



## Comparative study on the antifungal activity of hydroalcoholic extract of Iranian Propolis and Royal jelly against *Rhizopus oryzae*

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### ABSTRACT

**Introduction:** Mucormycosis is an opportunistic fungal infection. *Rhizopus oryzae* is major cause of mucormycosis in humans. This disease is the most common form of the acute fungal infection with rapid progress. Iranian Propolis extract and *Royal jelly* are honey bee products which have been used by human over the past centuries in traditional medicine. In this study the effects of Iranian Propolis and *Royal jelly* were investigated against *Rhizopus oryzae* and *Candida albicans*.

**Methods:** The used method in this study was microdilution. To perform it, the prepared dilutions of *Royal jelly* and alcoholic extract of Iranian Propolis were added to tubes containing Sabouraud dextrose broth culture media except to control group and then *Rhizopus oryzae* suspension was added to all microtubes. In the next stage, microtubes were maintained in incubator at 25°C for 48 hours and then 10 ml of the content of each microtube was transferred to Sabouraud dextrose agar media. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the agents were calculated.

**Results:** In this study, the MIC and MFC of Iranian Propolis alcoholic extract on *Rhizopus oryzae* were respectively 0.1 and 0.25 mg/ml and the MFC of *Royal jelly* on *Rhizopus oryzae* were respectively 100 ± 34 and 133 ± 46 mg/ml.

**Conclusion:** The results indicate that Propolis might be used as an ideal combination for the treatment of fungal infections like *Rhizopus oryzae*. However, clinical studies are needed to confirm the effects of these drugs.

### Implication for health policy/practice/research/medical education:

Propolis might be used as an ideal combination for the treatment of fungal infections, if clinical studies confirm the effects of these drugs.

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### Introduction

Mucormycosis is an opportunistic fungal infection caused by fungi in the order of mucorales and can cause acute fungal infection. These fungi have a wide geographical spread. *Rhizopus oryzae* is major cause of mucormycosis in humans. This disease is the most common form of the infection with rapid process (1-4). Therefore, with regard to the increasing incidence of fungal diseases and problems that exist with the use of classical medications and also due to incidence of some drug resistances, there

is a need to introduce new drugs with fewer side effects. So, more extensive researches should be performed in this regard (5).

Today, much attention has been paid to the use of therapies with natural and biological materials. *Royal jelly* and Propolis, the bee products, have been used in traditional medicine by humans over the past centuries (6,7). *Royal jelly* is one of bee products that is produced by incomplete digestion in the stomach of workers of honey bees. This material is secreted from hypopharynx and mandibular

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glands for feeding the queen bee (6,7).

Propolis is a plant resin around the hive and is produced by honey bees. This resin is used to block the gaps and disinfect the hive to strength the walls and body of the hive (8).

Several beneficial properties of this compound can be considered for human being. The extract of the Propolis contains flavonoids, tannins, steroids, alcohol and alkaloid. Overall, Propolis has antimicrobial effect with different spectrum and therefore, it might be considered as a potent candidate for treatment of several clinical scenarios (9).

The chemical composition of Propolis is very complex and more than 300 different compounds are identified in Propolis samples depending on the location and time of collection and the plants of the region. There are naturally 50% gum (including flavonoids and phenolic acids), 30% wax, 10% volatile oils, 5% pollen and 5% various organic compounds in composition of Propolis (6,10-12).

In recent years, several reports have been published about the specific antimicrobial characteristics of natural products to treat bacterial infections that Propolis is one of the considerable cases. Studies have also shown antibacterial activity of *Royal jelly* against bacteria and fungi (13-17).

On the other hand, the irregular use of antifungal drugs, has caused drug resistance against them and they also can have many side effects on human health. Thus, investigations to find natural antimicrobial drugs have increased in order to prevent side effects of drugs (18). Although several reports have been published on anti-inflammatory, antitumor, antiallergic (19), and antihypertensive agents (20,21), few information is available on the antimicrobial property of Propolis. Hence, the present study was conducted to investigate the antimicrobial property of Iranian Propolis and *Royal jelly* against *Rhizopus oryzae* and *Candida albicans*.

## Materials and Methods

*Rhizopus oryzae* with 5176 (SBC327.47) code was provided from Iranian Research Organization for Science and Technology (Tehran). First, *Rhizopus oryzae* was cultured on dextrose agar medium at 25°C. After 24 hours, for preparation of suspension of the *Rhizopus oryzae* fungus, some normal saline was poured onto the fungus colonies surface so that the medium was completely covered and then media were shaken to suspend the spores in it. Then, the supernatant was collected in a sterile tube with screw cap and an uniform similar suspension was prepared by vigorous shaking to separate hydrophobic spores from each other (3,5,22).

## Extraction

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 and powder was transferred into percolator and the extract

was prepared by the percolation method according to instruction no. 10 of German pharmacopeia. The obtained extract was distilled 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 (23,24). 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 National Clinical Laboratories Standards Committee. Two fold dilutions of extract was poured into first microtube and next dilutions were prepared serially (22).

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

*Royal jelly*: 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100, 110, 120, 130, 140, 150, 160, 180, 200, 220, 240 mg/ml.

Iranian Propolis extract: 0.015, 0.023, 0.0312, 0.046, 0.0625, 0.0937, 0.1, 0.187, 0.25, 0.375, 0.5, 0.75, 1, 1.5, 2 mg/ml.

