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Research Article

Enhancement of Fluconazole Activity by *Ocimum basilicum* Essential Oil in *Candida albicans* and *Pichia kudriavzevii*

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Abstract

Antimicrobial resistance (AMR) has intensified the challenge of treating fungal infections, particularly those caused by *Candida albicans* and *Pichia kudriavzevii*, which show increasing resistance to conventional antifungal agents. This scenario highlights the urgent need for new therapeutic approaches capable of enhancing existing treatments. In this study, we investigated the antifungal potential of *Ocimum basilicum* essential oil (OEOB) and its combined effects with standard antifungal drugs. In antifungal assays, isolated OEOB did not exhibit significant inhibitory activity against *Candida albicans* and *Pichia kudriavzevii*. However, when associated with fluconazole, OEOB promoted a marked reduction in fungal growth, demonstrating its potential to enhance the efficacy of conventional antifungal therapy. These findings indicate that OEOB acts as a promising adjuvant agent in antifungal treatments, contributing to improved therapeutic outcomes against resistant *Candida albicans* and *Pichia kudriavzevii* strains.

Keywords: *Antimicrobial resistance; Candidiasis; Antifungal activity.*

1. Introduction

Antimicrobial resistance (AMR) is one of the greatest threats to global public health. This resistance is driven by the misuse of antibiotics or the emergence of intrinsic, acquired, and adaptive mechanisms [1]. AMR contributes to 20% of global deaths from bacterial infections, a problem that has been further aggravated by the action of transmembrane efflux pumps, which expel antibiotics before they reach their targets [2].

A growing challenge in the hospital environment is represented by opportunistic fungi of the genus *Candida*, especially *Candida albicans* and non-*albicans*, which are highly relevant in immunosuppressed patients and in intensive care [3]. The prevalence of these infections, along with resistance to conventional antifungals such as amphotericin B and fluconazole, reinforces the urgent need for new therapeutic alternatives [4].

In this context, the search for natural medicines has gained prominence, driven by the continuous increase in bacterial and antifungal resistance [5]. Essential oils extracted from plants have stood out as promising sources of bioactive compounds, recognized for their antimicrobial, antifungal, analgesic, anti-inflammatory, anesthetic, and spasmolytic activity [6-8].

It is estimated that around 60% of essential oils extracted from plants have antifungal activity and 35% have antibacterial activity [9,10]. Among these plant species, *Ocimum basilicum* L., popularly known as basil, stands out, with its methanolic extract associated with anti-inflammatory, antioxidant, analgesic, antibacterial, and antifungal activities [11].

The objective of this study was to evaluate the antifungal potential of *Ocimum basilicum* essential oil (OEOB) against clinically relevant *Candida* species and to investigate its ability to enhance the activity of conventional antifungal drugs.

2. Materials and Methods

2.1 Collecting botanical material

Ocimum basilicum leaves were collected in the municipality of Crato-CE, Brazil, at geographical coordinates -7.272119s and -39.460795w, at 9 a.m. in March 2023. The material was identified by botanist Dr. José Weverton Almeida-Bezerra.

2.2 Essential oil extraction

The essential oil of *O. basilicum* (OEOB) was extracted using the hydrodistillation method. The dried leaves were crushed and transferred to a 5 L round-bottom flask containing 2 L of distilled water. The leaves were then boiled continuously for 2 hours using a Clevenger apparatus. The extracted essential oil was then stored in an amber glass bottle and kept refrigerated at 4 °C until used in biological assays.

2.3 Antifungical Activity

2.3.1 Strains and culture media

Two standard clinical strains were used in this study: *Candida albicans* INCQS 40006 and *C. krusei* (*Pichia kudriavzevii*) INCQS 40095, from the Culture Collection of the National Institute for Health Quality Control (INCQS) of the Oswaldo Cruz Foundation (Manguinhos, Rio de Janeiro, Brazil). For the culture of fungal strains to be used in the antifungal evaluation of OEOB, Sabouraud Dextrose Agar (SDA) and Sabouraud Dextrose Broth (SDB) culture media were used, following the manufacturer's guidelines for their respective preparations. Thus, the media were diluted in distilled water and subjected to an autoclave sterilization process at 121ºC for 15 minutes.

