Ministry of Higher Education and Scientific Research

University Of Basrah - College of Pharmacy

“ Synergism effects of Fluconazole with Vitamin E and Phellinus igniarius Mushroom Extract agenst Candida Albicans ”

BY

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الاهداء

إلي كل من أضاء بعلمه عقل غيره

أو هدى بالجواب الصحيح حيرة سائليه

فأظهر بسماحته تواضع العلماء

وبرحابته سماحة العارفين

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اهداء الى

الى الدكتور داوود جلوب البهادلي استاذ البحث الذي رافق خطواتنا الخيرة وساعدنا في ذلك و الصيدلاني عقيل وكل من كان له مساهمة في هذا العمل والجهد.

الشكر والتقدير

أشكر الله عز وجل وأحمده على توفيقه لأنجاز هذا العمل المتواضع والفضل و الشكر كله المكنا

كما أتقدم بالشكر الى عمادة كلية الصيدلة الموقرة

Abstract

Mushrooms have been widely used in traditional medicine for the treatment of various diseases. Today, their therapeutic value is scientifically studied and appreciated. Natural antioxidants are widely used in the life sciences. *Phellinus igniarius* is a historically used natural antioxidant containing a variety of active compounds. Phenols, particularly Inoscavin A and Hypholomine B, are found in the high concentrations. the well diffusion method to ascertain the antifungal activity of Phellinus igniarius and Fluconazole, both individually and in combination with Vitamin E. The well diffusion method demonstrated that the combination of *Phellinus igniarius* and fluconazole exhibited significantly higher antifungal activity(2.7)mm than ketoconazole alone (1.5)mm. *Phellinus igniarius* and vitamin E presents promising natural antifungal properties. Its synergistic action with fluconazole provides an effective approach to overcoming drug resistance and improving the treatment of fungal infections.Thus, in this study, we aimed to identify potential therapeutic effects of an extract of *Phellinus igniarius* as anti-Candida activity.

Introduction

Candida species are the most important factors of fungal infections in humans and animals. It is necessary to prepare antifungal or antimicrobial drugs because of increasing drug resistance. The natural treatment of diseases of bacterial origin using medicinal plants is important. In this study the effect of antimicrobial medicinal herbal extract powder solute and conventional antifungal drugs were evaluated on Candida albicans in vitro(1).

Natural antioxidants are widely used in the life sciences. *Phellinus igniarius* is a historically used natural antioxidant containing a variety of active compounds. Phenols, particularly Inoscavin A and Hypholomine B, are found in the high concentrations. Better quantitative methods are needed to perform quality control in order to support further research of this mushroom (2).

Mushrooms belonging to the genus Phellinus of the Hymenochaetaceae Basidiomycetes have been traditionally used as a medicine in Asia to treat inflammation and cancers, and their medicinal functions are currently being examined (3). The most outstanding finding of the medicinal function of the mushroom is that polysaccharides extracted from Phellinus linteus have a potent immune-modulating property, which is useful in the treatment of inflammation and cancers (4). Phellinus igniarius, another mushroom belonging to the genus Phellinus, has been also used as an herbal medicine in Asia and its immunoregulatory properties have been reported. Polysaccharides, especially -glucan, are believed to be responsible for the biological activity observed in these medicinal mushrooms (5).

Biological Activities of *Phellinus igniarius*

Phellinus igniarius displays a wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer properties (6). Its extracts contain various bioactive compounds such as phenolic compounds, polysaccharides, and triterpenoids, which contribute to its therapeutic potential. Among its diverse activities, the antifungal properties of Phellinus igniarius have attracted significant attention from researchers (4).

Antifungal Activity of Phellinus igniarius

The antifungal activity of Phellinus igniarius is attributed to the presence of bioactive compounds that disrupt fungal pathogens' cell wall and cell membrane integrity, leading to cell death (7). The antifungal properties of Phellinus igniarius have been found effective against various fungal species, such as *Candida, Aspergillus, and Trichophyton* (8).

Potentiation of Antifungal Activity with Fluconazole

Fluconazole, a widely recognized antifungal drug, is used to treat an array of fungal infections. The emergence of drug-resistant fungal strains has driven the need for innovative therapeutic strategies (9).

