Evaluation of Antifungal Effects of *Phellinus igniarius* Extract on *Candida albicans*

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ABSTRACT

One of the key goals in treating illnesses like candidiasis and other infections associated with Candida is the development of novel antifungal drugs manufactured from natural substances. Current treatments for illnesses like candidiasis and other infections associated with *Candida* have detrimental effects on human health. Thus, discovering novel antifungal medications from natural sources is urgently needed. This study w to was carried out to evaluate the efficiency of a powder extract of *Phellinus igniarius* against drug-resistant *Candida albicans* isolates. This plant is widely utilized in Asian countries and has shown promising pharmacological capabilities.The efficacy of *Phellinus igniarius* compared with ketoconazole alone and combined with it, A systemic candidiasis mouse model was used to assess *Phellinus igniarius'* efficacy in vitro and in vivo in treating systemic *Candida albicans* infections when compared to ketoconazole alone and when combined with it. In vitro minimum inhibitory concentrations (MICs) determined. The antifungal efficacy was determined by measuring the zone of inhibition in a well diffusion experiment. The extract showed strong inhibitory efficacy at ratios of 250:0.2, 500:0.2, and 1000:0.2 against *Candida albicans* resistance. In contrast, a modest synergistic impact was shown at only 125:0.2 and 0.2 ketoconazole ratios. In addition, the minimum inhibition concentration of each antifungal agent was shown to be decreased when combined with a powder extract of *Phellinus igniarius*, indicating the presence of synergistic interactions. The rate at which the wounds of the different groups of rats injected with candida healed differed significantly from one another. This group was used as the control and received only the vehicle as treatment. Another group was given ketoconazole as the only drug, while the remaining four groups were given *Phellinus igniarius* in combination with ketoconazole in varied dosages.The ratios of 250 to 0.2, 500 to 0.2, and 1000 to 0.2 provided the greatest synergistic impact. A minuscule synergistic impact was seen at ratios of 125 to 0.2. To manage infections caused by Candida species while minimizing their hazardous side effects, it is possible to adopt a therapy including combinations of powder extract of *Phellinus igniarius* and traditional antifungal medications.

Keywords: *Phellinus igniarius*, Antifungal activity, *Candida albicans, ketoconazole, synergistic effect; naturals products*

INTRODUCTION

More than 0.2 million individuals are diagnosed each year with dangerous fungal infections, and around 1.7 million lose their lives. Alarmingly, these figures keep rising and increasing. This is because advancements in medicine and culture over the past several decades have facilitated the spread of fungal infections. Additionally, multidrug-resistant fungi, such as the deadly strain Candida, have emerged due to the prolonged use of antifungal medicines for treatment and prevention in high-risk individuals. As a result, fungal diseases pose a growing threat worldwide [1].

The opportunistic infections of the genus Candida are a leading cause of illness and death worldwide [2]. Vaginitis, oral candidiasis, cutaneous candidiasis, candidemia, and systemic infections are also caused by Candida species [3]]. studies on Candida blood stream infections (BSIs) have expanded our knowledge, Candida species rated fourth overall among etiological agents in a large database of nosocomial BSIs in the United States, and was the third most common cause of such infections in intensive care units (ICUs) [4]. The yeast *Candida albicans* is the most common pathogen found in isolation, Progressive isolation of other species has occurred, particularly in people infected with the human immunodeficiency virus (HIV) [5]. These include *C. glabrata, C. parasitosis, C. tropicalis, C. krusei, C. lusitaniae, C. dubliniensis, and C.guilliermondii* ,there is a lack of knowledge about the pathophysiology of *Candida* species, and the number of infections is rising fast[6].

Candida infections must be managed by early identification and prophylaxis due to a constant rise in antifungal resistance [7].

Azoles are the most effective and widely used antifungal medicines for treating Candida infections. Antifungals treat fungal infections. However, not all species are equally sensitive to the same drugs. Among these are allylamines, polyenes, echinocandins, and nucleoside analogs [8]. In treating Candida infections, azole ketoconazole (KTZ) is often used because of its inexpensive cost, minimal toxicity, and extensive availability in various forms [9]. Identifying fungi's resistance mechanisms is essential for developing new Candida antifungals, however, many studies have documented Candida species' azole resistance, resistance has caused new fungal infections and the recurrence of previous ones; thus, a more effective medicine is needed, this makes Candida infection control difficult in modern hospitals. To solve this challenge, innovative therapies based on clinically proven drugs and novel antifungal chemicals must be discovered to offer safer, more effective treatments, novel antifungals are needed to stop drug resistance, many potent medications are under development, therefore there is hope, as the examine some potential novel therapies in early clinical trials.[10].

Commonly known as "*P. igniarius*," the fungus *Phellinus igniarius* has been a traditional medicine in Asia for centuries [11]. The pharmacological characteristics of *P. igniarius* have just come to light, these characteristics range from anticancer to anti-influenza virus to anti-inflammatory and antioxidant to immunomodulatory [12, 13]. Using the well diffusion method, methanol and aqueous extracts of the Phellinus fruit body were tested for antibacterial and antifungal activity against five human pathogenic bacteria and five fungal pathogens, revealing a valuable zone of inhibition. [14].