2.3.2 Cultivation of *Candida* strains and preparation of the solution for antifungal assays

Fluconazole (FLUCOMED) was used as a positive control for the antifungal assays. *Candida* strains were cultivated in Petri dishes containing SDA medium and incubated for 24 hours at 37 °C. Subsequently, fungal samples were added to tubes containing 4 mL of sterile 0.9% NaCl, after which the samples underwent a process of agitation and standardization following the McFarland 0.5 turbidity scale.

To prepare the solutions containing OEOB, OEOB was diluted (16.384 µg/mL) in dimethyl sulfoxide (DMSO, 1 mL) in the same way that fluconazole was diluted. To prepare the stock solution, the oil was diluted in 9 mL of Sabouraud dextrose (SDB) medium to achieve a concentration of 1024 µg/mL, according to the methodology of Moraes-Braga et al. [12].

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2.3.3 Determination of minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC), the broth microdilution method was used in 96-well plates. Each contained 100 µL of SDB, 10% fungal inoculum, and 100 µL of OBEO or fluconazole, with concentrations ranging from 2 to 1024 µg/mL. Microdilution was performed up to the penultimate row, as the last row served as a control for normal fungal growth without the addition of OBEO or fluconazole. Additional controls for the saline diluent and sterile medium were also prepared. The plates were then incubated at 37°C for 24 h and subsequently read in an ELISA spectrophotometer (Thermoplate®) at a wavelength of 630 nm. The results obtained from the ELISA reading were used to construct the IC_{50} curve [13].

2.3.4 Determination of Minimum Fungicidal Concentration (MFC)

Samples from each well of the plate were tested to homogenize the medium and transfer an aliquot to the Petri dish containing Sabouraud Dextrose Agar. After 24 h of incubation at 37 °C, the plates were analyzed for the formation of Candida yeast colonies. The concentration at which there was no growth of fungal colonies was considered the MFC of OEOB following the methodology proposed by Brito et al. [14].

2.3.5 Fluconazole modifier activity

OEOB was evaluated at subinhibitory concentrations (MC/8) to verify its ability to modulate the activity of fluconazole. The assay followed the methodology proposed by Silva et al. [15] with some adaptations. Sabouraud Dextrose Agar medium containing OEOB (MC/8) was added to 96-well plates with the subsequent addition of fluconazole (2–1024 µg/mL) and fungal samples, followed by serial microdilution. After incubation at 37 °C for 24 hours, absorbance readings were performed using an ELISA spectrophotometer (Termoplate® Kasuaki, China).

2.4 Statistical analysis

Statistical analyses were conducted using GraphPad Prism 9 software. Assay results were expressed as mean ± standard deviation. Statistical significance was determined using two-way ANOVA followed by Tukey's post-hoc test, considering a 95% confidence level. Mean inhibitory concentrations (IC_{50}) were obtained by non-linear regression.

3. Results and Discussion

OEOB alone did not significantly reduce fungal growth for either strain at all concentrations evaluated; growth remained at around 100%. However, as shown in Figure 1, when the oil was combined with fluconazole (FCZ), a significant reduction in fungal growth was observed, especially at lower concentrations (16 to 32 µg/mL), where the combination of OEOB + fluconazole was able to almost completely inhibit the growth of *C. albicans.*

On the other hand, in Fig. 2, in relation to the *C. krusei* (*Pichia kudriavzevii*)strain, the combination of OEOB with fluconazole (FCZ + OEOB) demonstrated a significant potentiating effect. The combination of OEOB with fluconazole resulted in a reduction in the growth of *C. krusei* starting at 2 µg/mL, showing a more pronounced effect than fluconazole alone at the same concentration. However, this combination was not able to completely inhibit fungal growth.

Based on the results of this study and previous research, such as that of Almeida et al. [16] and Miao et al. [17], it can be noted that the antifungal activity of OBEO against strains of the genus *Candida* varies. Considerable activity to little or no action was observed when the oil was used alone. This inconsistency can be explained, as suggested by Veloso et al. [18], by the chemical composition of OBEO, which describes that the concentrations of its active components can vary significantly due to genetic and environmental factors. This, in turn, directly influences its effectiveness against fungi.

