Original Article

Antifungal Effects of *Iranian Propolis* Extract and *Royal jelly* Against *Candida albicans In-Vitro*

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Abstract

Background: *Candida albicans* is the most important opportunistic fungal that can establish infection in susceptible individuals. *Iranian Propolis* and *Royal jelly* are bee products that are traditionally used against fungal infections. This study was aimed to evaluate the antifungal effects of Iranian Propolis extract and Royal jelly against *C. albicans in vitro.* **Methods:** Antifungal activities of the extracts were performed according to microbroth dilution method in 96-well microdilution plates. The amount of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) based on counting the number of fungal colonies (CFU) were evaluated for each of *Royal jelly* and *Iranian Propolis* extracts against *C. albicans* compared with the control group. **Results:** In this study, the MIC, MIC_{50}, and MFC of *Royal jelly* on *C. albicans* were, respectively, 80, 103 ± 25, and 160 ± 34 mg/mL and for the *Iranian Propolis* alcoholic extract were, respectively, 0.030 ± 0.015, 0.0618 ± 0.027, and 0.0833 ± 0.0599 mg/mL. **Conclusions:** The results indicate that both *Royal jelly* and *Iranian Propolis* alcoholic extract are effective against *C. albicans,* but the former species has higher antifungal activity. If the clinical trials confirm the results of this study, *Iranian propolis*, as a new antifungal agent by replacing chemical drugs, can be used to develop antifungal medicinal herbs.

Keywords: *Candida albicans, Iranian propolis, Royal jelly*

Introduction

Candidiasis is one of the most important and most common opportunistic fungal diseases in humans. *Candida albicans* is one of the most important of them that can establish infection in susceptible individuals. Candidiasis can be seen as acute, subacute or chronic in skin, nails, vaginal mucosa, bronchus, lung, and gastrointestinal tract. Host response against infection is usually mild irritation and inflammation, then chronic form, and acute purulent or granulomatous changes.[1] Studies have shown that various microbial pathogens such as *Candida* species have become resistant to antimicrobial drugs.[1-3]

Today, modern medicine has much attention to the use of natural materials and biological therapies. These remedies have been searched and their effects have been confirmed in the treatment of various diseases.[4] *Royal jelly* and *Propels* are of bee products that have been used in traditional medicine by humans over the centuries. *Propels* is a resin plant that is created by bees from plants around the hive. These resins are used to strengthen shoulders and disinfect the hive. *Royal jelly* is one of bee products that are produced by incomplete digestion in the stomach worker bee honey. This material and a lot of plants have antioxidant activities.[5]

Natural agents have been the main agent since ancient times to treat various diseases.[6] In recent years, several reports have been published about the characteristics of specific antimicrobial natural products to treat bacterial infections and none-infectious diseases. *Propolis* and *Royal Jelly* are two of these remarkable agents are traditionally used against bacterial and fungal infections.[7-9]

Important fungi as of multiple opportunistic infections in humans that have been published recently are *Candida* species. Over the past four decades, due to immunosuppressive diseases such as AIDS and types of hematologic malignancies and indiscriminate use of antibiotics and corticosteroids, systemic infections caused by strains of candidates have been raised

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as one of the most important causes of morbidity and mortality, especially in patients who have been admitted in hospitals.[5,10-13]

The indiscriminate use of antifungal drugs has caused the occurrence of drug resistance against them have increased their side effects on human health. Thus, in the past 10 years, trying to find natural antimicrobial and other drugs have been increased in order to prevent complications of chemotherapy and the economy. This is the same for other diseases, i.e., the lack of positive response to conventional drugs has increased the tendency for the use of natural agents.[11-13]

The spectrum of infections called candidiasis that is caused by various species of *Candida* can be acute or chronic, localized, or systemic. *C. albicans* is the most important factor for most of these infections.[13] Therefore, due to the increasing incidence of fungal diseases and problems that exist in the classical use of medications and because of the incidence of drug resistance, there is a need to introduce new drugs.[14] So, the antifungal effects of *Iranian propolis* extract and *Royal jelly* were investigated against *C. albicans in vitro.*

Methods

Extraction method

Preparation of Candida albicans suspensions

The standard strain *C. albicans* (code no. PTCC5027) used in this study was provided from Scientific Research Center of Iran. *Candida albicans* was cultured on Sabouraud dextrose agar medium (Merck, Germany) at 35°C. After 24 h, some colonies were transferred to 1 mL normal saline to prepare a solution with a concentration of 0.5 McFarland 1.5 × 106 CFU/mL (neobar slides were used for colony counting). The resulting solution was diluted to a ratio of 1:1000 and suspensions with concentrations of (0.5–2.5) × 10^3 CFU/ml were provided.[3]

