



Synergistic effect of ketoconazole with piperine against *Candida albicans*

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Abstract

It is well known that Ketoconazole is the most important antifungals and a member of the imidazole group. Piperine as a natural plant medicinal compound used to treat many diseases. The development of multi-drug resistance in microorganisms and the reduction of antibiotics to control and treat microbial infection has led to the ongoing investigation of new natural compounds as antimicrobial agents. In this project an attempt to decrease resistance of *Candida albicans* to Ketoconazole. The inhibitory effect of Ketoconazole alone and combination with piperine was determined by agar well diffusion assay against *Candida albicans*, isolated from patients with pulmonary infections. Piperine were screened in combination with Ketoconazole to evaluate their synergistic or antagonistic interactions against *Candida albicans*. It could be noticed that antifungal activity of Ketoconazole alone and piperine alone increase as the concentrations increases. The synergistic effect of piperine with Ketoconazole was observed at ratios 25: 100, 50: 100 and 100: 100 respectively. The antagonistic effect was observed at ratios 25:200,50:200 and 100: 200

Keywords: *Candida albicans*, ketoconazole, piperine

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INTRODUCTION

The incidence and prevalence of infection with pathogenic fungi has increased since the 1980s, especially in hospitals with infectious diseases and immunodeficiency (Arendrup *et al*, 2005). Ketoconazole is one of the most commonly antifungal agents and a member of the imidazole group that interferes with sterol synthesis in the cell membrane (Kucers, 2017). The development of multidrug resistance in microorganisms and decrease of antibiotics to combat and treat microbial infections have led to a continual investigation for novel natural compounds as antimicrobial agents. Highly mortality is recorded around the world annually as a result of multidrug-resistant pathogens (Abreu *et al*, 2017). Plants as a natural products origin include novel therapeutic drugs and highly effective antimicrobial agents (Subramani *et al*, 2017). The antimicrobial combination therapy is one of the most advances to fight microbial resistance in clinical practice (Van Vuuren *et al*, 2009). The synergistic effect may be herb-herb, herb-drug, plant secondary metabolite- drug or plant secondary metabolite-secondary metabolite (Kapoor and Singla, 2015). Bioactive products of plants as antibiotic adjuvants can also enhance the effect of antimicrobials drugs (Yang *et al*, 2018). Piperine is a new effective alkaloid in most species of *piper* (Piska *et*

al, 2018), that investigated for their antimicrobial and biological activities (Priya and Saravana Kumari 2016).

MATERIALS AND METHODS

Extraction of piperine

Black pepper (*Piper nigrum L.*) seeds were collected from local markets, Basra, Iraq. Finely ground black pepper (100g) was refluxed with ethanol (250 ml) for 90 minutes. Vacuum filtration was used to collect the filtrate, which was then evaporated. The residual oil was dissolved in 10% wt. KOH in ethanol (12.5ml). Water (100ml) was slowly added, forming a yellow precipitate. The solution was sealed and refrigerated overnight. The precipitate was collected by vacuum filtration and washed with cold ether (8ml). Recrystallization (3:2 acetone/ hexanes) afforded piperine as flaky, golden crystals (2.1g, 2.17%) (Zeashan *et al*, 2017).

Fungal isolate

Candida albicans isolated from patients with pulmonary infections in respiratory and chest disease centre, Basra, Iraq.

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Table 1. Antifungal activity of Ketoconazole against *Candida albicans*

Ketoconazole (mg)	IZ (mm)
100	30
200	41

Table 2. Antifungal activity of piperine against *Candida albicans*

Piperine (mg)	IZ (mm)
25	8
50	10
100	12

Table 3. Antifungal activity of Ketoconazole with piperine against *Candida albicans*

Piperin: Ketoconazole (mg)	IZ (mm)
25: 100	35
50: 100	35
100: 100	37
25: 200	30
50: 200	30
100: 200	30

Susceptibility test for combination of Ketoconazole with piperine against *Candida albicans*

The inhibitory effect of Ketoconazole alone and combination of Ketoconazole with piperine was determined by agar well diffusion assay against *Candida albicans*. A sterile cork-borer (5 mm diameter) was used to make wells in the Mueller-Hinton agar plates. A McFarland 0.5 standardized bacterial suspension is swabbed over the surface of a Mueller- Hinton agar plate. 50µl of the (100, 200 mg/ml) Ketoconazole solution, 50µl of the (25, 50, 100 mg/ml) piperine solution, 50µl of the (25, 50,100 mg/ml) piperine with 100 and 200mg/ml Ketoconazole, were added to each well. The plates were incubated overnight at 37°C (Kudi *et al*, 1999). Antifungal activity was recorded by measured the inhibition zone (Mahon *et al*, 1998).

RESULTS

The effect of Ketoconazole alone against *Candida albicans* listed in **Table 1** and the effect of piperine alone against *Candida albicans* listed in **Table 2**. It could be noticed that antifungal activity of Ketoconazole and piperine increase as the concentrations increases.

The combination of Ketoconazole with piperine at various concentrations was tested against *Candida albicans*. The synergistic effect was observed at ratios 25: 100, 50: 100 and 100: 100. The antagonistic effect was observed at ratios 25:200,50:200 and 100: 200 (**Table 3**).