Studies have shown that combining Phellinus igniarius with ketoconazole can enhance the antifungal efficacy of the drug. This promising approach may help combat drug-resistant fungal infections. The synergistic action of Phellinus

igniarius and fluconazole is attributed to their different modes of action, targeting distinct cellular structures in fungal pathogens (10)

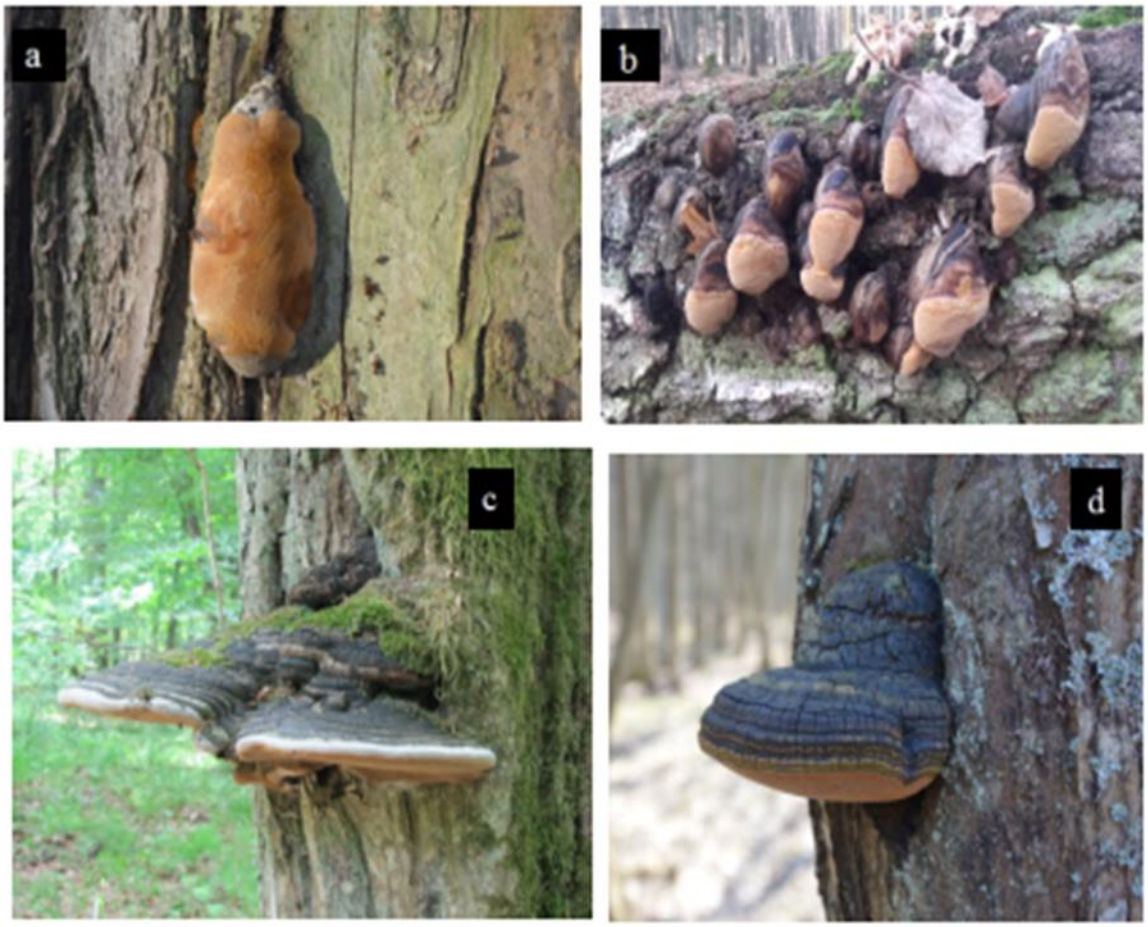

Figure 1: *P igniarius* growing in Bialowieza Forest in Poland. a) Young fruiting body on living *Salix fragilis*. b) On the dead trunk of *Betula pendula*. c) Over ten years old specimen on living *Carpinus betulus*,
d) Older specimen on living *Carpinus betulus*. Habitats: alluvial alder and ash forests, *Fraxino-Alnetum* (a) and oak and hornbeam forests, *Tilio-Carpinetum* (b-d)
All pictures are from the collection of Marek Wolkowycki

