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Comparative Effect of Honey Bee Venom and Common Antifungal Agents on *Candida albicans*

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Abstract

Fungal infections, can cause various symptoms in different forms, and are associated with high morbidity and mortality in vulnerable groups. one of the most significant fungal infection is candidiasis which caused by Candida**.** In recent years, there has been growing worried about the challenging of fungal diseases treatment and unwanted consequence of synthetic antifungal drugs. This necessitates the searching for new classes of broad-spectrum anti-fungal drugs to bring pharmaceutical characteristics and more efficient treatments. Bee venom (BV) is an antimicrobial agent which has been widely used to treat various diseases**.** the aim of the current study was to examine the antifungal properties of honey bee venom on *Candida albicans* to compare it to some common antifungal drugs (ketoconazole and amphotericin B). To perform this assay, the standard strains of *C. albicans* (ATCC10231) was used for antimicrobial assessment, and Disc diffusion assay and the microbroth dilution method were used to evaluate the anti fungal activity of (BV). The result of this study showed that BV, amphotericin B and ketoconazole had inhibitory effects on *C. albicans* which MIC of the were measured to be 118.2, 3.3 and 2 respectively, furthermore, Comparison of BV and common antifungal drugs demonstrated that, amphotricin B and ketoconazole have a better effect on *C. albicans*.

Keywords: *Candida albicans, bee venom (BV), antifungal, candidiasis, infections*

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Introduction

During recent decades, the consequences of Infectious diseases, have a significant impact on public health systems, throughout the world (Bhagawan et al., 2022; Bhagawan & Kusumawati, 2021; Ying et al., 2022). Fungal infections, can cause various symptoms in different forms, from mild to very serious and are associated with high morbidity and mortality in vulnerable groups with immunodeficiency or immunosuppression (Rayens & Norris, 2022) one of the most significant fungal infection is candidiasis which caused by Candida. The widely prevalent fungal species is *Candida albicans* that causes infections disease in humans and is a part of the normal flora in oral cavity, healthy microbiota, skin of most humans, and gastrointestinal tract and reproductive systems (Gavanji & Larki, 2017). Two clinical complications, including superficial and life-threatening systemic infections are caused by this opportunistic fungi (Mayer et al., 2013).

In recent years, there has been growing worried about the challenging of fungal diseases treatment and unwanted consequence of synthetic antifungal drugs because of the danger of drug resistance and negative side effect (Fuentefria et al., 2018; Houšť et al., 2020). This necessitates the searching for new classes of broad-spectrum anti-fungal drugs to bring pharmaceutical characteristics and more efficient treatments. due to effectiveness and milder side effects, traditional medicine has been widely used in fungal infections for treatment and plays a significant role in drug development (Gavanji, Larki, et al., 2014; Xie et al., 2018) Admittedly, Natural toxins have shown more reliable and secure outcome in antimicrobial assessments. Bee venom (BV) has been traditionally used to treat several diseases including rheumatism arthritis, central nervous system diseases (Alzheimer, Parkinson, and amyotrophic lateral sclerosis), and infectious diseases.

Bee venom therapy (BVT) is an alternative medicine approach which has been used for medicinal purposes for treatment of various diseases in different countries for more than 5000 (Lee et al., 2014). BV, released from the venom gland of female worker bees, and contains numerous bioactive peptides including melittin, adolapin, apamin, mast cell degranulating (MCD) peptide (Ceremuga et al., 2020; Gu et al., 2020), which melittin as the Main component of BV (40–60% w/w), hinders the fungal growth and plays a crucial role in antimicrobial properties. The other components of BV are enzymes, such as phospholipase A2 (PLA2) and hyaluronidase (Wehbe et al., 2019) that have different mechanisms as the immunotherapies agents to treat several diseases (Hossen et al., 2016; Kim et al., 2014; Soltan-Alinejad et al., 2022). Several studies have demonstrated that the main components (melittin and PLA2) of the venom, exhibited antimicrobial properties and can be used as anti-microbial agents, (Socarras et al., 2017; Wehbe et al., 2019). These compounds, target the cell membrane and cause the pore formation through the membranes which lead to the cell membrane cleavage and lysis and finally release of cellular contents (Leandro et al., 2015). Numerous research studies indicated that bee venom possesses significant antiviral activity against a range of enveloped, and non-enveloped Viruses (Bachis et al., 2010; Uddin et al., 2016). Based on a study, two principal compounds of BV (melittin and PLA2) have impressive antiviral potentials to inhibit human immunodeficiency virus (HIV) (Bachis et al., 2010; Hood et al., 2013; Wehbe et al., 2019).