DISCUSSION

In immunocompromised patients, *Candida albicans* is an opportunistic fungal pathogen and treated by anti-fungal drugs, it has become fatal. Increase in the antimicrobial resistance in *C. albicans* is a matter of concern since it is in the human micro biome (Kamal *et al*, 2018). Infections of antibiotic-resistant pathogens indicate the onset of increasing impendence to

humaneness. (Mark *et al*, 2019). Antibacterial resistance is not affected only to those patients who evolve bacterial infections; broad medical proceedings stand to be influenced. Antibiotic prophylaxis is generally used to prevent the evolution of infections, in immunocompromised patient's using chemotherapy and pre operatively for surgical procedures (Crader and Bhimji, 2018). The search of novel alternative compounds for combating the antimicrobial resistance must be part of any global response to this problem. One such hopeful avenue of this problem includes antibiotic resistance breakers (ARBs), making bacteria to desensitize to antibiotic resistant's (Kamal *et al*, 2018). Natural products of plant make an alternative therapy to develop the effect of Ketoconazole at low cost and without any important adverse effects (Sharma *et al*, 2010).

In our study, Ketoconazole and piperine were recorded antifungal activity against *Candida albicans*. This activity increase as the concentrations increases (**Table 1, 2** and **Fig. 1**). Ketoconazole are still active against *Candida albicans* and may have clinical applications against these organisms (Shan-shan *et al*, 2011). The antibacterial activity of alkaloids such as piperine has been already proven and many studies have documented that these agents play an important function throughout the treatment of most infectious diseases (Cushnie, 2014). The synergistic effect of Ketoconazole with piperine against *Candida albicans* was observed at ratios 25: 100, 50: 100 and 100: 100, while antagonistic effect was observed at ratios 25: 200, 50: 200 and 100: 200 (**Table 3** and **Fig. 1**). This could be attributed to combination between Ketoconazole (100mg/ml) and piperine in three concentrations used in this study improvement permeability of cell membrane; there the inhibition zones were larger. Piperine have the ability to improve the antibacterial activity of tetracycline and rifampicin (Mgbeahuruik *et al*, 2019). This result agreement with many studies which documented the synergistic effect of combination between Ketoconazole

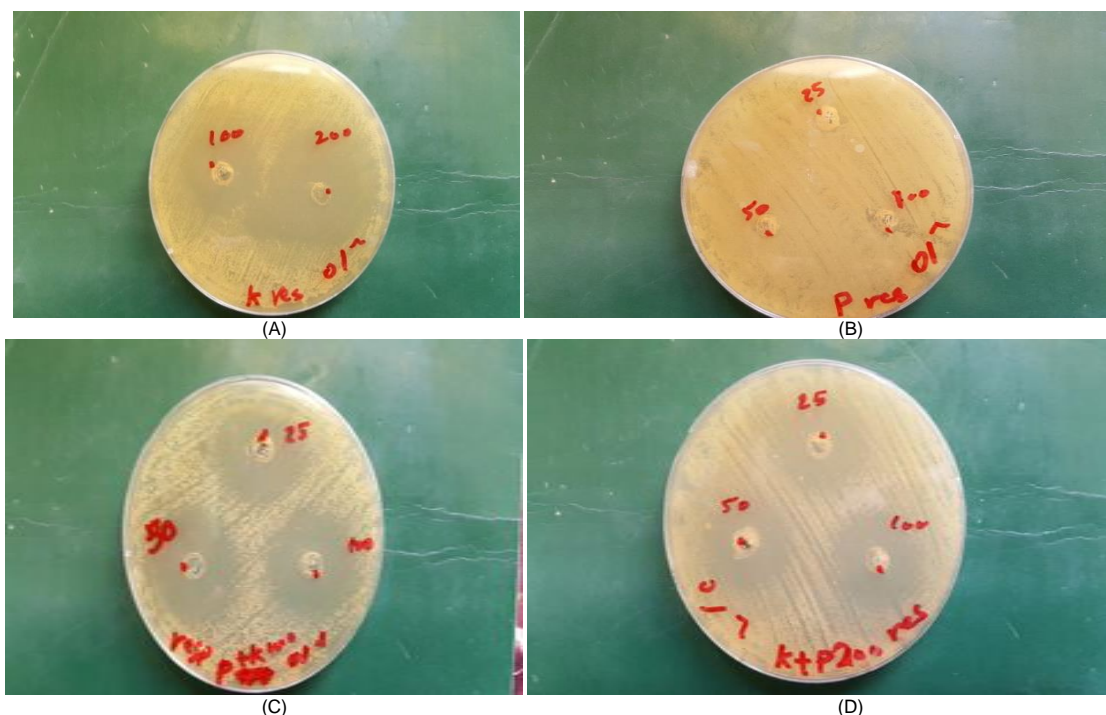


Fig. 1. Antifungal activity of Ketoconazole and piperine against *Candida albicans*.
 A: Ketoconazole alone B: Piperine alone C&D: Ketoconazole with piperine

with alkaloids and other alternative compounds against *Candida albicans* and other fungi (Amber *et al*, 2010, Zhou *et al*, 2012, Pyun and Shin, 2006, Giordani *et al*, 2001). In contrast, the combination between Ketoconazole (200mg/ml) and piperine in all concentrations used in this study reduced permeability of fungal cell and competed for the entry sites of cell membrane; there the inhibition zones were smaller.

CONCLUSIONS

The results of the current study documented that, Ketoconazole with piperine have synergistic activity against *Candida albicans* from patients with respiratory infections in different concentrations.

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