercitrin (alpha-oligoglucosyl quercetin 3-O-glucoside), quercetin 3-O-glucoside, alpha-oligoglucosyl rutin, and quercetin, when administered orally to mice. *J. Nat. Med.* **2013**, *67*, 881–886. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Yun, J.; Woo, E.R.; Lee, D.G. Effect of isoquercitrin on membrane dynamics and apoptosis-like death in *Escherichia coli*. *Biochim. Biophys. Acta Biomembr.* **2018**, *1860*, 357–363. [\[CrossRef\]](#)
56. Jayachandran, M.; Zhang, T.; Wu, Z.; Liu, Y.; Xu, B. Isoquercetin regulates SREBP-1C via AMPK pathway in skeletal muscle to exert antihyperlipidemic and anti-inflammatory effects in STZ induced diabetic rats. *Mol. Biol. Rep.* **2020**, *47*, 593–602. [\[CrossRef\]](#)
57. Rogerio, A.P.; Kanashiro, A.; Fontanari, C.; da Silva, E.V.; Lucisano-Valim, Y.M.; Soares, E.G.; Faccioli, L.H. Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma. *Inflamm. Res.* **2007**, *56*, 402–408. [\[CrossRef\]](#)
58. Jung, S.H.; Kim, B.J.; Lee, E.H.; Osborne, N.N. Isoquercitrin is the most effective antioxidant in the plant *Thuja orientalis* and able to counteract oxidative-induced damage to a transformed cell line (RGC-5 cells). *Neurochem. Int.* **2010**, *57*, 713–721. [\[CrossRef\]](#)
59. Yun, J.; Woo, E.R.; Lee, D.L. Isoquercitrin, isolated from *Aster yomena* triggers ROS-mediated apoptosis in *Candida albicans*. *J. Funct. Foods* **2016**, *22*, 347–357. [\[CrossRef\]](#)
60. Desnos-Ollivier, M.; Bretagne, S.; Boullie, A.; Gautier, C.; Dromer, F.; Lortholary, O.; French Mycoses Study Group. Isavuconazole MIC distribution of 29 yeast species responsible for invasive infections (2015–2017). *Clin. Microbiol. Infect.* **2019**, *25*, 634.e1–634.e4. [\[CrossRef\]](#)
61. Jorgensen, K.M.; Astvad, K.M.T.; Hare, R.K.; Arendrup, M.C. EUCAST susceptibility testing of isavuconazole: MIC data for contemporary clinical mold and yeast isolates. *Antimicrob. Agents Chemother.* **2019**, *63*, e00073–19. [\[CrossRef\]](#)
62. Sickles, E.A.; Greene, W.H.; Wiernik, P.H. Clinical presentation of infection in granulocytopenic patients. *Arch. Intern. Med.* **1975**, *135*, 715–719. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Ivanov, M.; Kannan, A.; Stojkovic, D.S.; Glamoclija, J.; Calhelha, R.C.; Ferreira, I.; Sanglard, D.; Sokovic, M. Flavones, flavonols, and glycosylated derivatives-impact on *Candida albicans* growth and virulence, expression of *CDR1* and *ERG11*, cytotoxicity. *Pharmaceuticals* **2020**, *14*, 27. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Xue, H.; Li, H.; Zhang, W.; Lu, D.; Chen, Y.; Yin, J.; Meng, Y.; Ying, X.; Kang, T. Pharmacokinetic study of isoquercitrin in rat plasma after intravenous administration at three different doses. *Braz. J. Pharm. Sci.* **2013**, *49*, 435–441. [\[CrossRef\]](#)
65. Kontoyiannis, D.P.; Lewis, R.E.; Alexander, B.D.; Lortholary, O.; Dromer, F.; Gupta, K.L.; John, G.T.; Del Busto, R.; Klintmalm, G.B.; Somani, J.; et al. Calcineurin inhibitor agents interact synergistically with antifungal agents in vitro against *Cryptococcus*

- neoformans* isolates: Correlation with outcome in solid organ transplant recipients with cryptococcosis. *Antimicrob. Agents Chemother.* **2008**, *52*, 735–738. [[CrossRef](#)] [[PubMed](#)]
66. Kim, S.; Woo, E.R.; Lee, D.G. Synergistic antifungal activity of isoquercitrin: Apoptosis and membrane permeabilization related to reactive oxygen species in *Candida albicans*. *IUBMB Life* **2019**, *71*, 283–292. [[CrossRef](#)] [[PubMed](#)]
67. Castanheira, M.; Messer, S.A.; Rhomberg, P.R.; Dietrich, R.R.; Jones, R.N.; Pfaller, M.A. Isavuconazole and nine comparator antifungal susceptibility profiles for common and uncommon *Candida* species collected in 2012: Application of new CLSI clinical breakpoints and epidemiological cutoff values. *Mycopathologia* **2014**, *178*, 1–9. [[CrossRef](#)]
68. Sanglard, D.; Coste, A.T. Activity of isavuconazole and other azoles against *Candida* clinical isolates and yeast model systems with known azole resistance mechanisms. *Antimicrob. Agents Chemother.* **2016**, *60*, 229–238. [[CrossRef](#)]
69. Pristov, K.E.; Ghannoum, M.A. Resistance of *Candida* to azoles and echinocandins worldwide. *Clin. Microbiol. Infect.* **2019**, *25*, 792–798. [[CrossRef](#)]