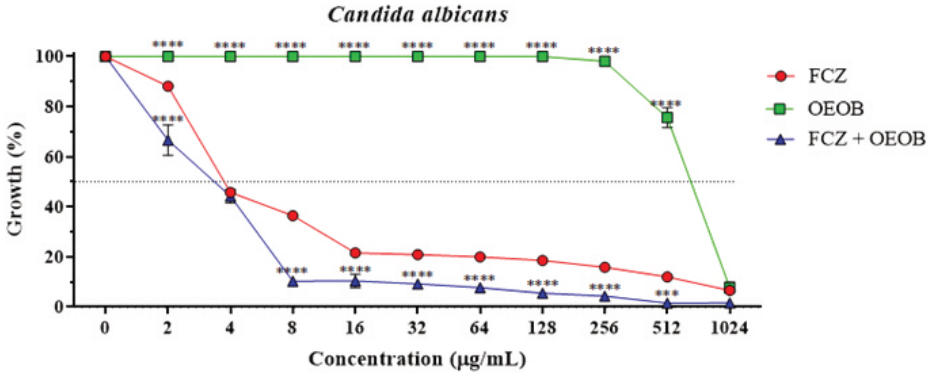

Figure 1. Cell viability curve of *Candida albicans* and IC_{50} value (dashed line) of different concentrations of *Ocimum basilicum* essential oil (OEOB), Fluconazole (FCZ), and their combination (FCZ + OEOB). *= p <0.05, *** = p <0.001, *** = p <0.0001. Bars indicate standard error of the mean (n=4).

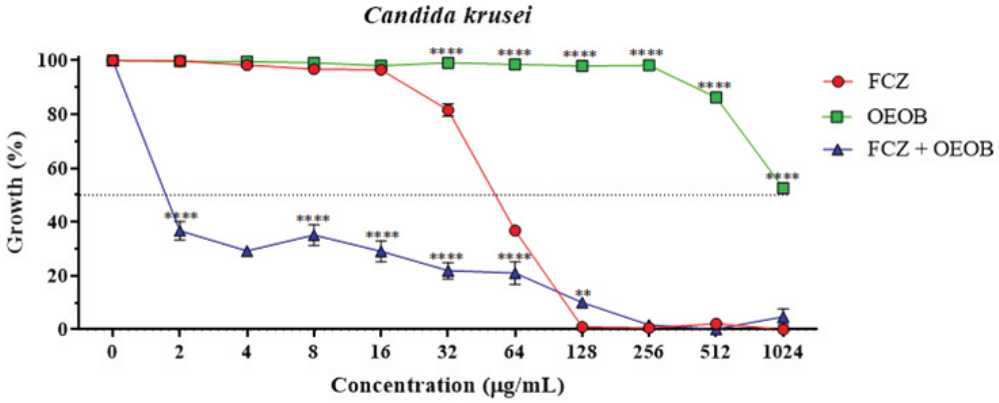

Figure 2. Cell viability curve of *Candida krusei* (*Pichia kudriavzevii*) and IC_{50} value (dashed line) of different concentrations of *Ocimum basilicum* essential oil (OEOB), Fluconazole (FCZ), and their combination (FCZ + OEOB). *= p <0.05, *** = p <0.001, *** = p <0.0001. Bars indicate standard error of the mean (n=4).

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However, the combination of OEOB with the antifungal fluconazole was able to synergistically reduce fungal growth, as seen in the data found in the study by Silva et al. [19], which suggest that the combination of OEOB, or isolated compounds such as geraniol and linalool, with fluconazole can result in a significant reduction in fungal growth in species of the genus *Candida*. This finding highlights the importance of a combined approach to overcoming challenges, considering the possibility of reducing the high concentrations of drugs that are associated with the emergence of major side effects [20].

This synergism is believed to occur due to damage to the cell wall and membrane of fungi, which facilitates the action of fluconazole. According to the study by Miao et al. [17], OEOB was able to damage the cell membrane of fungi, and in the study by Cardoso et al. [20], it was observed that OEOB was able to reduce ergosterol synthesis in *C. albicans* and *Cryptococcus neoformans*. Considering that the presence of ergosterol in the fungal membrane contributes to resistance and cell cycle regulation [21]. Facilitating drug entry is very promising, especially given the increased resistance of Candida species to azoles.

The results of this article indicate that OEOB can enhance the action of antibiotics and fluconazole against multidrug-resistant bacteria and *Candida* strains. This suggests that OEOB could be an adjunct to traditional therapies. However, the mechanisms of action and toxicity of OEOB need to be further investigated, as this study evaluated toxicity in only one model organism.