Material and Methods

Materials:

1. Phellinus Igniarius Extract (1000 mg)
2. Vitamin E powder (100 mg/wt), sourced from Pioneer Drugs Company
3. Fluconazole (100 mg/wt)
4. DMSO solution, used as a solvent
5. Isolated strain of *Candida albicans.*

Methods:

Our study utilized the well diffusion method to ascertain the antifungal activity of Phellinus igniarius and Fluconazole, both individually and in combination with Vitamin E. This method is a widely accepted technique for assessing the antimicrobial properties of various substance (11).

In this process, agar plates were first prepared and inoculated with the isolated strain of Candida albicans. Following the inoculation, wells were carefully created in the agar using a sterile borer. These wells served as reservoirs for the test samples (12).

The test samples, which included the Phellinus igniarius extract, Fluconazole, Vitamin E, and their combinations, were then introduced into the wells. Care was taken to avoid any contamination during this process.

Following the introduction of the test samples, the agar plates were incubated at a suitable temperature for a predetermined period. This allowed for the interaction between the test samples and the fungal strains (13).

Post incubation, the antifungal activity was evaluated by measuring the zones of inhibition around the wells. These zones, characterized by the absence of fungal

growth, indicated the effectiveness of the test samples against the fungal strains. Larger zones of inhibition were indicative of higher antifungal activity(14).

This methodology allowed us to not only assess the antifungal activity of Phellinus igniarius and Fluconazole individually but also evaluate their combined effect with Vitamin E, providing a comprehensive understanding of their potential use in antifungal treatments (15).

In this study, we embarked on an exploration of the antifungal potential of Vitamin E, and Phellinus igniarius extract solution. Our primary focus was to discern the impact of varying concentrations of Vitamin E on the antifungal activity of these combinations (16).

Preparation of Solutions:

Our initial step was the meticulous preparation of individual stock solutions for each of our key materials: Vitamin E, and Phellinus igniarius extract. We dissolved each material in DMSO solvent, creating uniform solutions that could be easily adjusted for concentration. From the Vitamin E stock solution, we derived a series of diluted solutions, each representing a different concentration. Starting from the stock concentration of 100% (equivalent to 0.01 gm), we prepared solutions with concentrations of 100%, 75%, 50%, 25%, 12.5%, and 6.25%.

Experimental setup

The experimental design involved the use of agar plates, each featuring three wells. These wells were filled with different combinations of the test materials, providing a platform to observe the antifungal effects of each combination (17).

The combinations tested were as follows:

1. Well 1: A combination of Phellinus igniarius extract solution and Fluconazole, serving as a control to benchmark the effects of the other combinations.

2. Well 2: A combination of Vitamin E and Phellinus igniarius extract solution, designed to investigate potential synergistic effects.

3. Well 3: A combination of Phellinus igniarius extract solution, Fluconazole, and Vitamin E, aimed at testing the hypothesis that Vitamin E could enhance the antifungal activity of the standard treatment.

Each agar plate was dedicated to testing a specific concentration of the Vitamin E solution. For ease of identification and accuracy, the plates were labeled according to the Vitamin E concentration being tested, ranging from 100% to 6.25%.

This experimental setup facilitated a systematic evaluation of the antifungal activity of the different combinations of materials at various concentrations of Vitamin E. The results provided a comprehensive understanding of their potential synergistic effects, paving the way for future research in this area."

Result and Discussions

The well diffusion method demonstrated that the combination of *Phellinus igniarius* and fluconazole exhibited significantly higher antifungal activity than ketoconazole alone, even against drug-resistant strains. This enhanced effect may lead to lower doses of fluconazole, reducing the risk of side effects and drug resistance. Moreover, using natural products like *Phellinus igniarius* could potentially minimize the environmental impact associated with the production and use of synthetic antifungal agents.the results showed that analyzed mushroom extract exhibit antimicrobial activity. With an increasing number of yeasts that have developed resistance to commercial antibiotics, extracts and derivatives from mushrooms hold great promise for novel medicines. Regarding the development of natural antimicrobials from macrofungi, further research could be focused on the identification and quantification of individual antimicrobial compounds in the selected mushroom extract.