Preparation of Iranian Propolis extract and Royal jelly

A total of 500 g of *Iranian propolis* raw material was purchased from a reputable grocery, and to prepare extract, raw material was crushed. The obtained powder was mixed with 80% ethyl alcohol in the ratio of 1:1, then powder was transferred into percolator, and the extract was prepared by the percolation method according to instruction no. 10 of German pharmacopoeia.[2] The obtained extract was distillated by the rotary vacuum evaporation until alcohol was removed completely. Also *Royal jelly* that was purchased and maintained in special vial was used in next stages to perform examinations. The needed dilutions of *Royal jelly* and *Propolis extract* were prepared in distilled water and ethanol (70%), respectively. Then, using the micro dilution method, different dilutions were prepared based on the method recommended by Clinical Laboratories Standards Committee M27-A3. Twofold dilutions of extract was poured into the first microtube and next dilutions were prepared serially.[2,3]

The concentrations of *Royal jelly* and *Propolis extracts* were prepared as follows:

Royal jelly: 320-20, 200- 25, 240-30, 280-35, 180-45, 220-55, 300-75, and 130-260 mg/mL,

Iranian Propolis extract: 4-0.015 and 3-0.023 mg/mL.

The used method in this study was the microdilution method. Antifungal activities of the extracts were performed according to the microbroth dilution method in 96-well microdilution plates.[2]

To study the effects of *Iranian propolis extract* and *Royal jelly* on *C. albicans*, 100 µL of each dilution of the extract was added separately to the socket of 96-well plates that contained 100 mL of RPMI 1640 (with glutamine, without bicarbonate; Bio IDEA, Iran). Then 100 mL of *Candida albicans* suspension in volumes equivalent to 2500 cells per mL were inoculated into the all sockets, except the control plates. The plates were kept at 35°C for 48 h in the incubator on the Shaker.

MIC determination

After incubation time, the amount of growth in the well containing the agent is compared visually with the amount of growth in the growth-control well (no antifungal agent) used in each set of tests; MIC is easily read as the lowest drug concentration that prevents any discernible growth.

MIC_{50} determination

Then, 10 µL of the contents of each socket was cultured on Sabouraud dextrose agar medium (Merck, Germany) and were placed for 48 h at 35°C. After this period, the number of colonies of growth in the plate is compared visually with the number of colonies of growth in the growth-control plate (no antifungal agent) used in each set of tests; a plate reduction of 50% in the number of fungal colonies (CFU) is considered as MIC_{50}.

Minimum fungicidal concentration (MFC) determination

The MFC was the lowest extract concentration that showed either no growth or fewer than three fungal colonies (CFU) to obtain approximately 99–99.5% killing activity.

Data analysis

The tests were performed in triplicate. Mean and standard deviation MICs, MIC_{50}, and MFCs were calculated for both plant extract.

Results

Results of Royal jelly

Table 1 shown the values of MIC, MIC_{50}, and MFC of *Royal jelly* on *C. albicans*, which were tested three times,

that in the first trial MIC, MIC_{50}, and MFC of *Royal jelly* were, respectively, 80, 100, and 180 mg/mL, in the second test were 80, 80, and 120 mL/mg, and in the third experiment were, respectively, 80,130 and 180 mg/mL.

The mean of MIC, MIC_{50}, and MFC of *Royal jelly*on *C. albicans* were, respectively, 80, 103 ± 25 and 160 ± 34 mg/mL [Table 1].

The values of MIC, MIC_{50}, and MFC of *royal jelly* on *C. albicans* in three times tested are compared in Figure 1.

The mean of MIC, MIC_{50}, and MFC values (mg/mL) of *Royal jelly* on *C. albicans* are compared in Figure 2.

Results of Iranian propolis

The values of MIC, MIC_{50}, and MFC of *Iranian propolis* on *C. albicans* were tested three times, that in the first trial, MIC, MIC_{50}, and MFC of *Iranian propolis* were, respectively, 0.023, 0.046, and 0.0625 mg/mL, in the second test were 0.046, 0.0935, and 0.125 mL/mg, and in the third experiment were, respectively, 0.023, 0.046 and 0.0625 mg/mL [Table 2].

The values of MIC, MIC_{50}, and MFC of *Iranian propolis* on *C. albicans* three times tested are compared in Figure 3.

