

Antibacterial, antifungal and antioxidant activities of bee glue ethanol and aqueous extracts

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Abstract

Food-borne pathogens is a group of micro-organisms that cause food-borne illness, thus the research for finding effective drugs against this infection is necessary. The purpose of this study determines antibacterial, antioxidant activity antifungal of bee glue ethanol and aqueous extracts. Antibacterial and antifungal activities of extracts were evaluated against food-born (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae* and *Candida albicans*) purchased from Boali hospital on by the well diffusion method. Broth serial dilution method was doing to find the Minimal Inhibitory Concentration (MIC) and the agar dilution method to find the Minimal Bacterial Concentration (MBC) of extracts. The antioxidant activity of the extracts assaying by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Ethanol extract of bee glue was found the most effective against *S. aureus* strains (inhibition zone=27±0.87 mm) than *E. coli* strains (inhibition

zone=5.5±0.37mm). Aqueous extract of propolis was found the most effective against *S. aureus* strains (inhibition zone=14.5±0.75 mm) than *E. coli* strains (inhibition zone=3.5±0.5mm) and *C. albicans* (inhibition zone=10±0.5 mm). The MIC and MBC values of the extracts ethanol and aqueous most effective against *B. cereus* strains were 0.3125, 0.625, 0.3125 and 1.25 mg/mL than *E. coli* strains 0.625, 1.25, 2.5 and 50 mg/mL. and also for *C. albicans* was 0.625, 1.25, 1.25 and 2.5 mg/mL. IC50 ethanol and aqueous extracts of propolis were 8±0.25 and 45±1.75, respectively. This study was to assess the Antibacterial, antifungal and antioxidant activities activities of bee glue extracts in *in vitro*.

Introduction

The honey bees can be considered as the collect bee glue from plant resin through biting off scraps. Bee glue is hard and fragile at room temperature and soft, malleable and sticky when heated. It can be extracted by the honey bees from the resin of plants in the inside of bee nest and used to repair honeycombs, and keep up disinfection of the beehive, prevent decay organisms inside the bee nest that dies.^{1,2} Furthermore, the variation in the composition of bee glue can be associated with the plant species, regional geography, and seasons, which were visited by the honey bees as well as the metabolized and secreted substances by them and also further added components during its production.³

Some biological properties such as antioxidant, antifungal, and antimicrobial activity, mainly due to of phenolic compounds, especially flavonoids, were demonstrated previously.⁴⁻⁶ Moreover, recently, the utilization of natural preservative ingredients such as natural waxes, plant extracts and essential oils in order to inhibition of growth of foodborne pathogens as well as spoiling molds in food products is highly regarded.⁷⁻⁸ Due to favorable properties, bee glue can be utilized in variety of applications, such as anti-wrinkle, anti-acne, anti-inflammatory, antiviral, anti-diabetic and leishmanicidal agents.⁹⁻¹⁷ In addition approaching of extracted bioactive compounds from medicinal plants sources as antimicrobial and antioxidants agents attracted notable attention.^{18,19} In this context, the bee glue extracts can be incorporated in food processing, nutraceuticals or cosmetic anti-aging formulations.

Hence, the goal of this study was undertaken to assess the antioxidant and/or antimicrobial activities of bee glue extracts an *in vitro* system.

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Materials and Methods

Preparation of extracts

Bee glue samples were collected from reclaimed land at Kandovan of Tabriz province, Iran 2012 and stored for 3 months at 20°C. Ten gram of sample and divided into two equal parts, each of them was cut into small pieces and extracted at room temperature with 100 mL of 80% ethanol (twice after 24 h) for ethanol extract and with 100 mL of water distillates (twice after 24 h) for water extract. The extracts evaporated under vacuum at 50°C until dryness.

Isolation and identification of *Candida albicans*

Candida albicans isolates was recovered from women with vaginitis attended to Yas Moheb hospital, Tehran, Iran. Swabs were taken from patient by using sterile cotton swabs with transport media. The samples were cultured on Sabouraud dextrose agar supplemented with chloramphenicol to prevent bacterial contamination and incubated at 37°C. The fungal culture was examined according to the colonies, cellular morphology and germ tube formation.