BV, as an anti-inflammatory agent (El Mehdi et al., 2022), has invoved in many signaling pathways to inhibit the Activator protein 1 (AP-1) and Translocation of nuclear factor kappa B NF-κB. BV, have demonstrated in vitro antifungal activity against a range of opportunistic pathogens, including *C. albicans*, *Trichophyton rubrum* and *T. mentagrophytes* (Park et al., 2018). Although, several research studies have indicated that BV is an anti -bacterial agent and antibacterial properties of Bee venom (BV) by various mechanisms has been studied, the mechanisms of antifungal action and

important insights about BV on fungi are not elucidated, Therefore, the aim of the current study was to examine the antifungal properties of honey bee venom on *C. albicans* to compare it to some common antifungal drugs.

Materials and Methods

1. Preparation of bee venom (BV)

In this study the honey bees were maintained at Tradidtional Medicine and Herbal Research Institute (Isfahan, Iran) and the bee venom collecting was done by using electrical shock without any hurt to them. they sting a glass plate and BV was collected and dried and kept in a freezer at -20 °C (El-Didamony et al., 2022)

2. Antifungal activity assays

2.1. Standard strains

The standard strains of *C. albicans* (ATCC10231) was used for antimicrobial assessment, and to perform this assay, the preparation of the lyophilized strains was done at Traditional Medicine and Herbal Research Institute (Isfahan, Iran) and they were grown on Sabouraud Dextrose Agar (SDA) and incubated for 2 days at 25 ◦C (Gavanji et al., 2015)

2.2. Anti-Candida activity of honey bee venom (BV) using disk diffusion method

The Sabouraud Dextrose Agar (SDA) or Sabouraud agar medium was used to evaluated the antifungal activity of bee venom (BV) on C. albicans. In the currect study, the *C. albicans* was grown and incubated on SDA for 48 hours, at 37°C prior to testing, after that two to three colonies inoculated to sterile saline and 0.5 McFarland [1×106 colony- forming units(CFU)/mL] is set for turbidity. In the next step, the preferred suspension was cultured and grown on dextrose agar medium. To perform the disk diffusion method, the blank disc(6/4mm) was used which contained bee venom (BV) concentrations of 60 µg/mL, 70 µg/mL, 80 µg/mL, 90 µg/mL, 100 µg/mL, 110 µg/mL, 120 µg/mL, 130 µg/mL, 140 µg/mL, 150 µg/mL, 160 µg/mL that dissolved in Dimethyl sulfoxide (DMSO) solvent. For positive control, the amphotericin B (10 µg) discs and ketoconazole (15 µg) discs and for negative control Dimethyl sulfoxide (DMSO) were used. All plates were incubated at37 ◦C for 72 hours the diameter of the inhibition zone for each group at 24 hours,48 hours, and 72 hours, was measured in millimeters (Saracino et al., 2022).

2.3. Detection of the minimum inhibitory concentration and minimal lethal concentration using the microbroth dilution method

The minimum inhibitory concentration(MIC) and minimal lethal concentration [i.e., minimal fungicidal concentration (MFC)] of honey bee venom (BV), ketoconazole and amphotericin B, against *C. albicans* were determined by using of microbroth dilution method. In the first step, the bee venom (BV) was diluted in Dimethyl sulfoxide (DMSO) and concentrations of 60-160 µg/mL were prepared. Sabouraud dextrose agar (SDA) medium was used in the test as the liquid medium. In the next step, 100 µL of each dilution was added to each well of plate (96-well), and microbial suspension of *C.* albicans with 10^4–10^5 CFU/mL concentration was added to each well and the plates were incubated for 24hours at 35 ◦C. After that, the MIC and MFC of BV, ketoconazole and amphotericin B were determined (Gavanji et al., 2015; Torabi et al., 2022).

Statistical Analysis

IZ between different concentration of BV, MIC and MFC between BV, ketoconazole and amphotericin B, were analyzed by one-way ANOVA followed by Tukey post hoc test using GraphPad Prism software version 7. Data were reported as Mean± standard deviation (SD).

