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Short Communication

Comparative Study on the Antimicrobial and Antioxidant Activity of Propolis

Bo Yeon Kim[1], Hyung Joo Yoon[1], Min Ok[2], Byung Rae Jin[1] and Kwang Sik Lee[1,*]

[1]College of Natural Resources and Life Science, Dong-A University, Busan 49315, Republic of Korea
[2]Nanum Bio Co., Ltd., Yeongcheon 38891, Republic of Korea

Abstract Propolis, a mixture of resinous substances gathered by honeybees from various plants, has garnered significant attention due to its wide-ranging activities. In this study, we compare the antimicrobial and antioxidant activities of two commercially available propolis samples obtained from different geographical locations. Using two powder-type propolis samples, Power King Propolis (PKP; collected in Korea) and Beehi Propolis (BP; primarily collected in Australia), we investigated their antimicrobial and antioxidant activities. Propolis exhibited antibacterial activity and protected mammalian cells against oxidative stress, leading to increased cell viability. Furthermore, the comparison of antimicrobial and antioxidant activities between the two propolis samples indicated higher values for PKP compared to BP. These findings indicate that the difference in the antimicrobial and antioxidant activities of propolis can be attributed to variations in its composition, which are influenced by the plant sources collected from different geographical locations.

Keywords Honeybee, Propolis, Antimicrobial activity, Antioxidant activity, Reactive oxygen species

INTRODUCTION

Propolis, an essential product of honeybees, has a rich history of use as a functional food for human health care. Raw propolis is a mixture collected by honeybees from various plant resources, consisting of plant resins, essential oils, beeswax, and pollen (Przybyłek and Karpiński, 2019; Šuran *et al*., 2021). In general, propolis contains a variety of organic compounds such as polyphenols, terpenes, and esters (Przybyłek and Karpiński, 2019; Šuran *et al*., 2021). Due to this composition, propolis exhibits diverse biological and pharmacological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Schnitzler *et al*., 2010; Ramanausjiene and Inkeniene, 2011; Franchin *et al*., 2016; Przybyłek and Karpiński, 2019).

The composition of propolis varies according to geographical regions, environmental factors, seasons, and the plant species collected (Bankova, 2005; Bankova *et al*., 2006; Souza *et al*., 2016; Rufatto *et al*., 2018). Honeybees produce propolis from various plant sources, such as poplar, birch, willow, spruce, oak, fir, pine, and acacia (Przybyłek and Karpiński, 2019). Additionally, honeybees use the secretions of *Xanthorrhoea* in Australia and *Baccharis*, *Araucaria*, and *Eucalyptus* in Brazil as sources for propolis. On the other hand, the chemical composition of propolis also depends on the types of solvents, their ratios, and the extraction procedures (Šuran *et al*., 2021). Due to the existence of several types of propolis with different chemical compositions, standardization for propolis has been proposed based on plant origin and chemical composition (Bankova, 2005).

While propolis is composed of over 500 bioactive molecules, which are primarily secondary metabolites derived from plants (Huang *et al*., 2014), and its composition is dependent on the plant sources available in dif-

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*Corresponding author. E-mail: leeks@dau.ac.kr

ferent geographical locations (Wang *et al*., 2016), it exhibits similar biological and pharmacological activities. In this study, we compare the antimicrobial and antioxidant activities of two commercially available propolis samples, which are similar types of propolis obtained from different geographical locations.

MATERIALS AND METHODS

1. Propolis

Two commercially available powder-type propolis samples were obtained from BN Care Co., Ltd. (Icheon, Korea): Power King Propolis (PKP), collected in Korea, contains 600 mg of total flavonoids per 60 g of total propolis and Beehi Propolis (BP), primarily collected in Australia, contains 676 mg of total flavonoid per 60 g of total propolis. The powder-type propolis samples were dissolved in distilled water at 37°C for 30 min and then filtered using a 0.45-μm Millipore filter (Sartorius Stedim Biotech., Goettingen, Germany). In this study, we used the filtered propolis samples for further assays.

2. Antimicrobial activity assay

The Gram-positive bacterium *Bacillus thuringiensis* 656-3 and the Gram-negative bacterium *Escherichia coli* DH5α were assayed in this study. The antimicrobial activity of propolis was assessed using a liquid growth inhibition method as previously described (Lee *et al*., 2016). Briefly, bacterial inocula (1×10^2 cfu per well) were incubated with serial diluted samples of propolis in a 96-well plate. The plate was then incubated in Luria-Bertani (LB) medium at 37°C for 24 h with shaking at 220 rpm. The growth inhibition caused by the propolis samples was evaluated by measuring the absorbance at 595 nm using a microplate reader (Bio-Rad Model 3550, Bio-Rad). The minimal inhibitory concentration (MIC), which indicates 50% inhibition of bacterial growth by the propolis samples, was determined.

