

*Full Length Research Paper*

# Evaluation of antimicrobial activity of propolis and nanopropolis against *Staphylococcus aureus* and *Candida albicans*

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This study was carried out in order to evaluate the antibacterial and antifungal activity of propolis and nanopropolis, against *Staphylococcus aureus* and *Candida albicans* collected from *Ferula ovina* (Boiss.) Taleghan, Iran. Agar well diffusion method was employed to determine the antimicrobial activity of propolis and nanopropolis. The nanopropolis was prepared by milling media method. Most of the nanopropolis size was under the 100 nanometers. There were significant differences between propolis and nanopropolis in inhibition of *S. aureus* and *C. albicans* ( $p < 0.001$  and  $p < 0.05$ ), respectively. Findings of this study indicated that natural nanoparticles have the potential to be used efficiently in the control of bacterial and fungal diseases.

**Key words:** Nanopropolis, propolis, antimicrobial, *Staphylococcus aureus*, *Candida albicans*.

## INTRODUCTION

Propolis (natural antibiotic) is substantially a resinous collected by honeybees (*Apis mellifera*) from buds or the other different parts of plant exudates and finally after combination with wax and the other compounds from bee metabolism can be produced in hive. The chemical composition of propolis depends on a series of factors such as; the specific local flora at the site of collection and climatic characteristics. Propolis can be collected by bees from different parts of some trees like pine, oak, poplar, chestnut (Valle, 2000; Bankova et al., 2000; Trusheva et al., 2006). Most compounds of Propolis consist of about 40-45% resin, 25-30% fatty acid, 10% essential oil, 5% pollen and 5% minerals and organic compounds (Krell, 1996). Honeybee uses propolis against some pathogenic microorganisms in the hive, and

traditionally propolis has been used in some places as a remedy for controlling of some infectious diseases (Faten et al., 2002). At present, Propolis is in use in pharmaceutical industries and related as a modern medicine (Gebara et al., 2002). There are significant papers that reported there is a relationship between chemical composition of plants and quality of propolis produced by bee (Marcucci, 1995; Bankova et al., 2000). *Ferula ovina* (Boiss.) is a medicinal plant from (Apiaceae) family. Traditionally *Ferula* has been used as folk medicine for treatment of some diseases such as of digestive disorders, rheumatism, headache, arthritis, diabetes, toothache, etc. (Dehghan et al., 2007), there consists of 133 species of *Ferula* genus distributed in the Mediterranean area and central Asia (Hansen et al., 2001; Rios and Recio, 2005). The Iranian flora comprises 30 species of *Ferula*, which some of them are endemic, in Persian *Ferula* is known as "Koma" (Javidnia et al., 2005). In survey on the Iranian propolis by principal component analysis, Iran, Spain and Portugal propolis

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was evaluated in the same group (Sawaya et al., 2010). Regarding to importance of this valuable plant, in this research the propolis was collected from *Ferula* in Talegan, Iran. The role of nanotechnology is to help in increasing the effective of materials using the change in its size and this matter can result into having a better efficacy in various fields such as bioscience and medicine (Mirkin and Taton, 2000).

Accordingly, resistant strains in the world are increasing rapidly (Chopra, 2007). In order to increasing the effective and quality of propolis against gram-positive bacteria (*Staphylococcus aureus*) and yeast (*Candida albicans*) which are considered a useful model for antimicrobial activity, nanopropolis was used. Since in present literatures there was not any registered study that is related to the antimicrobial properties of propolis and nanopropolis extracted from *Ferula* genus; this study was therefore carried out with the aim to evaluate their antimicrobial activities.

## MATERIALS AND METHODS

### Propolis and nanopropolis preparation

The Propolis samples were cut into small pieces and dissolved in ethanol with a ratio of 3:10 (30 g of propolis in 100 ml of 96% ethanol%,  $\rho = 0.8051$  to  $0.8124 \text{ g/cm}^3$ ). Then, the propolis samples were kept for 7 days at room temperature and in a dark place. After 7 days shaking, the ethanolic extract of propolis (EEP) was filtered (Gonsales et al., 2006). The nanopropolis was prepared by using milling media method (balls/rods) (Eskandarany, 2001).