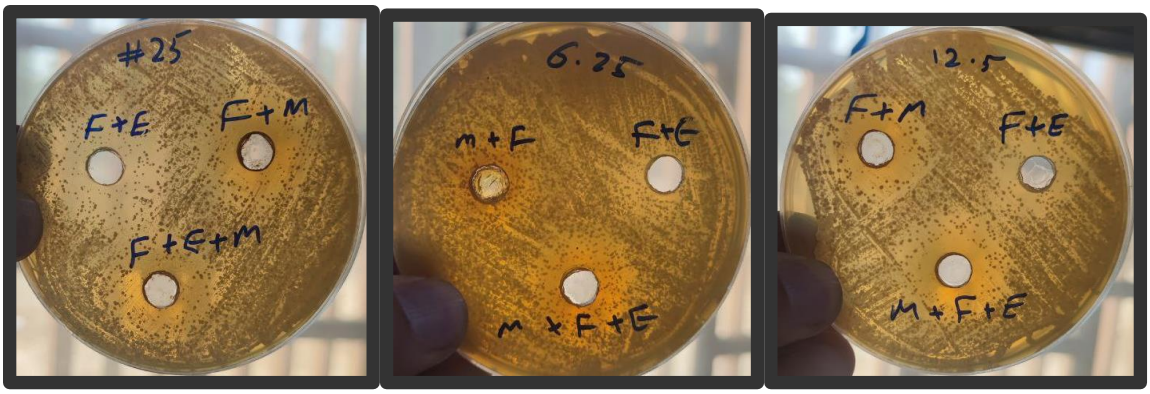

Figure 4.26 Inhibition zone in (mm) by anticandidal effect of Fluconazole, Phellinus igniarius extract solution at different concentrations of Vitamin E (µg/ml) against C. albicans (A). 0.01 µg/ml (B) 0.0075 µg/ml. (C) 0.00375 µg/ml. (D0.0009375 µg/ml. (E) 0.0001171875 µg/ml (F) 7.32 ng/ml

Represents the inhibition zone in (mm) of different concentrations of vitamine E (µg/ml) against pathogenic Candida spp. :

F= Fluconazole only

F+E =Fluconazole + vitamin E

F+M=Fluconazole + Phellinus igniarius extract solution

F+M+E= Fluconazole + Phellinus igniarius extract solution + vitamin E

Inhibition zone in (mm) by anticandidal effect of Fluconazole, Phellinus igniarius extract solution at different concentrations of Vitamin E (µg/ml) against C. albicans (A). 0.01 µg/ml (B) 0.0075 µg/ml. (C) 0.00375 µg/ml. (D0.0009375 µg/ml. (E) 0.0001171875 µg/ml (F) 7.32 ng/ml

No.	Vit.E conc.	100%	75%	50%	25%	12.5%	6.25%
1	F	1.5	-	-	-	-	-
2	F+E	2	1.8	1.5	2.5	2	1.8
3	F+M	1.7	-	2	2.5	2	-
4	F+M+E	2.1	2.7	2.3	2.2	2.5	2.2

Conclusion

Phellinus igniarius and vitamin E presents promising natural antifungal properties. Its synergistic action with fluconazole provides an effective approach to overcoming drug resistance and improving the treatment of fungal infections. The well diffusion method demonstrates the potential of this combination against various fungal strains, including drug-resistant ones. Further research is required to better understand the underlying mechanisms and optimize the therapeutic potential of this potent combination.

Reference

1.

de Oliveira Santos GC, Vasconcelos CC, Lopes AJO, de Sousa Cartágenes M do S, Filho AKDB, do Nascimento FRF, et al. Candida Infections and Therapeutic Strategies: Mechanisms of Action for Traditional and Alternative Agents. Frontiers in Microbiology. 2018 Jul 3;9(9).