Disk diffusion test

The aqueous extracts were tested against *Staphylococcus aureus* ATCC 25913, *Escherichia coli* ATCC 8739, *Bacillus cereus* PTCC 1709 and *Vibrio cholerae* PTCC 1611. The microorganisms were cultured in BHI (Brain Heart Infusion) for 18 hours at 37°C, and resuspended in 0.5 MacFarland Standard (5×10^8 CFU/mL) and inoculated directly in boards with Mueller-Hinton Agar (Merck). After inoculation of each microorganism, the diffusion method was used, putting 10 µL of essential oil on paper disks (6 mm of diameter) at 37°C for 24 hours, after which time the zone of inhibition were measured. Triple plates were used for each concentration.^{20,21}

Determination of antimicrobial activity by agar diffusion method

Petri plates containing 25 mL of Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for *C. albicans* were used. Agar media were seeded with a 24 h old culture of the microorganism strains (by sterile cotton swab dipped into the broth of these microorganisms). Four wells (5 mm diameter) were cut into the agar by cork borer and 0.1 mL of the crude bee glue extracts was applied in each well. The inoculum size was adjusted so as to deliver final inoculum of approximately 10^8 colony forming unit (CFU)/mL, compared with the turbidity of a sample of the 0.5 McFarland standards. Incubation was performed at 37°C for 24 h. The assessment of antibacterial and antifungal activity was based on measurement of the diameter of the inhibition zone formed around the well. Streptomycin was used as a reference antibacterial agent and nystatin as a reference antifungal agent.²²

DPPH assay

The hydrogen atom or electron donation abilities of the corre-

sponding extracts and some pure compounds were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This spectrophotometer assay uses stable radical DPPH (Sigma, Aldrich) as the reagent.²³ Briefly, 50 µL of the extracts (various concentrations) were added to 5 mL of the DPPH solution (0.004% methanol solution). After 30 min incubation at room temperature, the absorbance was read against pure methanol at 517 nm. The radical-scavenging activities of the samples were calculated as percentage of inhibition according to the following equation:

$$\% \text{ DPPH radical scavenging} = \frac{[(\text{control absorbance (blank)} - \text{sample absorbance}) / (\text{control absorbance})] \times 100}$$

Extract concentration providing 50% inhibition (IC 50) was calculated from the plot of inhibition percentage against extract concentration. All tests were done in triplicate. Values (mean±SD) of the extracts were compared with those of BHT using student's t-test. A P value less than 0.05 were statistically considered significant.²⁴

Statistical evaluation

All analyses and results were presented as mean±standard deviation. The statistical analyses of data were performed by using SPSS version 16.0 (IBM SPSS Inc., Chicago, IL). P<0.05 was considered as the significant difference.

Results and Discussion

Susceptibility test by broth and agar micro dilution or bee glue extracts were performed. The Minimal Inhibitory Concentration (MIC) e Minimal Bacterial Concentration (MBC) value of bee glue aqueous and extracts For *Staphylococcus aureus*, *E. coli*, *Vibrio cholerae* and *Bacillus cereus* showed in Table 1. The Table 2 shows antimicrobial activity ethanol extract of bee glue against the all the bacterial strains, used in this study (*S. aureus*, *E. coli*, *V. cholerae* and *B. cereus* with an inhibitory zone of 27±0.87 mm, 5.5±0.37 mm, 20±1.04 mm, 21±1.32 mm, respectively). The Table 3 shows antimicrobial activity aqueous extract of bee glue against the all the bacterial strains, used in this study (*Staphylococcus aureus*, *E. coli*, *Vibrio cholerae* and *Bacillus cereus* with an inhibitory zone of 14.5±0.75 mm, 3.5±0.5 mm, 14±1.30 mm, 5.5±0.32 mm, respectively). According to the results (Tables 1 and 2) extracts of bee glue revealed much antimicrobial activity against some food pathogenic bacteria. Antibacterial susceptibility was evaluated using classical microbiological techniques with disk diffusion, MIC and MBC determination.