4. Conclusion

Ocimum basilicum essential oil (OBEO) demonstrated a notable synergistic effect when combined with fluconazole, significantly enhancing antifungal activity against Candida strains. These results suggest that OBEO may serve as a promising adjuvant in antifungal therapy, improving the efficacy of conventional drugs and contributing to strategies aimed at overcoming fungal resistance. Nonetheless, further studies are required, particularly *in vivo* experiments and pharmacokinetic assessments, to confirm its safety profile and clinical applicability. The observed activity reinforces the potential of plant-derived essential oils as valuable sources of new therapeutic agents against resistant fungal infections.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Antonelli, G., Cappelli, L., Cinelli, P., Cuffaro, R., Manca, B., Nicchi, S., ... & Schiavetti, F. (2021). Strategies to combat antimicrobial resistance: The example of Escherichia coli and Pseudomonas aeruginosa. International Journal of Molecular Sciences, 22 (9), 4943. https://doi.org/10.3390/ijms22094943.

2. Tambat, R., Mahey, N., Chandal, N., Verma, D. K., Jangra, M., Thakur, K. G., & Nandanwar, H. (2022). A microbe-derived efflux pump inhibitor of the resistance-nodulation-cell division protein restores antibiotic susceptibility in Escherichia coli and Pseudomonas aeruginosa. ACS infectious diseases, 8(2), 255-270. 10.1021/acsinfecdis.1c00281

3. Moran C, Grussemeyer CA, Spalding JR, Benjamin DK Jr, Reed SD. Candida albicans and non-albicans bloodstream infections in adult and pediatric patients: comparison of mortality and costs. Pediatr Infect Dis J. 2009;28(5):433-435. doi:10.1097/INF.0b013e3181920ffd

4. Batista Filha, A. J. A., dos Santos, J. A. B. ., de Almeida, K. A., Lopes, R. Q., de Sá, S. . ., & Macedo, G. B. . (2025). Essential oils and alcoholic extracts: a comparison of antifungal activity against Candida spp. Aracê, 7(6), 30213-30226. https://doi.org/10.56238/arev7n6-067

5. Brito, G. B., & Trevisan, M. (2021). The misuse of antibiotics and the imminent risk of bacterial resistance. Revista Artigos.Com, 30, e7902-e7902.

6. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils - a review. Food and Chemical Toxicology, 46 (2), 446-475.IOPPublishing, 2020. p. 012060. 10.1088/1742-6596/1524/1/012060Morais-Braga, M. F. B., Sales, D. L.,C302-313. https://doi.org/10.1016/jsjbs.2015.09.028

7. Bilia, A. R., Guccione, C., Isacchi, B., Righeschi, C., Firenzuoli, F., & Bergonzi, M. C. (2014). Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evidence-based complementary and alternative medicine: eCAM, 2014, 651593. https://doi.org/10.1155/2014/651593

8. Fronza, M., Porto, F. V. D. A., Pessoa, I. S., & Endringer, D. C. (2021). Essential oils as an alternative source of income in silvopastoral systems. In Integrated Production Systems: Research and Technology Development (Vol. 1, pp. 165-183). Editora Científica Digital. 10.37885/210605119

9. Maia, T. F., Donato, A. D., & Fraga, M. E. (2015). Antifungal activity of essential oils from plants. Revista Brasileira de Produtos Agroindustriais, 17(1), 105-116.

10. Abdallah, EM, Alhatlani, BY, de Paula Menezes, R., & Martins, CHG (2023). Back to nature: medicinal plants as promising sources of antibacterial drugs in the post-antibiotic era. Plants, 12 (17), 3077. https://doi.org/10.3390/plants12173077.