Results and Discussion

One of the most significant social and clinical problem is antifungal resistance which depends on numerous microbial and host factors that can cause the serious infectious diseases (White et al., 1998) so new strategies are needed to manage, evaluate and development of new antimicrobial drugs (Gavanji, Mohammadi, et al., 2014; Zinner, 2005). Numerous studies have reported that natural products have exerted the potential antimicrobual activities. BV as a natural toxin has long been traditionally used to treat various diseases, furthermore, several research shown that BV possess antimicrobial properties against a variety of pathogens (Han et al., 2016; Uddin et al., 2016). In the current study, antibacterial properties of Bee venom (BV) agaist *C. albicans* was evaluated that the result showed that BV play a crucial role to inhibit this pathogen (Table 1). The statistical analysis indicated that honey bee venom, with different concentrations at the times of 24, 48 and 72 h had inhibitory effect against *C. albicans* which lethal toxic of BV at higher concentrations was increased ($p < 0.0001$). Also the result showed that 60 µg/ml of bee venom had no effect on C. albicans and the significant effect of honey bee venom on C. albicans, was emerged starting at 70 µg/mL, after 24 hours of exposure (Figure. 1)

Table 1. Antifungal activity of of bee venom (BV) using the disc diffusion method

Bee venom (µg/ml)	*Candida albicans*		
	Mean±SD		
	24	48	72
60	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
70	12.60±1.35^{b}	13.00±1.00^{b}	13.90±1.01^{b}
80	12.87±2.58^{b}	14.67±0.58^{bc}	15.20±0.43^{bc}
90	14.00±0.50^{bc}	16.00±1.00^{cd}	17.00±1.00^{cd}
100	14.93±0.86^{bcd}	15.93±0.97^{cd}	17.33±0.58^{de}
110	14.77±1.25^{bcd}	17.60±0.53^{de}	18.27±0.64^{de}
120	15.03±0.95^{bcd}	17.93±0.11^{def}	18.10±0.36^{de}
130	16.90±0.82^{cd}	18.67±0.58^{efg}	18.93±0.90^{ef}
140	16.73±1.42^{cd}	19.67±0.58^{fg}	20.47±0.50^{fg}
150	17.63±0.71^{d}	20.10±0.96^{g}	20.77±0.68^{fg}
160	17.93±0.11^{d}	20.50±0.50^{g}	20.87±0.23^{g}

Data are presented as mean±SE.
$^{a-g}$Different letters on every column represent meaningful difference ($p < 0.05$).

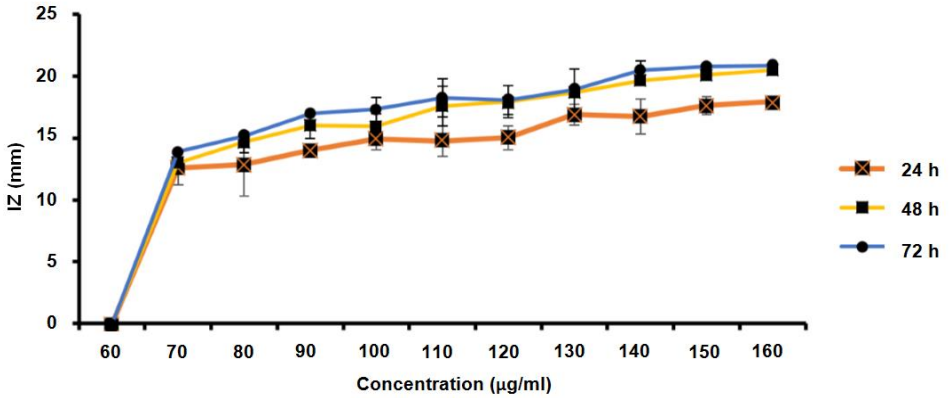

Figure 1. Comparison of inhibition zone of bee venom in different tims (24, 48 and 72h)

The comparison of IZ between the BV (160 µg/ml) and two common fungicidal drugs (amphotericin B (10 µg) and ketoconazole (15 µg)) showed that the BV and ketoconazole had higher IZ on *C. albicans* and amphotericin B indicated a low effect on C. albicans compared to BV and ketoconazole (Figure 2, $p < 0.05$). Also ketoconazole showed a better effect on *C. albicans* compared with the Bee Venom and amphotericin B (Figure 2, $p < 0.05$).

