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Research Article

Total phenolic content, antibacterial and antiradical properties of bee bread from Turkey

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ABSTRACT

Pollen grains, honey, and lactic acid bacteria are combined to make bee bread, which serves as the hive's one of the sources. This study aimed to evaluate a Turkish bee bread concerning the total phenolic content, antiradical, and antimicrobial activity against *Bacillus cereus, Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Salmonella Typhimurium*. Folin-Ciocalteu method and DPPH test were used to determine the total phenolic content (TPC) and antiradical activity of the aqueous extract of bee bread, respectively. Antibacterial activity of the extract on the bacteria was evaluated using minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The TPC of the bee bread was found to be 24.45 ± 3.75 g GAE/mg. The DPPH assay results indicated that the water-soluble extract of bee bread (1 mg/mL) had a scavenging activity of 3.40±2.99%. Additionally, it showed an antibacterial effect on *S. aureus, E. coli, S. Typhimurium*, and *B. cereus* at the different concentrations (6.25 to 25 mg/mL). Overall, it was revealed that the bee bread had high total phenolic content and antiradical activity. Also, it showed antibacterial activity on all of the tested bacteria. This research contributes to the knowledge of the bioactive properties of this unexplored natural material.

INTRODUCTION

The species *Apis mellifera*, commonly known as honeybees, complete their dietary requirements by gathering nectar and pollen. The primary source of carbohydrates for bees is nectar, while pollen serves as a source of proteins, lipids, vitamins, and minerals. Bee bread refers to a blend of bee pollen, bee salivary enzymes, and honey or nectar that undergoes a process of fermentation within the honeycomb cells of a hive. The substance is utilized as sustenance for both the worker bees and the developing larvae (Sobral et al., 2017). Numerous studies of bee bread's chemical composition have shown that it usually contains water, protein, free amino acids, carbohydrates, fatty acids, minerals, vitamins, and many other bioactive compounds such as kaempferol, rutin, quercetin, luteolin, rosmarinic acid. The composition of bee bread varies depending on parameters such as, geographical area, the climatic conditions of the plants producing honey and seasonal changes (Mohammad et al., 2020; Bayram et al., 2021; Tutun et al., 2021; Ćirić et al., 2022). The chemical compositions that occur due to these conditions make bee bread a potential functional food with different bioactive components as well as being food source for bees (Bakour et al., 2022). Bee bread has a wide range of biological properties including antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer (Didaras et al., 2020; Khalifa et al., 2020; Tutun et al., 2021). These biological properties are closely related to the chemical composition of bee bread. Bee bread is regarded as a dietary supplement owing to its biological impacts. In recent times, there has been a surge in interest regarding the utilization of diverse bee bread and its products for the treatment of numerous illnesses (Khalifa et al., 2020). Anatolia serves as a connecting link between three continents owing to its geographical location. The diverse flora and varying climatic conditions across different regions make it highly beneficial for beekeeping activities (Kambur and Kekeçoğlu, 2020). Turkey has 75% of the world's honey plants flora. Of the 11,500 species of flowering plants found in European countries, more than 9,000 are found in Turkey, of which 3,000 are endemic (Suna, 2019). Despite the significance of honey production among beekeepers in Turkey, the production of bee pollen and bee bread does not receive sufficient attention. In addition, studies on the content and biological activities of bee bread produced in different regions are very limited. This study aimed to determine the total phenolic content, antiradical, and antibacterial properties of bee bread collected from the Ankara province of Turkey.

MATERIAL and METHODS

Sampling and extraction

Samples of fresh bee bread were obtained from apiaries located in three different points of Ankara province, in the inner Anatolia region of Turkey. Samples were stored at 4 °C until testing. Aqueous extraction was carried out by adding 50 g of the homogenized sample to 200 mL of distilled water at room temperature and left in the dark for 2 days. Subse-

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quently, the samples were filtered through a filter paper, and the filtrates were concentrated at 40 °C in rotary evaporation (RV10, IKA®, Germany). The concentrated extracts received the process of evaporation and were subsequently subjected to drying in a freeze-dryer (Martin Christ, Alpha 1-2LD Plus, Germany) to obtain a crude extract. The lyophilized extract was stored in a tube at +4 °C in the dark for further analysis (Akhir et al., 2017).

Total phenolic content (TPC)

TPC was determined in aqueous extracts of bee bread by using the Folin–Ciocalteu method which was described by Pelka et al. (2021). The extract was dissolved in distilled water to obtain a concentration of 1 mg/mL. A volume of 50 µL of Folin-Ciocalteu reagent diluted (1:10, v/v) with high purity deionized water was added to a 96-well plate containing 10 µL of the extract solution. Following a 5-minute incubation period, 40 µL of Na_2CO_3 (7.5%) solution was added to the mixture. Following the stirring, 100 µL of high-purity water was added to the mixture and the volume reached 200 mL. The mixture was then allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction was detected at 725 nm using a spectrophotometer (MultiskanGo, ThermoScientific). For constructing the calibration curve, fresh Gallic acid standard solutions (3.13 to 200.00 µg/mL) were prepared. Ethanol was used as a blank. The quantification of total phenolic content in the extracts was conducted by expressing the content as milligrams of Gallic acid equivalent (GAE) per gram of the extract. The measurements were conducted in duplicate (Pelka et al., 2021).