To study the effects of Iranian Propolis extract and *Royal jelly* on *Rhizopus oryzae*, 1.5 cc microtubes were used. Then, 500 µl of prepared dilutions of Iranian Propolis and *Royal jelly* were added to tubes containing 500 µl of sabouraud dextrose broth culture media except to control group and then 50 µl of *Rhizopus oryzae* was added to all microtubes. In the next stage, microtubes were maintained in incubator at 25°C for 48 hours and then 10 ml of the content of each microtube was transferred to sabouraud dextrose agar culture media and again maintained at 25°C for 48 hours and finally the minimum fungicidal concentration (MFC) was determined for *Rhizopus oryzae* (3,24).

Effects of *Royal jelly* and Iranian Propolis extract on *Rhizopus oryzae* were compared, minimum inhibitory concentration (MIC) and MFC of them were determined and finally the results of MIC and MFC of *Royal jelly* and Iranian Propolis on *Rhizopus oryzae* were analyzed.

## Results

### Results of Iranian Propolis

Table 1 shows the MIC and MFC values of alcoholic extract of Iranian Propolis tested on *Rhizopus oryzae* for 3 times. In every experiment MIC and MFC values of alcoholic extract of Iranian Propolis on *Rhizopus oryzae* were respectively obtained 0.1 and 0.25 mg/ml.

The mean of MIC and MFC of alcoholic extract of Iranian Propolis on *Rhizopus oryzae* were respectively 0.1 and 0.25 mg/ml (Table 1).

### Results of *Royal jelly*

Table 2 represents the values of MIC and MFC of *Royal jelly* on *Rhizopus oryzae* in 3 times of test that in the first trial MIC and MFC values of *Royal jelly* were respectively 60 and 80 mg/ml, in the second test 120 and 160 mg/ml respectively and in the third test 120 and 160 mg/ml, respectively.

**Table 1.** MIC and MFC values (mg/ml) of Iranian propolis on *Rhizopus oryzae*

Experiments	MIC	MFC
First test	0.1	0.25
Second test	0.1	0.25
Third test	0.1	0.25
Mean	0.1	0.25

Abbreviation: MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

**Table 2.** MIC and MFC values (mg/ml) of *Royal jelly* on *Rhizopus oryzae*

Experiments	MIC	MFC
First test	60	80
Second test	120	160
Third test	120	160
Mean $\pm$ SD	100 $\pm$ 34	133 $\pm$ 46

Abbreviation: MIC, minimum inhibitory concentration; MFC, minimum fungicidal concentration.

The mean of MIC and MFC of *Royal jelly* on *Rhizopus oryzae* were respectively 100  $\pm$  34 and 133  $\pm$  46 mg/ml (Table 2).

The MFC of samples containing ethanol extract of Iranian Propolis was 0.25 mg/ml and were 133 mg/ml in samples containing *Royal jelly* on *Rhizopus oryzae*.

## Discussion

*Royal jelly* and Propolis are of honey bee products and has been used in traditional medicine by humans over the past centuries (6,7). In a study Jamali and colleagues (9) have suggested that the physical character of Propolis generally has been used by honey bees to protect their hive, but several beneficial properties of this compound could be considered for human being. Their findings concerning the chemical analysis of the Propolis exhibited the presence of flavonoid, tannin, steroid, alcohol and alkaloid in extracts. Overall, Propolis has antimicrobial effect with different spectrum and therefore, it might be considered a potent candidate for treatment of several clinical scenarios.

The main chemical compounds present in Propolis are silver, mercury, copper, iron, calcium, silis, flavonoids, and phenolic compounds. Propolis contains some volatile oils, terpenes and bee wax, too (9,10).

Ownagh and colleagues (25) expressed that the ethanol extract of Propolis has an inhibitory effect on all tested fungi and the MIC of Propolis was 62.5-500  $\mu$ g/ml for dermatophytes (*Microsporum canis*, *Microsporum gypseum*, *Microsporum nanum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*) and 62.5-125  $\mu$ g/ml for nondermatophytes (*Candida albicans*, *Aspergillus niger*, and *saccharomyces*). Twenty-six compounds were identified by gas chromatography-mass spectrometry analysis. The chemical composition of

ethanol extract of Propolis included considerable amount of flavonoids (25).

In another study the antifungal activity of alcoholic extract of Propolis was examined and its impact on experimental *Candida* keratitis showed that 1000  $\mu$ g/ml concentrations of Propolis could completely treat *Candida albicans* keratitis (18). The inhibitory effect of alcoholic extract of Propolis against *Candida albicans* was also examined. Half of the fermented strains were affected by inhibitory effect of 0.25 g/dl concentration of Propolis extract and minimum fungicidal concentration of extract was determined 0.25 g/dl in more than 50% of the isolates (5). In another study the minimum inhibitory concentration of Propolis on *Candida rugosa*, *Aspergillus niger* and *Rhizopus oryzae* fungi was found to be 2.5 mg/ml (26). In our study, the mean of MIC and MFC of *Royal jelly* on *Rhizopus oryzae* were respectively 100  $\pm$  34 and 133  $\pm$  46 mg/ml that are in agreement with the results of other studies. The means of MIC, and MFC of Iranian Propolis alcoholic extract on *Rhizopus oryzae* were 0.1 and 0.25 mg/m, respectively. These results are in consistent with the results of previous studies which worked on other fungi. Therefore Iranian Propolis extract has antifungal effect on *Rhizopus oryzae* and might be used as an antifungal agent for treatment of fungal infections.

## Conclusion

The results of this study show that alcoholic extract of Iranian Propolis has higher effect compared to *Royall jelly* in agreement with the results of other studies. So Propolis might be used as an antifungal agent for treatment of fungal infections like *Rhizopus oryzae*. If clinical studies also confirm the effects of these drugs, they might be used to treat fungal infections.

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## Authors' contributions

All the authors wrote the manuscript equally.

## Conflict of interests

The authors declared no competing interests.

## 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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