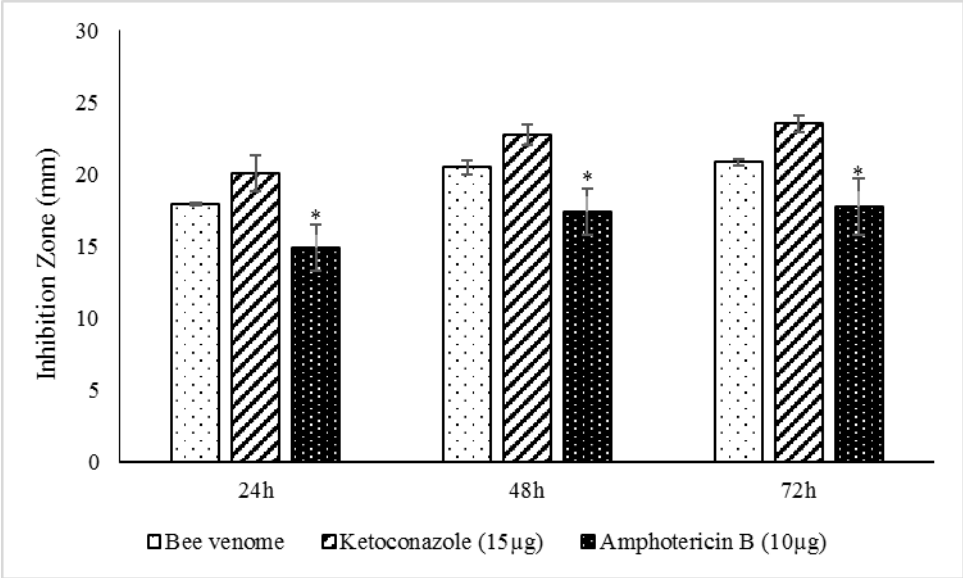

Figure 2. Comparison effects of BV and common anti-fungal on the *Candida albicans*

Comparison of MIC between BV and common antifungal drugs on *C. albicans* showed that the BV and two antifungal drugs (amphotericin B and ketoconazole) had effect and inhibited the *C. albicans* which MIC of the BV, amphotericin B and ketoconazole were measured to be 118.2, 3.3 and 2 µg/mL respectively, also the result indicated that ketoconazole had had the highest inhibitory effect against this pathogen ($p < 0.05$). the result of MFC showed that amphotericin B and ketoconazole had the better effect compared with BV ($p < 0.01$). The MFC value on *C. albicans* was measured as 153 µg/ml for BV and 5.6 and 7.8 µg/ml for ketoconazole and

amphotericin B respectively (Table 2). In 2016, Lee reported that Bee Venom (BV) and Sweet Bee Venom (SBV) had inhibitory effects against clinical isolates of *C. albicans* which MIC rates were varied from 62.5 to 125 µg/mL and for SBV MIC values were from 15.63 to 62.5 µg/mL (Lee 2016). A comparison indicated that our results are compatible with those obtained by Lee and MIC values of Bee Venom (BV) were in the same rate, but Sweet Bee Venom (SBV) exhibit sastronger inhibitory on *C. albicans* in comparison to our result. Another research by Park demonstrated that Bee Venom and its major components, including melittin, apamin and BV based mist (BBM) had inhibitory activity against *Trichophyton rubrum* (Park et a., 2018). In 2013, Ali examined the effect of Bee Venom (BV) on isolates of C. albicans which MIC value was 40 µg/mL (Ali 2013).

Table 2. MIC and MFC values for BV and common anti-fungal on the *Candida albicans*

		(µg/ml)		
n	Component	MIC	MFC	Pathogens
1	honey bee venom	118.2	153	
2	Ketoconazole	2	5.6	*Candida albicans*
3	Amphotericin B	3.3	8.7	

Kimand co workers examined the effect of honey Bee Venom (BV) on the growth of Trichomonas vaginalis, and they observed an inhibitory effect on this pathogen, and the result of this study showed that BV with 75 ppm concentration, completely inhibited the growth of T. vaginalis (Kim et al., 2014). Also another research in 2015, demonstrated that bee venom and melittin exhibited antimicrobial effect on *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, which MIC values of BV were measured 100, 500, 60, 300, 60 µg/mL respectively (AL-Ani et al., 2015). A comparison of our results with those (AL-Ani et al., 2015), showed that BV possessed a higher inhibitory effect on isolated *C. albicans*, and these result indicated that antimicrobial properties of Bee Venom (BV) is generally because they contain various compounds also several parameterssuch as antimicrobial Resistance (AMR) and conditions for antimicrobial testing methods and other factors are effective on antimicrobial activity.

However, this experience demonstrated that Bee Venom (BV) has antifungal activity against *C. albicans* but threre are various limitations includiong toxicological assay, find the molecular mechanisms and other significant parameters to suggest the BV in the clinical treatments.

Conclusion

The drug resistance and adverse effects of chemical antimicrobial substances has become an important public health challenge. Therefore, discovering new antimicrobial agents from natural sources play a significant role in Treatment of fungal infections. The present study indicated that that amphotricin B and ketoconazole have a better effect on *C. albicans* in comparison to BV. Based on the acceptability of BV, further researches on toxicological reactions, mechanism of actions, clinical trials and other important parameters are needed.

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