The Table 4 shows antioxidant activity ethanol and aqueous extracts. In this study antioxidant activity was measured and compared with that of butylated hydroxytoluene (BHT) using DPPH method. The high absorbance values indicated that the sample pos-

Table 1. Determination of MIC and MBC value (µg/mL) for aqueous and ethanol extract of propolis against microorganisms.

Test	<i>S. aureus</i>		<i>V. cholera</i>		<i>B. cereus</i>		<i>E. coli</i>		<i>C. albicans</i>	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
MIC (µg/mL)	1250	625	1250	312	625	312.5	1250	625	1250	625
MBC (µg/mL)	2500	1250	1250	625	1250	312.5	50000	2500	2500	1250

essed significant antioxidant activity. According to the result (Table 4), there was significant difference between antioxidant activity of ethanolic and aqueous extract of bee glue. Also there were significant differences between extracts and BHT ($P < 0.05$).

The scavenging activity on DPPH radicals has been widely used by scientists to determine the free radical-scavenging activity; therefore we study the antioxidant activity of the extracts of bee glue using DPPH method. The extracts exhibited a remarkable anti-oxidant activity ($LC_{50} = 8 \pm 0.25 \mu\text{g/mL}$ and $45 \pm 1.75 \mu\text{g/mL}$).

Many results found by several researchers have shown that extracts of bee glue have effect antibacterial and antioxidant. A research report by Musa *et al.* (2012) showed that antibacterial activity of propolis (bee glue) ethanolic extract more effective than aqueous extract.²⁵ Also, the previous survey by da Silva *et al.* (2010) reported that antibacterial activity of propolis methanolic extract more effective than ethanolic extract.²⁶ The similar conducted study by Cottica *et al.* (2011) reported that antioxidant activity low ethanol content extracts more effect than pure ethanol extract.²⁷

The previous study by Hegazi and Abd El Hady (2002), showed that antioxidant activity of Propolis samples. El-Saff propolis had a higher antioxidant activity than Ismailia propolis, while the Ismailia propolis had a higher antibacterial activity against *E. coli*, than El-Saff propolis.²⁸

According to a number of different studies was performed by other scientists, phytochemical compound that present in propolis extracts such as phenolic compounds and flavanoids can to associated with antioxidant and antibacterial activity.²⁹

Table 2. Comparison of average inhibitory halo diameter (mm) of various bacterial strains for ethanol extract of Propolis.

Bacterial strain	Samples	Min	Max	Mean \pm SD
<i>Staphylococcus aureus</i>	3	26	28.5	27 \pm 0.87
<i>Escherichia coli</i>	3	4.5	6	5.5 \pm 0.37
<i>Vibrio cholera</i>	3	19.5	21.5	20 \pm 1.04
<i>Bacillus cereus</i>	3	20	21.5	21 \pm 1.32
<i>Candida albicans</i>	3	27	28.5	27 \pm 0.58

Table 3. Comparison of average inhibitory halo diameter (mm) of various bacterial strains for aqueous extract of propolis.

Bacterial strain	Samples	Min	Max	Mean \pm SD
<i>Staphylococcus aureus</i>	3	13.5	15	14.5 \pm 0.75
<i>Escherichia coli</i>	3	3	4	3.5 \pm 0.5
<i>Vibrio cholera</i>	3	12.5	15	14 \pm 1.30
<i>Bacillus cereus</i>	3	4.5	6.5	5.5 \pm 0.32
<i>Candida albicans</i>	3	12	13.5	13 \pm 0.75

Table 4. The content of DPPH ($\mu\text{g/mL}$) in aqueous and ethanol extract of propolis ($P < 0.05$).

Sample	IC50 $\mu\text{g/mL}$
Ethanol	8 \pm 0.25
Aqueous	45 \pm 1.75
BHT	5.1 \pm 0.19

Conclusions

In this study, both extracts showed significant antibacterial, antifungal and antioxidant activities. The extracts may effective in other gram-positive and gram-negative bacteria as well as may effective as antioxidant in food. Therefore it can good alternative and satisfactory artificial preservatives used in the food industry today.

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