The mean of MIC, MIC_{50}, and MFC of *Iranian propolis* extract on *C. albicans* were, respectively, 0.030 ± 0.015, 0.0618 ± 0.027, and 0.0833 ± 0.0599 mg/mL and are compared in Figure 4.

Comparison of inhibitory and fungicidal effects of plant extracts

The logistic regression test showed that there were significant differences between the inhibitory and fungicidal effects of each of the plants extracts ($P < 0.05$). *Iranian propolis* extract had the greatest inhibitory and fungicidal effects on *C. albicans*, then *Royal jelly* had the least antifungal effect [Figure 5].

Table 1: MIC, MIC_{50}, and MFC values (mg/mL) of *royal jelly* on *Candida albicans*

Experiments	MIC	MIC_{50}	MFC
First test	80	100	180
Second test	80	80	120
Third test	80	130	180
Mean and standard deviation	80	103±25	160±34

Table 2: MIC, MIC_{50}, and MFC values (mg/mL) of *Iranian propulsion Candida albicans*

Experiments	MIC	MIC_{50}	MFC
First test	0.023	0.046	0.0625
Second test	0.046	0.0935	0.125
Third test	0.023	0.046	0.0625
Mean and standard deviation	0.030±0.015	0.0618±0.027	0.0833±0.0599

Discussion

Chemical analysis of the *propolis* exhibited the presence of flavonoid, tannin, steroid, alcohol, and alkaloid in extracts. The main chemical classes present in *propolis* are silver, mercury, copper, manganese, iron, calcium, vanadium, silis, flavonoids, phenolics, and aromatic compounds. However, *propolis* contains some volatile oils, terpenes, and bee wax.[14,15] Phenolic compounds have been shown to have antibacterial and antifungal activities. These compound also have antioxidants effective in a wide series of diseases.[16,17] Hence, antifungal effects of Propolis extract and Royal jelly might be, in part, due to these agents. Albeit, there are other natural sources which possess phenolic compounds

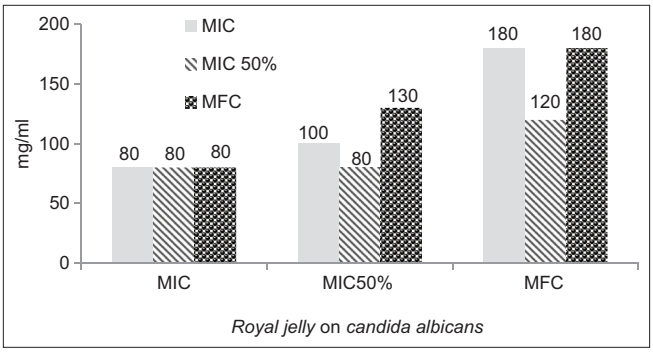

Figure 1: Comparison the values of MIC, MIC_{50}, and MFC of Royal *jillion Candida albicans* in three times tested

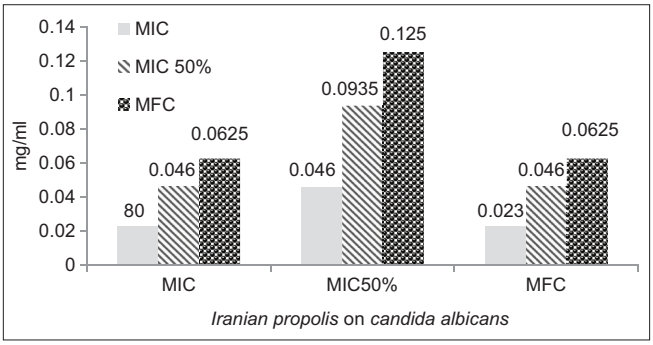

Figure 2: Comparison the mean of MIC, MIC_{50}, and MFC values (mg/mL) of *Royal jelly* on *Candida albicans*

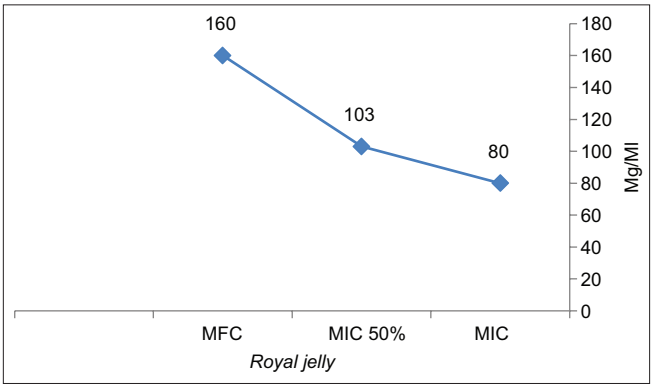

Figure 3: Comparison the values of MIC, MIC_{50}, and MFC of *Iranian propolis* on *Candida albicans* in three times tested