The effect of antioxidants on antifungal agent's reaction medium can be studied to improve its therapeutic efficacy and stability according to Biémont [15]. Polysaccharides from *P. igniarius* have been shown to exhibit natural antioxidant properties, according to a study by (Ming-Yeou LungJie-Chung Tsai).[16]. Therefore, the aim of this study to show that *Phellinus igniarius*, both on its own and in combination with ketoconazole, is effective against fungal infections.

1.1 Materials and Methods:

No.	Materials	Origin
1	Ketoconazole	Pioneer Co./Iraq
2	Willow Bracket (Phellinus igniarius) Mushroom Extract Powder	Hard Rhino/USA
3	Sabouraud dextrose aga	TM Media /India
4	DMSO solution	TM Media /India
5	An isolated strain of Candida albicans, OK631832 C. albicans isolates.	The University of Basrah College of Science and Biology

1.2 Well diffusion method:

This study utilized the well diffusion method to ascertain the antifungal activity of *Phellinus igniarius* and Ketoconazole. This method is widely accepted for assessing various substances' antimicrobial properties.

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 [17].

The test samples, including the *Phellinus igniarius* extract, ketoconazole, and their combinations, were 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 at 35 ◦C for 24 hours. This allowed interaction between the test samples and the fungal strains [18].

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 [19].

This methodology allowed us to individually assess the antifungal activity of Phellinus igniarius and ketoconazole and evaluate their combined effect, providing a comprehensive understanding of their potential use in antifungal treatments [20].

CANDIDA ALBICANS SUSCEPTIBILITY TESTING USING KETOCONAZOLE AND PHELLINUS IGNIARIUS IN COMBINATION. (IN VITRO):

Agar-well diffusion assays were used to compare the effectiveness of ketoconazole alone against *Candida albicans* to the effectiveness of ketoconazole combined with *Phellinus igniarius* powder. Wells were drilled into Mueller-Hinton agar plates using a sterile cork borer of 5 mm diameter. Swabs of a fungal suspension with a McFarland standardization factor 0.5 contaminate a Mueller-Hinton agar plate. For the initial activity test of *Phellinus igniarius* powder, 50µ litter of the 0.2 mg/ml ketoconazole solution was added to each well, followed by 50µ Litters of the 125, 250, 500, and 1000 mg/ml *Phellinus igniarius* with 0.2 mg/ml Ketoconazole as combinations.

Table 1. compound used in well diffusion method:

Group	Compounds
A	Vehicle control (Negative control)
B	KTZ 0.2 mg /0.05 ml. (Positive control)
C	Phe. 1000 mg / (0.05) milliliters with KTZ (0.2 mg) / (0.05 ml).
D	Phe. 500 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).
E	Phe. 250 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).
F	Phe. 125 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).

Where Phe. = *Phellinus igniarius, and* KTZ. = Ketoconazole

Overnight incubation at 37 degrees Celsius [21] lead to in the antifungal activity being measured by the size of the inhibitory zone [22].

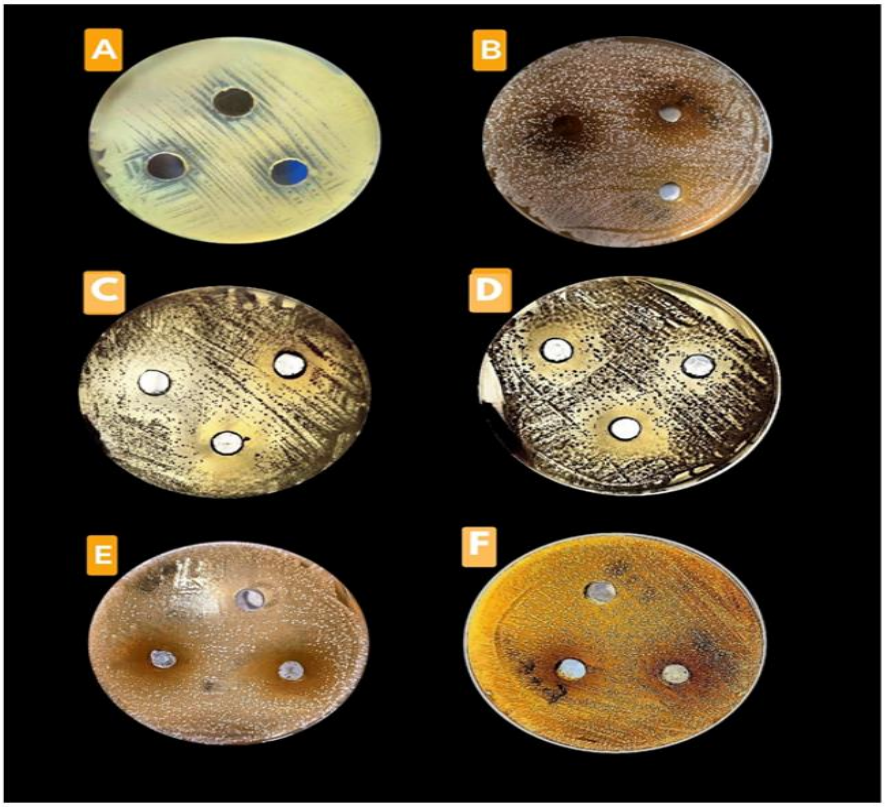
PHELLINUS IGNIARIUS WITH KETOCONAZOLE ON (ANIMAL MODEL'S) IN VIVO:

The Mice were shaved on the back, and two injection sites were chosen at random. The mice were then randomized into seven groups with a total of six individuals in each group. The *Candida albicans*, OK631832 yeast stock suspension, which contained between one and five times 106 cells/ml, was injected into the groups (A, B, C, D, E, and F), respectively. The treatment is prepared by dissolving (0.2 grams of ketoconazole) in 2 milliliters of dimethyl sulfoxide (DMSO), and finishing the solution with glycerin for the positive control group. For the other groups, the treatment is prepared by dissolving (0.2 grams of ketoconazole) and specific different concentrations (1000,500,250,125) mg of *Phellinus igniarius* powder.

Phellinus igniarius powder, 50µl of the 0.2 mg/ml ketoconazole solution was added to each well, followed by 50µl of the 125, 250, 500, and 1000 mg/ml *Phellinus igniarius* with 0.2 mg/ml ketoconazole as combinations. The following are the specifics: Phe. = Phellinus igniarius, KTZ. = Ketoconazole (A) Vehicle negative control, (B) KTZ 0.2 mg /0.05 ml (positive control). (C) Phe. 1000 mg / (0.05) milliliters with KTZ (0.2 mg) / (0.05 ml). (D) Phe. 500 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml). (E) Phe. 250 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml). (F) Phe. 125 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).

RESULT AND DISCUSSION

Ketoconazole and *Phellinus igniarius*, at varying doses, were put through a series of tests to see how well they worked against Candida albicans. The ratios 250: 0.2, 500: 0.2, and 1000: 0.2 all showed the highest levels of synergy when compared at ratios of 125:0.2 and KTZ alone, a marginally significant synergistic effect was detected Figure [1]. It has been shown that the antifungal activity of ketoconazole and *Phellinus igniarius rises* to a higher level as the concentrations rise. While in figure [2]. shows the effects of *Phellinus igniarius* alone against *Candida albicans*.



Phe. = *Phellinus igniarius*, KTZ. = Ketoconazole

Figure [1] Activity of Ketoconazole or Ketoconazole in combination with *Phellinus igniarius* Against *C. albicans* by the agar well diffusion method.

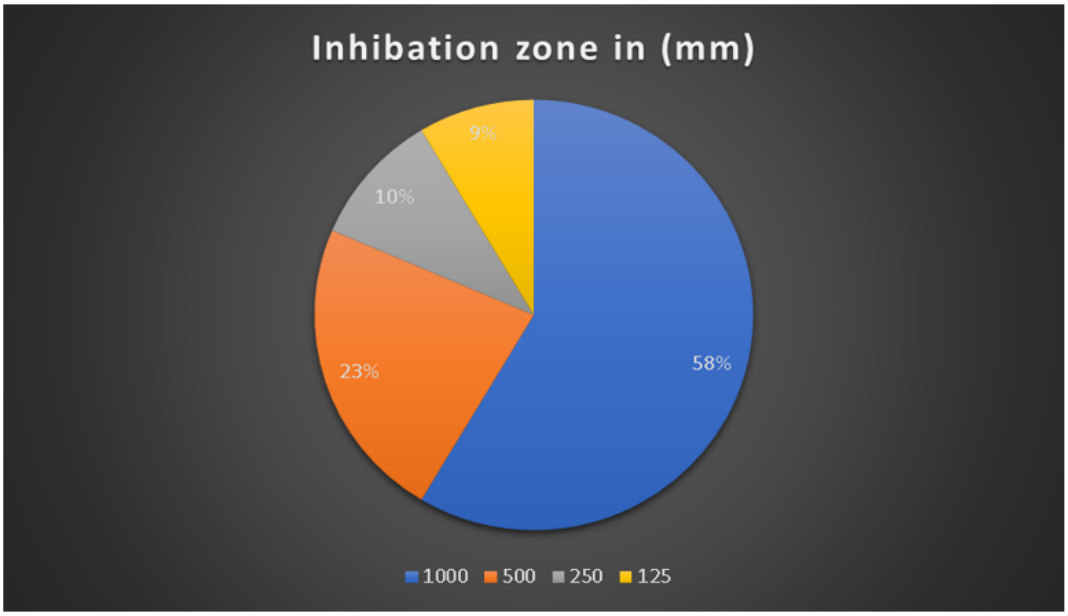
(A) Vehicle control, (B) KTZ 0.2 mg /0.05 m Positive control.