2.

Shou D, Dong Y, Wang N, Li H, Zhang Y, Zhu Y. Simultaneous Quantification of Antioxidant Compounds in Phellinus igniarius Using Ultra Performance Liquid Chromatography-Photodiode Array Detection-Electrospray Ionization Tandem Mass Spectrometry. Gill AC, editor. PLOS ONE. 2016 Sep 30;11(9):e0163797.

3.

Sonawane H, Arya S, Ghole V, Apte K, Shelke D, Chaskar M. Hypoglycemic and anticataract activity of crude exopolysaccharides of medicinal mushroom Phellinus badius on streptozotocin-induced diabetic rats and goat eye lenses respectively. Bioactive Carbohydrates and Dietary Fibre. 2020 Oct;24(October 2020, 100241):100241.

4.

Chen W, Tan H, Liu Q, Zheng X, Zhang H, Liu Y, et al. A Review: The Bioactivities and Pharmacological Applications of Phellinus linteus. Molecules [Internet]. 2019 May 16 [cited 2020 Jun 28];24(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6572527/

5.

Kachrimanidou V, Papadaki A, Alexandri M, Poulios V, GonouZagou Z, Kopsahelis N. Sepedonium sp. and Phellinus sp. Novel Isolates: Growth Pattern and Production of PolysaccharideProtein Complexes on Conventional and Grape Pomace Substrates. Waste and Biomass Valorization [Internet]. 2023; Available from: https://doi.org/10.1007/s1264902202017z

6.

Zapora E, Wolkowycki M, Bakier S, Zjawiony JK. Phellinus igniarius: A Pharmacologically Active Polypore Mushroom. Natural Product Communications. 2016 Jul;11(7):1934578X1601100.

7.

Palmieri D, Ianiri G, Del Grosso C, Barone G, De Curtis F, Castoria R, et al. Advances and Perspectives in the Use of Biocontrol Agents against Fungal Plant Diseases. Horticulturae. 2022 Jun 25;8(7):577.

8.

Lin L, Xu J. Fungal Pigments and Their Roles Associated with Human Health. Journal of Fungi. 2020 Nov 12;6(4):280.

9.

Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bignell EM, Bowyer P, et al. Tackling the emerging threat of antifungal resistance to human health. Nature Reviews Microbiology. 2022 Mar 29;20.

10.

Aabed K, Mohammed AE. Synergistic and Antagonistic Effects of Biogenic Silver Nanoparticles in Combination With Antibiotics Against Some Pathogenic Microbes. Frontiers in Bioengineering and Biotechnology. 2021 Apr 20;9.

11.

Manandhar S, Luitel S, Dahal RK. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. Journal of Tropical Medicine. 2019 Apr 2;2019:1–5.

12.

Naseem S, Douglas L, Konopka J. Candida albicans Agar Invasion Assays. BIO-PROTOCOL. 2020;10(16).

13.

Berkow EL, Lockhart SR, Ostrosky-Zeichner L. Antifungal Susceptibility Testing: Current Approaches. Clinical Microbiology Reviews. 2020 Apr 29;33(3).

14.

Sanchez Armengol E, Harmanci M, Laffleur F. Current strategies to determine antifungal and antimicrobial activity of natural compounds. Microbiological Research. 2021 Nov;252:126867.

15.

AV Holanda M, R da Silva C, B de A Neto J, G do AV Sá L, BSA do Nascimento F, D Barroso D, et al. Evaluation of the antifungal activity in vitro of midazolam against fluconazole-resistant Candida spp. isolates. Future Microbiology. 2021 Jan;16(2):71–81.

16.

Campanini M, Renata Evangelista Tavares, Ricardo D, Lorena Rigo Gaspar, Hosana Maria Debonsi. Photoprotective potential of metabolites isolated from algae-associated fungi Annulohypoxylon stygium. 2018 Jan 1;178:316–22.

17.

Vallières C, Raulo R, Dickinson MB, Avery SV. Novel Combinations of Agents Targeting Translation That Synergistically Inhibit Fungal Pathogens. 2018 Oct 4;9.