### The assay for antibacterial and antifungal activity

#### Bacterial and fungal strains

The bacterial strains (*S. aureus*) as gram- positive, and yeast (*C. albicans*) as fungal strain were supplied by Razi Vaccine and Serum Research Inst. Iran.

#### Agar well diffusion test

The Mueller-Hinton broth and Sabouraud's glucose broth were employed for in vitro evaluation of antibacterial and antifungal activities, respectively. The Ethanol 96% was used as control in separated plates. The concentrations of the propolis extracts used in the study were ranged from: 1, 2 and 4 mg/ml. The only concentration used for nanopropolis was 1 mg/ml. After the plates were solidified at room temperature, wells were made in the agar with sterile steel cylinders in 1mm dimension. All of the suspensions of the gram positive bacteria and the yeast were spread on to different plates. Then, the same 40  $\mu\text{l}$  of undiluted extracted propolis and nanopropolis was added into the wells of plates. For control were used the same amount of ethanol 96%.The plates were incubated for 24 h at 37°C and 72 h at 25°C for tested the bacteria and the yeast, respectively. Finally, diameters of inhibition zones around the wells were measured. All the plates of treatment were performed in triplicate.

#### Characterization of nanoparticle

The nanopropolis was measured by using scanning electron

microscopy (SEM) and particle size analysis.

### Statistical analysis

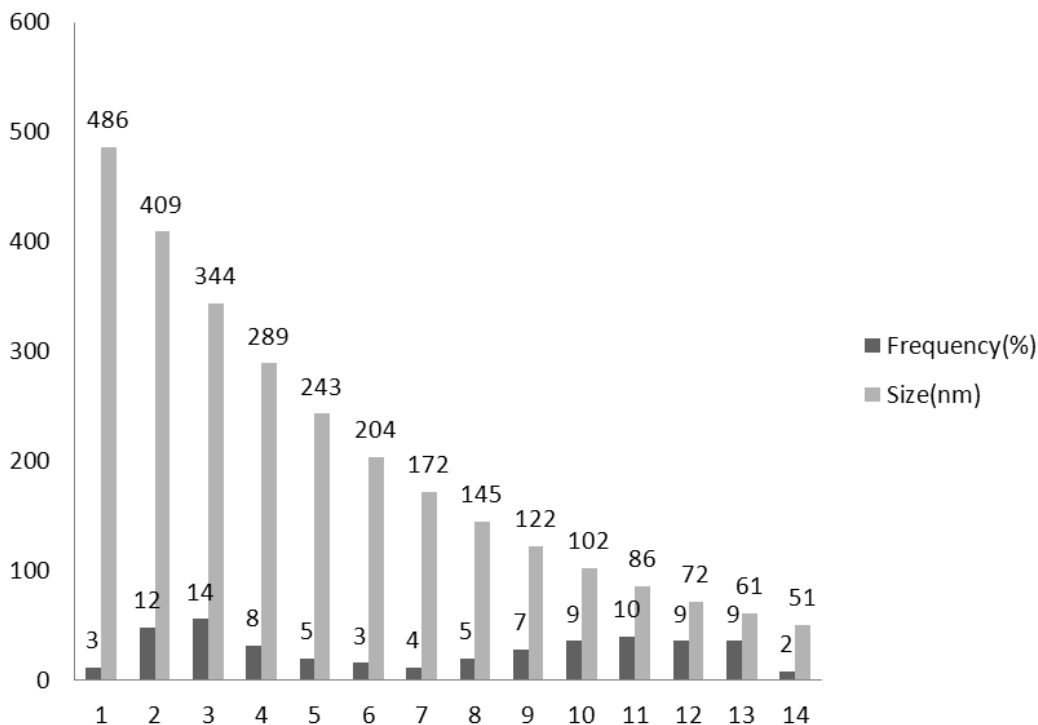
One-way ANOVA and the post test of Tukey were used to analyze the data.  $P < 0.05$  was considered as a significant level.