(C) Phe. 1000 mg /0.05 ml with KTZ 0.2 mg /0.05 ml.

(D) Phe. 500 mg /0.05 ml with KTZ 0.2 mg /0.05 ml.

(E) Phe. 250 mg /0.05 ml with KTZ 0.2 mg /0.05 ml.

(F) Phe. 125 mg /0.05 ml with KTZ 0.2 mg /0.05 ml.

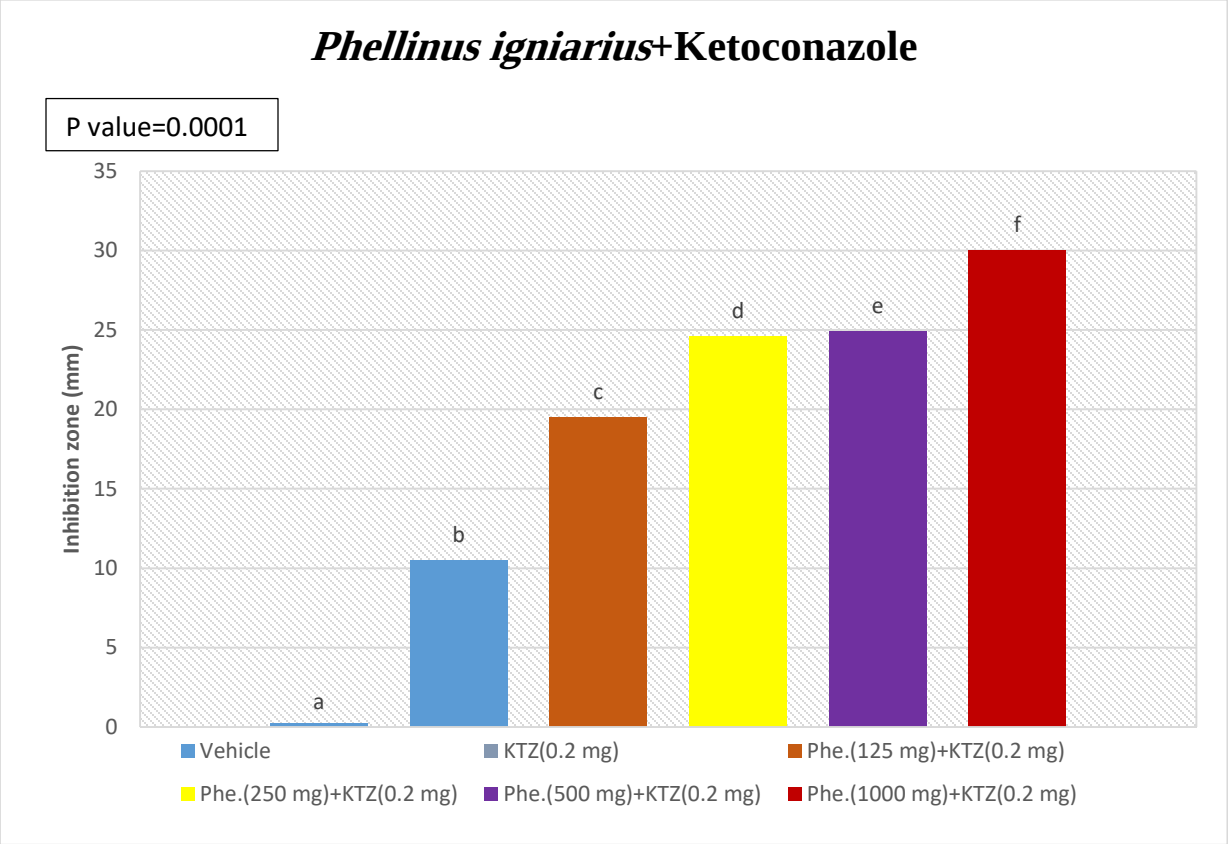


Phe. = *Phellinus igniarius*

Figure [2] Activity of *Phellinus igniarius* Against *C. albicans* by the agar well diffusion method.

- **Phe. 1000mg / (0.05 ml)**
- **Phe. 500mg / (0.05 ml)**
- **Phe. 250mg / (0.05 ml)**
- **Phe. 125mg / (0.05 ml)**

The effects of *Phellinus Igniarius* alone against *Candida Albicans*, was shown in figure [2]. the effect of *Phellinus Igniarius* was increasing by increasing the concentration of *Phellinus Igniarius*; the concertation of Phe. 1000 mg /0.05 ml show the best effect of inhibition zone in (mm) 58%, while the lowest effect obtained by the concentration Phe. 125 mg /0.05 ml (9%), as shown in (figure. 2) and table [2]



Phe. = *Phellinus igniarius*, KTZ. = Ketoconazole

Figure [3] Activity of Ketoconazole or Ketoconazole in combination with *Phellinus igniarius* to counteract *Candida albicans* using agar well diffusion.

