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Extended Abstract

Physicochemical and Antimicrobial Properties and Determination of Phenols and Flavonoids Content of Propolis from Bee Hives in Khorasan Razavi Province

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Abstract

In this research, the physicochemical and antimicrobial properties of propolis from honeybee beehives around Mashhad and the content of active compounds in propolis were determined by high pressure liquid chromatography (HPLC) both quantitatively and qualitatively. Physicochemical properties of the propolis sample (such as ash, moisture, soluble solids and insoluble solids and existing metal elements) were measured. The total phenolic and flavonoids compounds in the ethanolic extract of propolis were 40.126 mg/g (gallic acid) and 26.46 mg/g (quercetin), respectively. Antimicrobial tests showed that the minimum inhibitory concentration (MIC) of the extract against *Staphylococcus aureus* was 100 mg/mL, while in the applied concentration MIC did not achieve against *Escherichia coli*. Also, the results of the minimum bactericidal concentration (MBC) test indicated that propolis extract on *Staphylococcus aureus* had only inhibitory effects. Evaluation of the content of phenolic and flavonoid compounds in propolis extract by HPLC indicated that the flavonoid compounds included flavones (13.33 mg/g), flavonoids (6.375 mg/g), flavonols (8.235 mg/g) and flavanones (16.825 mg/g). Based on the results, propolis can be used in various food and pharmaceutical industries.

Keywords: Antimicrobial activity, Chromatography, Flavonoids, Phenolic compounds, Propolis

Introduction

Propolis is a product of bee honey and it is an effective antimicrobial agent in the prevention of the incidence and prevalence of diseases in hive (Kumazawa, Hamasaka, & Nakayama, 2004). This substance contains gum or resin of herbs, wax, essential fatty acids, pollen, organic compounds, vitamins and minerals. The amount and type of propolis composition is different depending on the place and time of collection and its production method (Kumazawa *et al.*, 2004). Moreover, it is also influenced by the geographical origin and weather conditions of that area (Socha *et al.*, 2011; Tosic, Stojanović, Mitic, Pavlović, & Alagić, 2017). The aim of this study was to investigate the physicochemical and anti-microbial characteristics of propolis of honeybees in the northern part of Mashhad (Khorasan Razavi

province) and also to identify its effective compounds by chromatography.

Materials and methods

The propolis was prepared from the areas around Mashhad (north of Mashhad, the mountains of a Hezar Masjed, Khorasan Razavi). The standards of phenolic and flavonoids for chromatography were purchased from Sigma-Aldrich Company. The culture media of Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) were purchased from Himedia Laboratories, LLC (France). *Staphylococcus aureus* (PTCC 1764) and *Escherichia coli* (PTCC 1330), *Aspergillus Niger* (PTCC 5011), and *Saccharomyces cerevisiae* (PTCC 2601) were obtained from the Center of Culture Collection (IROST, Iran).

Preparing propolis

Crude propolis was milled. The obtained powder was sieved (No. mesh 40) and stored in a glass container at 4 °C until the tests were carried out. Propolis alcoholic extract was prepared by maceration method at the time of 24 h according to the previous study (Razavizadeh & Niazmand, 2019).

Characterization

The moisture of crude propolis as well as the amount of soluble and insoluble solids in propolis sample were done according to Dias, Pereira, & Estevinho (2012). The pH, wax and ash content of propolis were measured according to the method of (Dias *et al*., 2012). The measurement of metallic elements in the ash of the sample was carried out by atomic absorption (GBC, Sens AA Dual, Australia), (Tosic *et al*., 2017).

The total phenolic compounds were measured in terms of Gallic acid according to the Folin-Ciocaltue colorimetric method (Lima, Lopes, Rossetto, & Vianello, 2009). The measurement of flavonoids was carried out on the basis of a colorimetric test in terms of Quercetin (Popova, Silici, Kaftanoglu, & Bankova, 2005). The content of antioxidant compounds was determined according to Wang, Sun, Cao, Tian, & Li (2008) method.

Micro dilution method was used to determine the minimum inhibition concentration (MIC). Each bacterial strain of *Staphylococcus Aureus* and *Escherichia Coli* in a Müller-Hinton broth were prepared to obtain 0.5 McFarland equivalent to a concentration of 1×10^6 (CFU/mL) from each of the bacterial strains of *S. aureus* and *E. Coli*. Also, ethanolic extract of propolis was obtained at concentrations of 100 mg/mL sequential dilutions in the broth medium. In microplate 96 well, 100 μL of different dilutions of the extract were poured. Then, 95 μL of broth medium and 5 μL of bacterial suspension were added. Two wells were considered as negative controls, one containing 200 μL MHB with ethanol 70% and bacteria and the other containing 200 μL MHB and bacteria, and a well containing medium, bacteria and antibiotic were considered as a positive control. The microplates were covered and incubated at 37 °C. After 24 h, the opacity was read at 630 nm by Elisa reader (AWARENESS, technologies INC, Stat fax 2100, USA), (Aamer, Abdul-Hafeez, & Sayed, 2014).