## RESULTS

The results obtained from the size of nanopropolis characterized using SEM were shown in Figure 1. As shown in this figure, the sizes of particles under 100 nm were between 51 and 86 nm. About 30% of the particles had a size under 100 nm while 9% of those had a 102 nm size. The highest particle size of propolis was 486 nm which was included 3% of total nanopropolis. There was nanopropolis with the sizes ranged between 122 to 409 nm, so that 58% of total nanopropolis was included (Figure 1). The results of antimicrobial activities propolis and nanopropolis samples collected from ovina (Boiss.) against *S. aureus* and *C. albicans* was summarized in (Table1). The largest inhibition zones obtained by nanopropolis against *S. aureus* were seen from 24 to 26mm, whilst for yeast *C. albicans* were from 18 to 21mm. The lowest inhibition zone was related to propolis which ranged from 11 to 13mm (Table1, and Figure 2). There were significantly differences between inhabitation zones of propolis and nanopropolis in *S. aureus* ( $p < 0.01$ ) and *C. albicans* ( $p < 0.05$ ) (Table1). There was not any significant difference between inhabitation zones of different concentrations of propolis against the gram positive bacteria and the yeast. There was not any inhibitory in the control group zone (96% ethanol, v/v).

## DISCUSSION

Studies carried out on essential oil composition obtained from *Ferula* species have showed that this compound type has antioxidant activity and antimicrobial properties (Dehghan et al., 2007). Moreover, various species of *Ferula* genus have been traditionally used for treatments of digestive disorders, rheumatism, headache, arthritis, diabetes, toothache (Dehghan et al., 2007; Yahya et al., 1998; Ferrari et al., 2005; Hilan et al., 2007; Javidnia, 2005; Kartal et al., 2007; Khajeh et al., 2005; Maggi et al., 2009). Therefore, in this research the propolis samples were collected from herbal plant. The most important problems for treatment of many infection diseases by antimicrobial drugs, especially intracellular infection, are difficulty to transport antimicrobials through cell membranes (Zhang et al., 2010). Since, nowadays drug delivery systems based on nanoparticle are increasingly approved for clinical uses and variety of diseases. Therefore some various formulations prepared are currently under clinical tests (Zhang et al., 2008; Wagner et al., 2006). Nanoparticles are defined as particles that



**Figure 1.** The size of nanopropolis produced from the plant *Ferula ovina* collected from Taleghan region.

have dimensions in a range from 1 to 100 nm, and this small size can result in special physicochemical properties (Buzea et al., 2007). Nanotechnology involves the production, manipulation and usage of materials ranging in size from less than a micron to that of individual atoms (Mohanpuria et al., 2008). The results indicated that the nanopropolis particles prepared range were in nanometer size (Figure 1). In this study, the milling media was used to produce nanoparticle because it has been reported so effective and economical in comparison to the other techniques (Eskandarany, 2001). Several studies have reported that the control (96 and 70% ethanol, v/v) did not show inhibitory zones against any of the microorganisms tested (Katircioglu and Mercan, 2006; Gonsales et al., 2006). These results show that the antimicrobial activity of ethanol extract of propolis was due to propolis constituents (Gonzales et al., 2006). In our study, there were not any inhibition zones in control. Under the condition of this study, it seems that for further and complementary studies there is not necessary to use ethanol as a control. The Results revealed that propolis and nanopropolis extracted from *Ferula ovina* have a strong antimicrobial activity against *S. aureus* and *C. albicans* yeast (Figure 2). In some studies, the inhibition diameters zones of propolis against *S. aureus*, which were considered from 8 to 13 mm, 10 to 14 mm and also 0 to 11 mm ranged between 0 to 10 mm for *C. albicans* have been reported (Gonsales et al., 2006; Marghitas et al., 2010). In this study the range of inhibition diameters zones of propolis against *S. aureus*