The graph shows the anticandidal effects of the combination of 50 µ litter of the 125,250,500 and 1000 mg/ml *Phellinus igniarius* with 0.2 mg/0.5ml Ketoconazole on *candida albicans*. The statistically significant difference (P value = 0.0001). This means that the difference in inhibition zone between the different concentrations of *Phellinus igniarius* is statistically significant.

The mean inhibition zone of *Phellinus igniarius* (1000mg) was the highest (23.75±0.29), and the lowest was of concentration (125 mg) was (14.4±0.4), this relation was statistically significant (ANOVA test F= 491.1013, P value = 0.0001), as shown in table [3].

Table 2. Antifungal activity of *Phellinus igniarius* against *Candid albicans*.

***Phellinus igniarius* (mg)**	**Inhibition zone (mm)**			**Mean± SD**
1000	**23.5**	**23.5**	**23**	**23.75±0.29**
500	**20.2**	**20.3**	**20**	**20.1±0.15**
250	**17.7**	**17.4**	**17**	**17.3±0.35**
125	**14.8**	**14.4**	**14**	**14.4±0.4**

ANOVA test F= 491.1013, P value = 0.0001

Table 3. Antifungal activity of ketoconazole with *Phellinus igniarius* against *Candida albicans*.

***Phellinus igniarius* (mg): Ketoconazole (mg)**	**Inhibition zone (mm)**			**Mean± SD**
1000:0.2	**30.9**	**30.5**	**30**	**30.46±0.45**
500:0.2	**24.9**	**24.8**	**24**	**24.5± 0.49**
250:0.2	**24.6**	**24.1**	**24**	**24.2±0.32**
125:0.2	**19.8**	**19.5**	**19**	**19.4± 0.4**

SD; Standard deviation, ANOVA analysis; F=349.1813, P value =0.0001

In order to test the synergistic effect of the ketoconazole and *Phellinus Igniarius*, with most effective concentration; the following concentrations were tested on agar. The best effect obtained by the concentration of Phe. 1000 mg /0.05 ml with KTZ 0.2 mg /0.05 ml. with inhibition zone of (30.46±0.45mm), and the lowest found with the concentration of 125:0.2 with (19.4± 0.4mm), the difference

between groups was statistically significant, as shown in table [3], and figure [3].