Antiradical activity

The radical scavenging activity of the extract was assessed by using (2,2-Diphenyl-1-picrylhydrazyl) assay according to the method reported by Kahraman et al. (2022). Briefly, a 50 µL of aqueous extract at a concentration of 1 mg/mL or ascorbic acid dissolved in ethanol (50 µg/mL) was mixed with 150 µL of a 200 µM methanolic DPPH solution in a 96-well plate. The mixture was incubated at room temperature for 30 minutes under dark conditions. Absolute methanol was used as blank. The results were measured at 517 nm in absorbance using a microplate reader (Multiskan Go, Thermo Scientific). The DPPH radical scavenging activity (%) was calculated as follows: DPPH scavenging activity (%) = [(Ac – As) / Ac] × 100, where Ac represented the absorbance of the control group [DPPH + Methanol without sample], As indicated the absorbance of the sample group [DPPH + Sample (extract)].

Antimicrobial activity

Bacterial strains

The strains of *Bacillus cereus*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Salmonella* Typhimurium were obtained from the stock culture collection of Burdur Mehmet Akif Ersoy University's Department of Food Hygiene and Technology Laboratory. The bacterial strains were inoculated onto tryptic soy agar (TSA, Merck, Germany) and incubated at 37 °C for 18-24 h.

Microdilution method

The microdilution method was used to determine bee bread's minimal inhibitory concentration (MIC) values against *S. aureus* and *E. coli*, *S.* Typhimurium, and *B. cereus* (CLSI, 2006). The bacterial inoculums were prepared from the bacterial culture grown in TSA (Merck, Germany) at 37 °C for 18 h and adjusted to 0.5 McFarland (≈ 1.5 x 10^8 CFU/mL) in 0.9% sterile saline buffer. The serial dilutions of the samples at the concentrations of 25, 12.50, 6.25, 3.12, 1.56, 0.78, and 0.39 mg/mL were prepared in Mueller Hinton broth (CM0337, Oxoid). These dilutions were subsequently transferred to the wells of 96-well plates. A 20 µL of each bacterial inoculum was added to the wells, and the plates were incubated for 24 hours at 37 °C (Keyvan et al., 2022). After the incubation period, 20 µL of 2,3,5-triphenyl-tetrazolium chloride (1% TTC, Sigma-Aldrich) was added to the wells for each bacterial species to determine the MIC value. The plates were left for incubation at 37 °C for 3 hours. Red color shift in those wells showed the presence of metabolically active microorganisms (Karpinski, 2019). The minimum bactericidal concentration (MBC) value was obtained by subculturing the MIC dilutions. A 10 µL of the suspension was collected from each well and plated onto the Mueller Hinton agar (CM0337, Oxoid) plates. After the incubation for 24 hours, at 37 °C, the lowest extract concentration that inhibits bacterial growth was noted as the MBC value (Zielińska et al., 2021; Kahraman et al., 2022).

RESULTS

In the current study, the total phenolic content of the aqueous extract of the bee breed was determined using the Folin-Ciocalteu reagent. The TPC of bee bread was found to be 24.45 ± 3.75 µg of GAE/mg of dry extract. The scavenging activity of the extract has been determined by using a DPPH assay. The assay results demonstrated that the aqueous extract (1 mg/mL) of bee bread has shown to be 3.40 ± 2.99% scavenging activity. Ascorbic acid used as standard at the 50 µg/mL concentration had 89.76 ± 1.00% scavenging activity.

Table 1. MIC and MBC values of the aqueous extract of bee bread against tested bacteria.

	S. aureus	*E. coli*	*S.* Typhimurium	*B. cereus*
MIC (mg/mL)	6.25	6.25	6.25	12.50
MBC (mg/mL)	25.00	12.50	12.50	25.00

MIC: minimal inhibitory concentration, MBC: minimum bactericidal concentration

In the current study, the MIC values of bee bread extract against three bacteria were determined via the microdilution method. Subcultures of MIC dilutions were used to obtain MBC values. The MIC value against *B. cereus* was 12.50 mg/

mL. The MIC values against three other bacteria were determined at 6.25 mg/mL. The MBC values against *E. coli* and *S.* Typhimurium were 12.50 mg/mL. The MBC values against two other bacteria were determined at 25.00 mg/mL. The MIC and MBC values for the bee bread extract's antibacterial effects on *S. aureus*, *E. coli*, *S.* Typhimurium, and *B. cereus* are shown in Table 1.