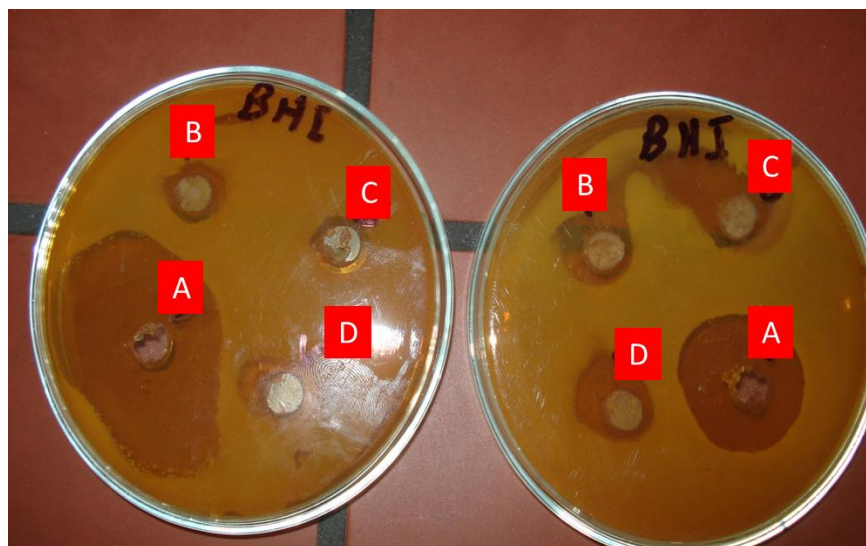
and *C. albicans* were from 11 to 13 mm and 16 to 18 mm, respectively (Table 1). Meanwhile, the inhibition diameters zones of nanopropolis against *S. aureus* and *C. albicans* were from 24 to 26 mm and 19 to 21 mm, respectively (Table 1). Results show that nanopropolis was more effective than the Tetracycline, with 20 mm diameter inhibition zone, against *S. aureus* (Gonsales et al., 2006). Nanopropolis has found to be more effective than propolis in antimicrobial activity (Table 1).

Moreover, the antimicrobial activity of nanopropolis against gram positive bacteria was stronger than the yeast. It seems that the reasons for being more effective in antibacterial activity from antifungal activity of nanopropolis can be search in characteristic of the cell wall, differences present in membrane of bacteria and yeast, the antibacterial activity and the thickness of peptidoglycan layer (Shockman and Barret, 1983). Nowadays silver nanoparticles are the most prevalent nanomaterial used in consumer products. The new developments in technology, silver nanoparticles usually entail some hazard as well as advantage to a society (Aitken et al., 2009). In some of studies demonstrate that nanosilver is toxic to mammalian liver cells (Braydich et al., 2005). The resistant strains are increasing developed in all over the world and some studies showed the propolis could break the resistance produced by some strain such as *S. aureus* and *Enterococcus faecium* (Kilic et al., 2005). According to these kinds of reports in this study, propolis and Nanopropolis were used as a natural antimicrobial substance. As dangerous effects of

**Table 1.** Antimicrobial activity of propolis and nanopropolis prepared from *Ferula oventia* plant against *Candidia albicans* and *Staphylococcus aureus*.

Samples	inhibition zone diameters(mm)	
	Yeast	Gram positive bacteria
	<i>Candidia albicans</i>	<i>Staphylococcus aureus</i>
Propolis	18 16 17	12 13 11
Nanopropolis	18 2119*	26 25 24**
Ethanol96%	0 0 0	0 0 0

\* Significant difference between propolis and nanopropolis;  $P < 0.05$ ; \*\*Significant difference between propolis and nanopropolis;  $P < 0.01$ .



**Figure 2.** Antibacterial activity of nanopropolis and ethanolic extract of propolis (EEP) prepared from *Ferula oventia* against *Staphylococcus aureus* at the 1% concentration of nanopropolis (A) and with 1, 2 and 4% concentrations of propolis (B, C and D), respectively.

nanosilvers has been reported (Chopra, 2007) propolis and nanopropolis could be replaced as an appropriate antimicrobial substances. Based on this study, it is likely that nanopropolis could efficiently reduce the drug cross resistance against harmful organisms bearing diseases in future.

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