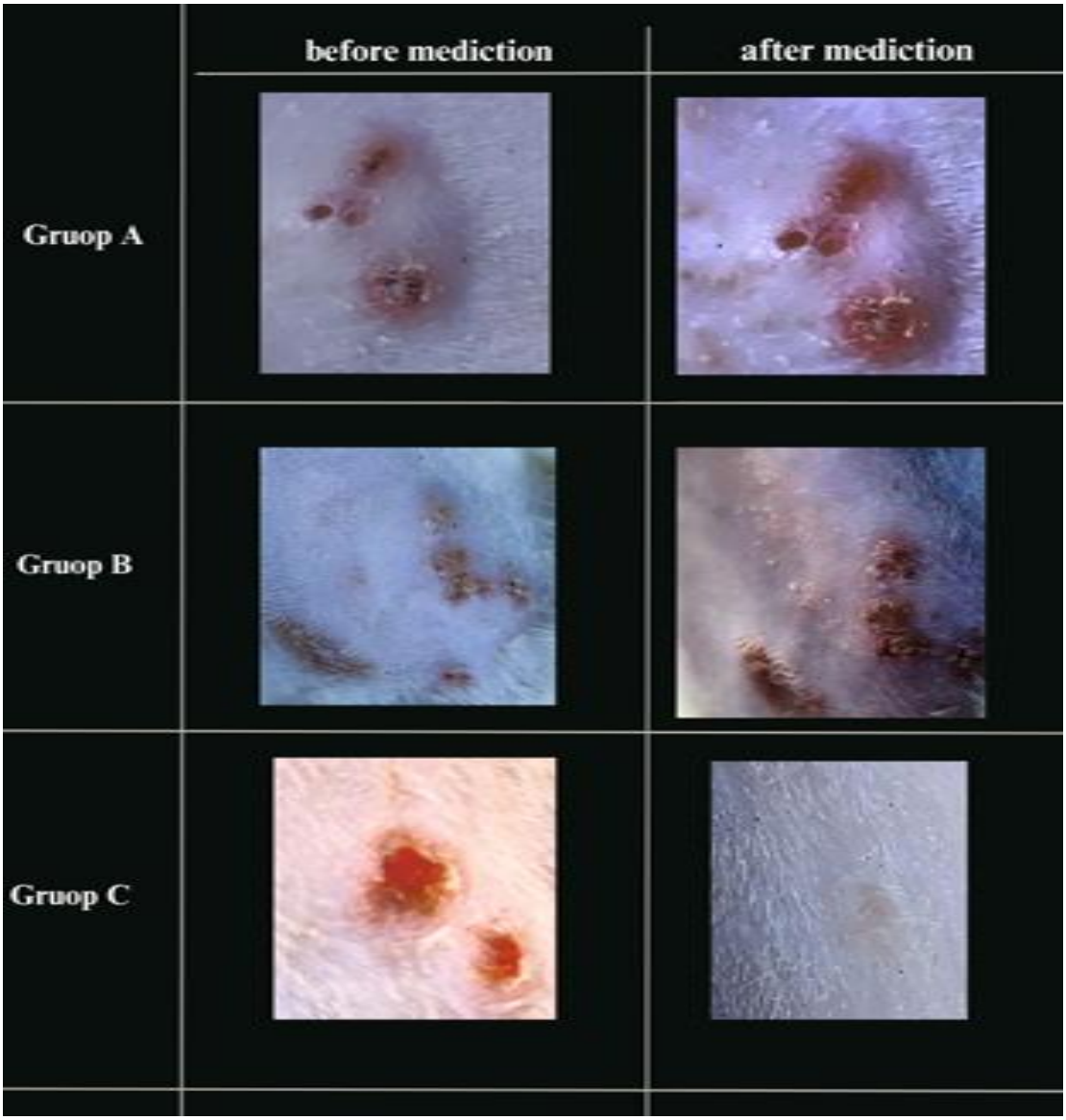


Figure [4.1] Rat skin before and after treatment fungal infection with *Candida albicans*.

(A) Vehicle negative control DMSO, (B) KTZ [positive control] 0.2 mg /0.05 ml. (C) Phe. 1000 mg / (0.05) milliliters with KTZ (0.2 mg) / (0.05 ml).

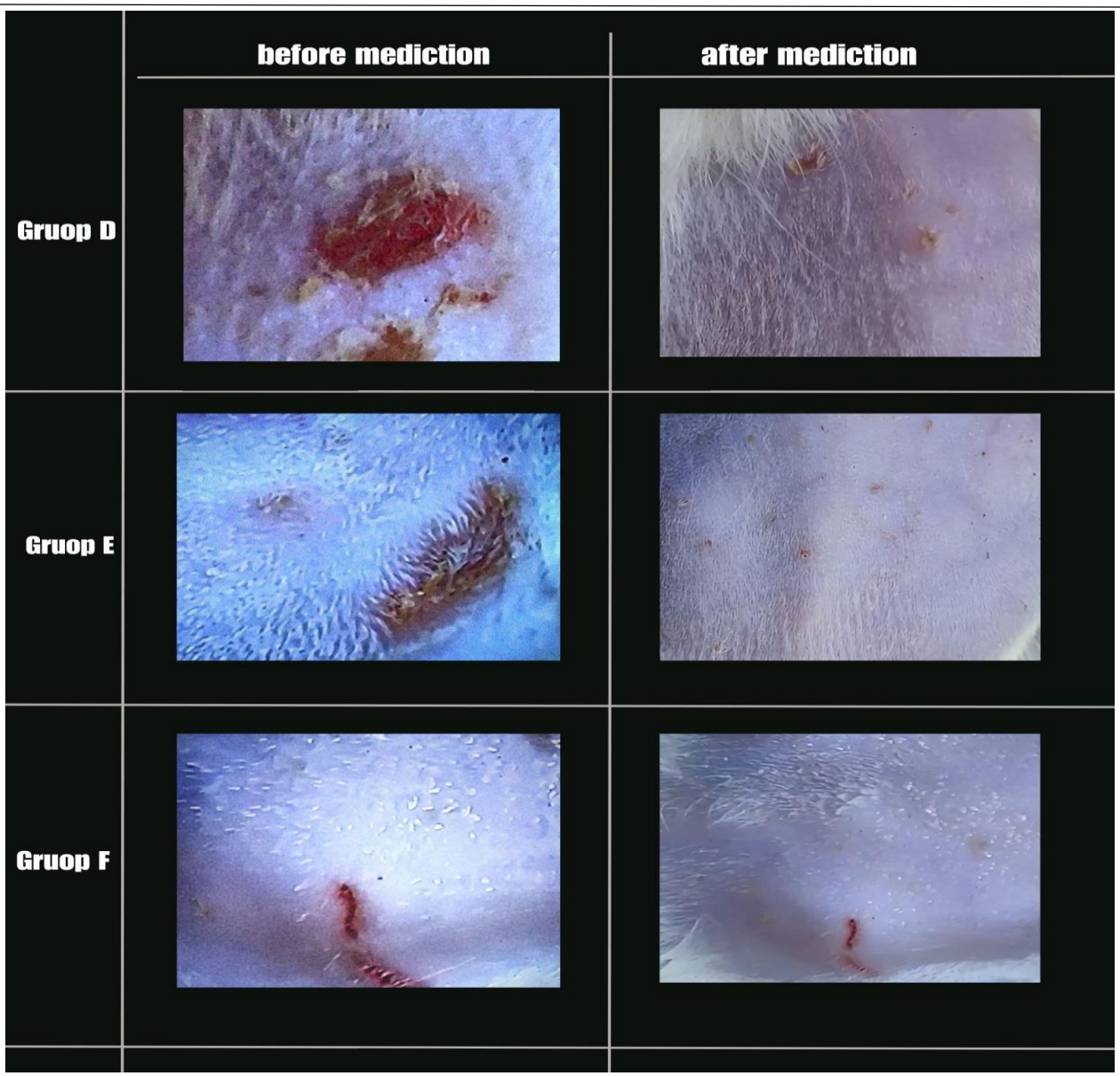


Figure [4.2] Rat skin before and after treatment fungal infection with *Candida albicans*

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(D) Phe. 500 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml). (E) Phe. 250 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).(F) Phe. 125 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).