For determining the Minimum Bactericidal Concentration (MBC), 100 μL of the wells that MIC obtained were moved and cultured in plates containing MHA media. The plates were incubated at 37 °C for 24 h. The non-growth of the bacteria indicated MBC (Ristivojević *et al*., 2016).

Separation and identification of phenolic compounds of propolis extract were done using HPLC equipment (Agilent, HPLC 1100, America) with diode array detector at 260 nm.

For statistical analysis, a completely randomized design was used in the form of factorial experiments. The comparison of mean values was done using Tukey test at 95% confidence level (P<0.05). All tests were performed in three replications.

Results and discussion

The physicochemical properties of the propolis, such as moisture, ash, wax, soluble and insoluble solids and pH, are comparable to those reported by other researchers (Saturnino da Silva AraÚJo *et al.*, 2016; Socha *et al.*, 2011).

Based on the results, the most important metal elements in the propolis sample from Khorasan Razavi province were calcium, magnesium, iron, potassium and sodium.

The total amount of phenolic compounds and as well as the flavonoids in the propolis sample were 40.126 mg/g (gallic acid) and 26.46 mg/g (quercetin), respectively. Also, the antioxidant content 60.768% was obtained. The comparison of the reported results indicated low levels of phenolic and flavonoid compounds in the propolis sample. Considering the fact that the propolis has been collected from the mountainous areas of a Hezar Masjed, it is also probable that the province of Khorasan Razavi has been facing declining rainfall and atmospheric precipitation in recent years. Probably, the climatic and geographical conditions of Khorasan Razavi province have affected the propolis characteristics in the region.

The results of antimicrobial tests showed that MIC for propolis extract against *S. Aureus* was 100 mg/mL, while up to this concentration the MIC was not found for *E. Coli*. Also, no bacterial growth was detected by MBC test for *S. Aureus*. The results of these tests indicated that the alcoholic extract of propolis on gram-positive bacteria (*S. Aureus*) was more effective than gram negative bacterium (*E. Coli*). It was concluded that propolis extract against *S. Aureus* has only an inhibitory effect and no bactericidal effect.

Table (1) shows the isolated and detected compounds in the propolis extract. The number of detected compounds from the extract of propolis was 15. The highest amount of compounds identified was related to flavonoids (flavanones, flavones, flavonols and flavonoids) (76.22%), while the amount of phenolic compounds (including Catechin and Gallic, ferulic and caffeine acids) was 23.68%. The highest amount of flavonoids compounds was for flavanones with a mean value of 28.68% includes Pinocembrin and Naringenin. Therefore, based on the results published in the sources, it is expected that this sample will show an inhibitory effect on bacteria (*Bacillus subtilis, Proteus vulgaris and B. vulgaris*) and some fungi (Ghisalberti, 1979).

Table 1. Phenolic and flavonoid compounds separated and identified by HPLC method in the propolis extract (mean value ± SD)

Group of Compounds		Number Pic	Name of composition	Concentration (mg/g)
Phenolic compounds		1	Gallic acid	1.36±0.06
		2	Caffeic acid	4.87±0.02
		3	Catechin	1.98±0.05
		5	Ferulic acid	5.70±0.02
Flavonoids compound	Flavonoid	4	Epicatechin	1.89±0.01
		8	Coumaric Acid	4.49±0.10
	Flavone	7	quercetin-3-methyl-ether	1.70±0.03
		10	Apigenin	4.64±0.03
		12	Luteolin	2.10±0.04
		13	Chrysin	4.92±0.20
	Flavanone	9	Naringenin	8.18±0.06
		14	Pinocembrin	8.65±0.11
	Flavonol	6	Quercetin	1.83±0.03
		11	Camphorol	1.16±0.06
		15	Galangin	5.26±0.020

Conclusions

In this study, the physicochemical and anti-microbial properties and the content of active ingredients in propagates were evaluated. The results indicated that the anti-microbial activity of propolis was influenced by the amount of phenolic and flavonoid compounds present in the sample. So that, the propolis sample showed more antibacterial effects on gram-positive bacteria. In general, this study showed that propolis has a high potential for being used as an antimicrobial or preservative agent in food, as well as antimicrobial supplements for food and food-medications.

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