11. Fachriyah, E.; Wibawa, Pj; Awaliyah, A. Antibacterial activity of basil oil (Ocimum basilicum L.) and basil oil nanoemulsion. In: Journal of Physics: Conference Series. IOP Publishing, 2020. p. 012060. 10.1088/1742-6596/1524/1/012060

12. Morais-Braga, M. F. B., Sales, D. L., Carneiro, J. N. P., Machado, A. J. T., Dos Santos, A. T. L., de Freitas, M. A., Martins, G. M. A. B., Leite, N. F., de Matos, Y. M. L. S., Tintino, S. R., Souza, D. S. L., Menezes, I. R. A., Ribeiro-Filho, J., Costa, J. G. M., & Coutinho, H. D. M. (2016). Psidium guajava L. and Psidium brownianum Mart ex DC.: Chemical composition and anti - Candida effect in association with fluconazole. Microbial pathogenesis, 95, 200–207. https://doi.org/10.1016/j.micpath.2016.04.013

13. Morais-Braga, M. F., Carneiro, J. N., Machado, A. J., Sales, D. L., Dos Santos, A. T., Boligon, A. A., ... & Coutinho, H. D. (2017). Phenolic composition and medicinal usage of Psidium guajava Linn.: Antifungal activity or inhibition of virulence?. Saudi journal of biological sciences, 24(2), 302-313. https://doi.org/10.1016/j.sjbs.2015.09.028

14. Brito, D. I. V., Morais-Braga, M. F. B., Cunha, F. A. B., Albuquerque, R. S., Carneiro, J. N. P., Lima, M. S. F., Coutinho, H. D. M. (2015). Phytochemical analysis and antifungal activity of the essential oil from Lippia sidoides Cham. leaves and Thymol against Candida spp. strains. Revista Brasileira de Plantas Medicinais, 17(4 suppl 2), 836-844. https://doi.org/10.1590/1983-084X/14_060

15. Silva, JT. Da Costa, Meenezes, SA, Novais, MHG, Farias, NS, Costa, AR, Felix, FSH, ... & Almeida-Bezerra, JW Potential of the β-Myrcene-Rich essential oil of Astronium Urundeuva (M. Allemão) engl. (anacardiaceae) to potentiate fluconazole activity and inhibit morphological transition in Candida Species (2025). Current Pharmaceutical Biotechnology. http://dx.doi.org/10.2174/0113892010359736250707113515.

16. Almeida, LFD, Cavalcanti, YW, Castro, RD, & Lima, EO. (2012). Antifungal activity of essential oils against clinical samples of Candida albicans isolated from HIV-positive patients. Revista Brasileira De Plantas Medicinais, 14 (4), 649–655. https://doi.org/10.1590/S1516-05722012000400012

17. Miao Q, Zhao L, Wang Y, Hao F, Sun P, He P, Liu Y, Huang J, Liu X, Liu X, Deng G, Li H, Li L, Tang Y, Wang L, Feng M, Jia W. Microbial metabolomics and network analysis reveal fungistatic effect of basil (Ocimum basilicum) oil on Candida albicans. J Ethnopharmacol. 2020 Oct 5;260:113002. doi: 10.1016/j.jep.2020.113002

18. Veloso, R. A., Castro, H. G., Barbosa, L. C. A., Cardoso, D. P., Chagas Júnior, A. F., & Scheidt, G. N. (2014). Content and composition of the essential oil of four accessions and two cultivars of basil (Ocimum basilicum L.). Revista Brasileira de Plantas Medicinais, 16, 364-371. https://doi.org/10.1590/1983-084X/12_180

19. Silva, V. A.., Pereira da Sousa, J., de Luna Freire Pessôa, H., Fernanda Ramos de Freitas, A., Douglas Melo Coutinho, H., Beuttenmuller Nogueira Alves, L., & Oliveira Lima, E. (2016). Ocimum basilicum: Antibacterial activity and association study with antibiotics against bacteria of clinical importance. Pharmaceutical biology, 54(5), 863-867. https://doi.org/10.3109/13880209.2015.1088551

20. Cardoso, N. N., Alviano, C. S., Blank, A. F., Romanos, M. T. V., Fonseca, B. B., Rozental, S., ... & Alviano, D. S. (2016). Synergism effect of the essential oil from Ocimum basilicum var. Maria Bonita and its major components with fluconazole and its influence on ergosterol biosynthesis. Evidence-Based Complementary and Alternative Medicine, 2016(1), 5647182. https://doi.org/10.1155/2016/5647182

21. Eliaš, D., Tóth Hervay, N., & Gbelská, Y. (2024). The biosynthesis and regulation of ergosterol impact the antifungal resistance and virulence of Candida spp. stresses, 4 (4), 641-662. https://doi.org/10.3390/stresses4040041