ASSESSMENT OF THE MEDICATION'S EFFICACY AGAINST CANDIDA SPECIES IN LIVING SUBJECTS IN VIVO.

The rate at which the wounds of the different groups of rats injected with candida healed differed significantly from one another. This group was used as the control and received only the vehicle as treatment. Another group was given ketoconazole as the only drug as positive control, while the remaining four groups were given *Phellinus igniarius* in combination with ketoconazole in varied dosages. (A) Vehicle negative control, (B) KTZ 0.2 mg /0.05 ml (positive control). (C) Phe. 1000 mg / (0.05) milliliters with KTZ (0.2 mg) / (0.05 ml). (D) Phe. 500 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml). (E) Phe. 250 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml). (F) Phe. 125 mg / (0.05) milliliters with KTZ (0.2 mg)/ (0.05 ml).

The effectiveness of ketoconazole and *Phellinus igniarius* in combination with *Candida albicans* was evaluated using a range of concentrations.

The ratios of 250 to 0.2, 500 to 0.2, and 1000 to 0.2 provided the greatest synergistic impact. A minuscule synergistic impact was seen at ratios of 125 to 0.2.

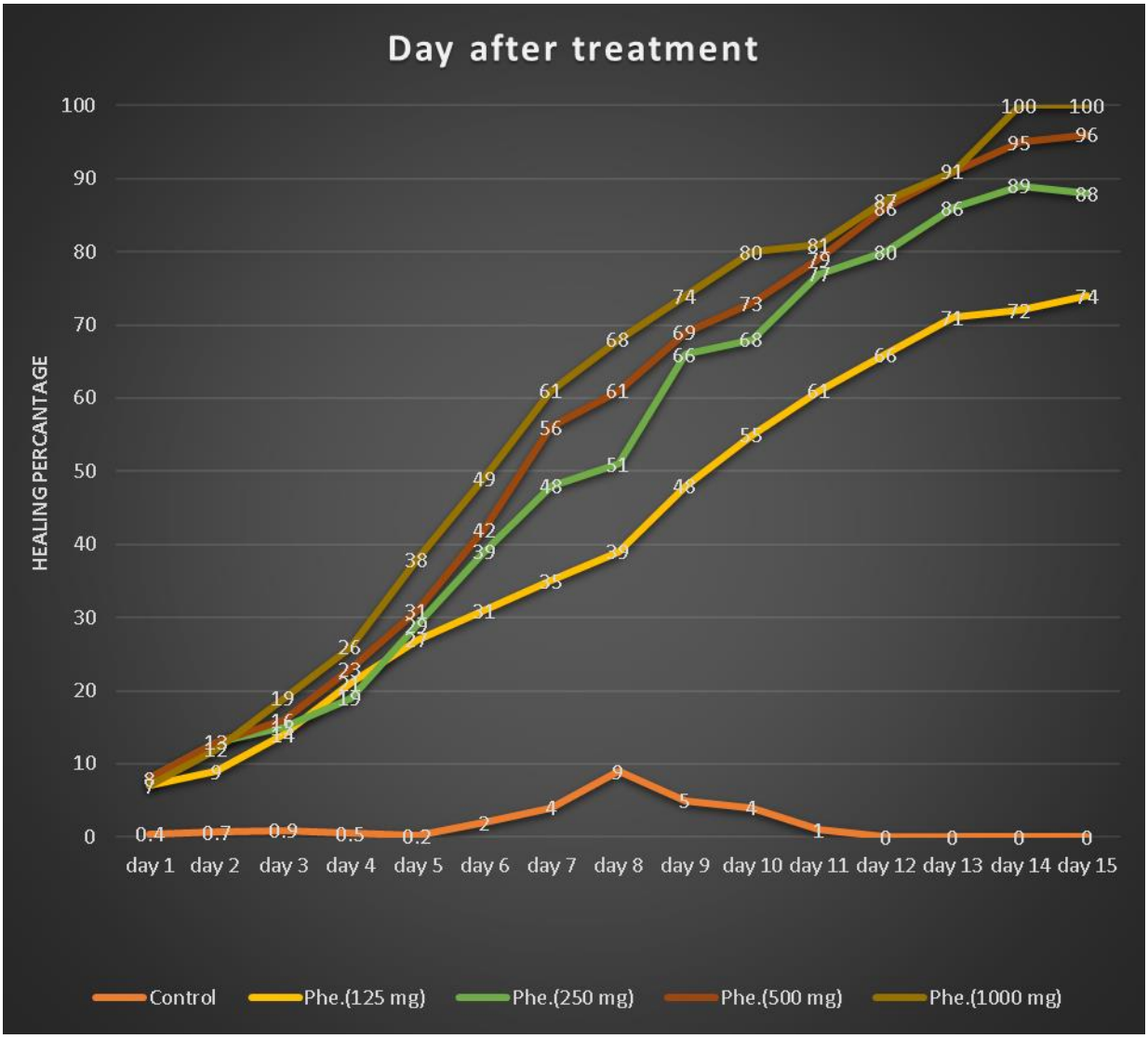

Figure [5]. The healing time of the different groups receiving different concentrations of *Phellinus igniarius* plus ketoconazole of 0.2 mg in relation to healing percentage.

As a result of the infection, the rats' wounds became acute, suppurative, and vividly red.

During the treatment, which lasted from 0 to 15 days, our findings revealed that the groups with varied doses of *Phellinus igniarius* in conjunction with ketoconazole underwent an exceptionally speedy recovery process, Figure[4.1],[4.2],[5]. In contrast, the group of rats treated with ketoconazole only showed a marginal recovery and a substantial loss of weight Fig (4.1)

The rats who were not treated (the negative control group) did not experience any recovery and instead saw excessive weight loss, ultimately leading to death. Fig. (4.1)

Most of these antimycotics' effectiveness appears to be associated with the amount of ROS generation; consequently, targeting the oxidative stress defense mechanism of fungal cells with certain chemicals can increase the amount of ROS accumulation and, hence, fungicidal activity. Compounds that can promote oxidative damage or ROS accumulation by ROS-inducing antimycotics (but not

oxidative defense-related proteins) can also be used in combination therapy. Various findings describe a synergistic augmentation of the efficacy of azoles and amphotericin B against *Candida albicans*, based on an increase in oxidative stress caused by substances such as curcumin, a natural antioxidant. [23].

During our research, ketoconazole and *Phellinus igniarius* showed antifungal efficacy against *Candida albicans*. The concentration of this activity causes it to increase as seen in Tables 3., and Figure [3]. The antifungal medication ketoconazole continues to be effective against *Candida albicans* and may have practical implications in the fight against these organisms (24). Ketoconazole and *Phellinus igniarius* were found to have a synergistic effect against *Candida albicans*, with the highest synergistic effect being observed at ratios of 250:0.2, 500:0.2, and 1000:0.2. In contrast, only a small synergistic effect was observed at ratios of 125:0.2, and 0.2 ketoconazole only. Table 3 and Figure [3,[4.1] and [4.2].