DISCUSSION

Bee bread is a nutrient-dense substance that contains high levels of proteins, vitamins, and polyphenols, including phenolic acids. Phenolic compounds are considered to be a vital human dietary component and the largest contributors to the antioxidant potential of natural foods as well as other health benefits (Kumar and Goel, 2019). Several papers reported that the TPC in bee bread ranged from 2.1 to 25.4 mg of GAE/g (Ivanišová et al., 2015; Zuluaga et al., 2015; Suleiman et al., 2021). In the current study, the TPC of bee bread has been found as 24.45 ± 3.75 µg of GAE/mg of dry extract, corroborating previous studies.

The scavenging activity of the extract has been determined by using a DPPH assay. The assay results demonstrated that the aqueous extract (1 mg/mL) of bee bread has shown to be 3.40 ± 2.99% scavenging activity. Ascorbic acid used as standard at the 50 µg/mL concentration had 89.76 ± 1.00% scavenging activity. According to the findings of Dervişoğlu et al. (2022), the ethanolic extract from bee bread exhibited varying degrees of inhibition, ranging from 20.15 ± 0.68% to 93.18 ± 0.44%, depending on the concentration (ranging from 25 to 200 mg/mL). Akhir et al. (2017) reported that 70% ethanolic and hexane extract of bee bread exhibited percentage of inhibition 93.60 ± 0.03 and 83.81 ± 0.05, respectively. Another study showed that significant decreases in DPPH activity of bee bread from Malaysia were detected in bee bread water extract (7.62 ± 0.13%) relative to bee bread hot water extract (8.47 ± 0.01) and bee bread ethanolic extract (85.79 ± 0.40%) (Suleiman et al., 2021). The ethanolic extract of bee bread showed higher antioxidant properties due to higher phenolic and flavonoid contents compared to its aqueous extract (Othman et al., 2019). In the current study, the low antiradical activity of the aqueous extract may be due to the geographical characteristics of the region where bee bread is produced and the extraction method that changed the chemical composition of the extract.

In this study, MIC and MBC were used to determine the extract's antibacterial activities on *S. aureus, E. coli, S.* Typhimurium, and *B. cereus*. Pelka et al. (2021) observed an antibacterial effect on *S. aureus* at 2.5% (v/v) MIC concentration of bee bread. It has been reported that bee bread has a higher antibacterial effect on Gram-positive bacteria than pollen (Pelka et al., 2021). In a study conducted in Malaysia, the MIC_{50} values of bee bread were reported to be 1.914 µg/mL, 1.923 µg/mL, 1.813 µg/mL, and 1.617 µg/mL on *Klebsiella pneumonia, E. coli, Shigella,* and *S.* Typhi, respectively (Suleiman et al., 2021). In another study carried out in Ukraine, the antibacterial effect of bee bread with a MIC value of 6.40 µg/mL was determined on *E. coli* and *S. enterica* (Ivanišová et al., 2015). The application of different extraction methods and the diversity of plant flora may have been effective among the reasons for the varied results in MIC and MBC values in this study.

There are few studies about the water extract of bee bread. In a study conducted by Urcan et al. (2018), a concentration of 33% and 16.66% of bee bread water extract exhibited high inhibitory effects on *S. aureus*, whereas the partial inhibitory effect on *E. coli*, *S.* Enteritidis, *P. aeruginosa* and *B. cereus*. Sawicki et al. (2022) examined the antimicrobial effect of methanolic extract from bee bread and reported that the MIC values against to *S. aureus, L. monocytogenes, E. coli, E. faecalis* and *S.* Typhimurium ranging between 50% - 15% (v/v). Another study on ethanolic and hexane extract of stingless bee bread showed that MIC for *B. subtilis, S. aureus, E. coli* and *Salmonella* ranged from <6.67 to 33.33 µL/mL (Akhir et al., 2017).

CONCLUSION

Bee bread, which is made of pollen blended with honey and digestive enzymes from bees, is a treasured bee product that has been ignored. The results of this study showed that the bee bread from Ankara has antiradical activity and antibacterial effects against the foodborne pathogens.

DECLARATIONS

Ethics Approval

Ethics committee approval is not required since humans/animals were not used in our study

Conflict of Interest

Authors do not have any conflict of interests to disclose nor do they endorse the use of any product/technology/service over the other.

Consent for Publication

Not applicable

Competing Interest

The authors declare that they have no competing interests.

Author contribution

Idea, concept and design: NK, HAK, HT, EK

Data collection and analysis: NK, MSU, MMK, HAK, HT, EK

Drafting of the manuscript: NK, HAK, HT, EK

Critical review: NK, HAK, HT, EK

Data Availability

Not applicable.

Acknowledgments

Not applicable.

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