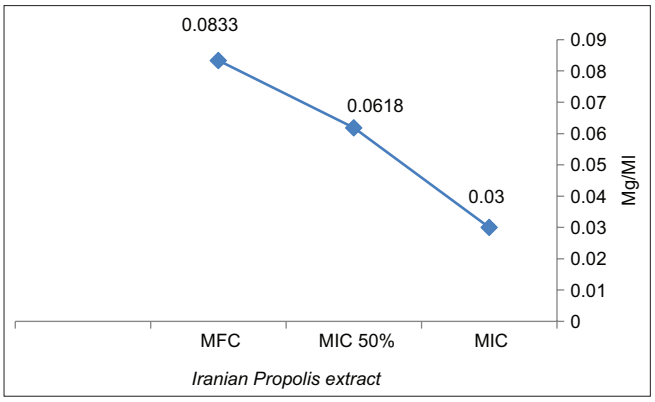

Figure 4: Comparison the mean of MIC, MIC_{50}, and MFC of *Iranianpropolis* extract on *Candida albicans*

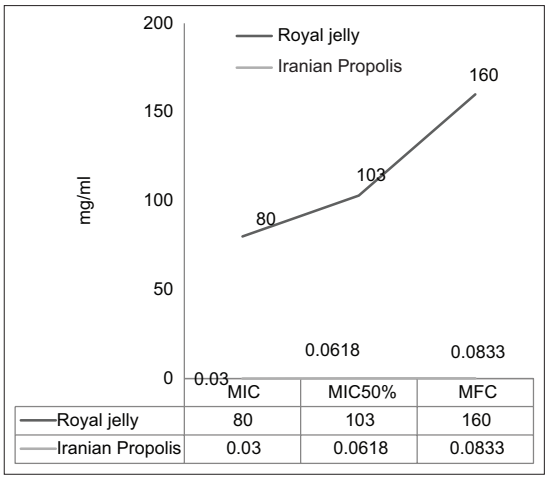

Figure 5: Comparison the mean of MIC, MIC_{50}, and MFC values (mg/mL) of *royal jelly* and alcoholic Iranianpropolis extract on *Candida albicans*

and, hence, might have antifungal activities.[17,18] Most importantly, because phenolic compounds and antioxidants are effective on various diseases, these products might be useful for other problems that the users may suffer from.[19]

Although several reports have been published on anti-inflammatory, antitumor, antiallergic, anticancer, stimulation of humoral and cell-mediated immunities, and antiblood pressure properties, the available information on the antimicrobial property of *propolis* is scarce. Hence, the present study was conducted to investigate the antimicrobial property of *Iranian propolis* on some pathogenic microorganisms.

In our study, the mean of MIC, MIC_{50}, and MFC of *Iranian propolis* extract on *C. albicans* were, respectively, 0.030 ± 0.015, 0.0618 ± 0.027, and 0.0833 ± 0.0599 mg/mL, which they are consistent with the results of previous studies. These results may indicate that *Iranian propolis* extract has antifungal effect against the *C. albicans* and may be used as an antifungal agent for the treatment of fungal infections.

As it was mentioned, propolis has antioxidant activity and antioxidants usually have positive effects on various conditions, including infectious diseases.[20,21] Therefore, propolis may have other positive effects on other diseases, which worth examining.[17-21]

Conclusions

The mean of MIC, MIC_{50}, and MFC of *Royal jelly* on *C. albicans* in the present study were, respectively, 80, 103 ± 25, and 160 ± 34 mg/mL, and the mean of MIC, MIC_{50}, and MFC of *Iranian propolis* extract on *C. albicans* were, respectively, 0.030 ± 0.015, 0.0618 ± 0.027, and 0.0833 ± 0.0599 mg/mL. The results of this study show antifungal efficacy of *Royal jelly* on *C. albicans* that with results of other studies correspond that indicate antifungal activity of these materials. As this search showed, *Royal jelly* had less antifungal activity than *propolis*. So *propolis* can be used as an ideal combination for the treatment of fungal infections. Antifungal effects of *propolis* are probably due to available flavonoids, phenolics, tannins, silver, mercury, copper, and aromatic compounds.[19-21]

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Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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Conflicts of interest

There are no conflicts of interest.

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