The anti-oxidant activities of *Phellinus igniarius* may be responsible for the enhanced antifungal activity seen with a combination of ketoconazole and *Phellinus igniarius* [16]. The existence of bioactive molecules like phenolic compounds, flavonoids, and polysaccharides, for example, is a significant factor in the antioxidant activities of the substance. It has been demonstrated that these chemicals can neutralize free radicals and prevent oxidative stress from occurring [25].

CONCLUSION

In conclusion, the present study highlights the antifungal potential of both ketoconazole and *Phellinus igniarius* against *Candida albicans*, with their efficacy demonstrated to be concentration-dependent. The synergistic investigation of their combined effects reveals pronounced enhancement at specific concentration ratios, notably 250:0.2, 500:0.2, and 1000:0.2, and subtle synergism at 125:0.2 and 0.2 KTZ only. This observation suggests a strategic avenue for optimizing antifungal therapies that warrant further exploration.

The clinical relevance of ketoconazole in *Candida albicans* management remains apparent, although the emergence of resistance necessitates the exploration of complementary approaches. Notably, the interactive augmentation of ketoconazole's antifungal activity by *Phellinus igniarius*, facilitated through the antioxidative potential of the latter, represents a promising adjunctive strategy against resistant strains.

The mechanistic insight into Ketoconazole's mode of action, involving inhibition of fungal cytochrome P450 and the discerned influence of antioxidants on membrane phospholipid arrangement, furnish valuable perspectives for rational drug design and therapeutic advancement. By capitalizing on the inherent antioxidant properties of *Phellinus igniarius*, which encompass phenolic compounds, flavonoids, and polysaccharides, the quest for efficacious and resilient antifungal interventions takes a constructive step forward.

In light of the documented clinical challenges posed by *Candida albicans* infections in immunocompromised populations, pursuing novel antifungal strategies remains imperative This research aids in the foundation of understanding by elucidating the potential synergy between established antifungal agents and natural antioxidant sources, offering a strategic direction for future investigations and therapeutic innovations. The presented the findings emphasize how important it is that a multifaceted approach in combating fungal infections and reaffirm the prospect of merging conventional pharmacotherapy with natural agents to address the evolving landscape of antifungal resistance.

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