3. Cell viability assays

Cell viability in response to propolis samples was assessed using a murine fibroblast cell line NIH 3T3, following the protocols outlined in Park *et al*. (2019). The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma), supplemented with 10% FBS (Gibco BRL), at 37°C in an atmosphere containing 5% CO_2. For the MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, NIH 3T3 cells (2×10^4 cells/well of 96-well plates) were incubated with serial dilutions of propolis samples (0, 1, 100, or 1000 μg per mL of medium) and H_2O_2 (50 μM) for 24 or 48 h. Subsequently, NIH 3T3 cells were treated with 50 μL of MTT reagent (BioVision, Milpitas, CA, USA) for 4 h. Cell viability was evaluated by measuring absorbance at 590 nm using a microplate reader (Bio-Rad Model 3550).

4. Measurement of reactive oxygen species (ROS) and iron levels

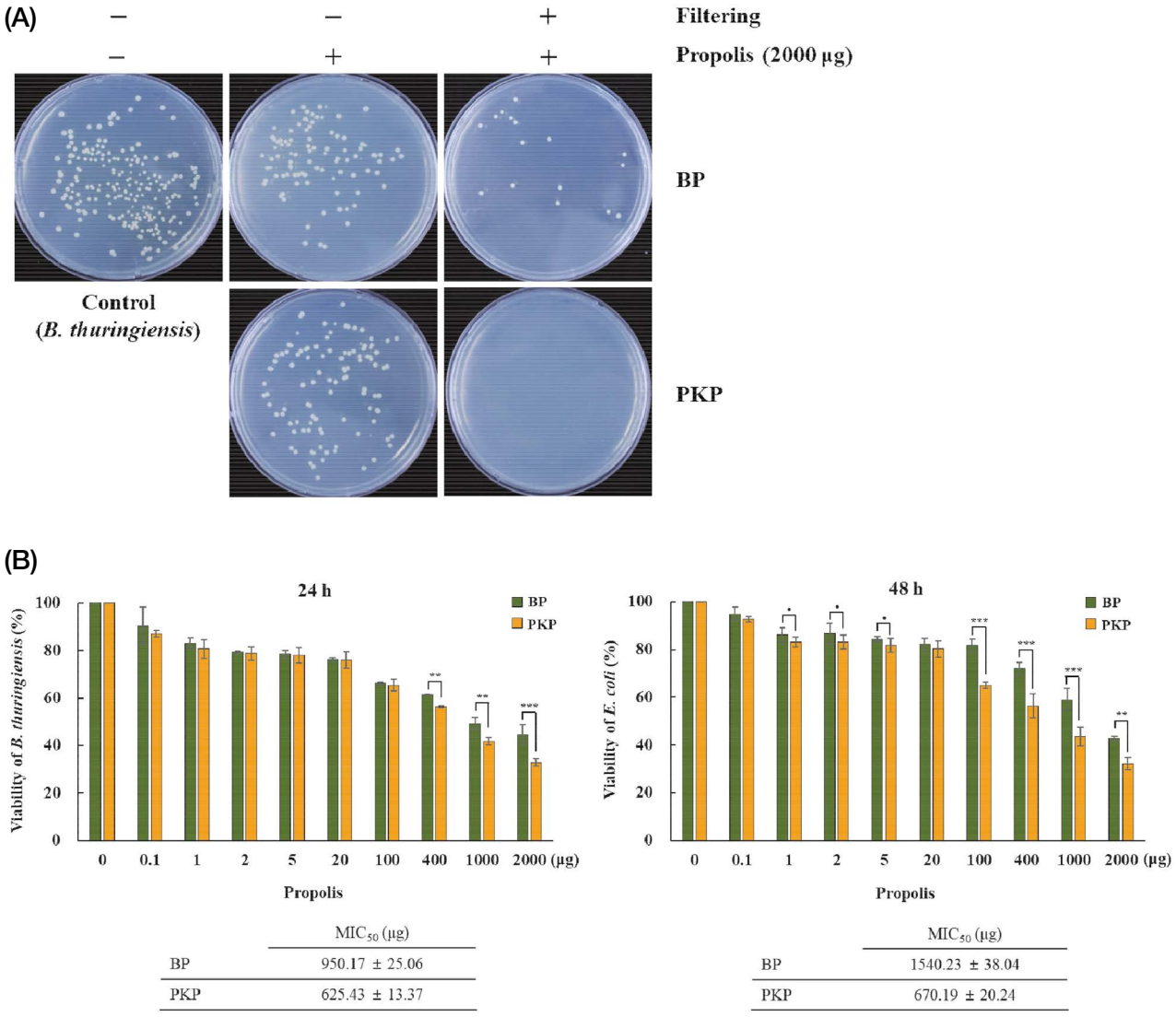
NIH 3T3 cells (2×10^4 cells/well of 96-well plates) were incubated with serial dilutions of propolis samples (0, 1, 100, or 1000 μg per mL of medium) and H_2O_2 (50 μM) for 24 or 48 h as described above. The levels of ROS and iron in the cell culture medium were quantified using the OxiSelect In Vitro ROS/RNS Assay Kit (Green Fluorescence; Cell Biolabs, Inc., San Diego, CA, USA) and Iron Colorimetric Assay Kit (BioVision Inc., Milpitas, CA, USA) following the manufacturers' instructions. The experiments were performed three times using independent samples.

5. Statistical analysis

Results are presented as the mean ± standard deviation (SD) of triplicates. The data were analyzed using a two-sample t-test by R package. Statistically significant differences are denoted with asterisks as follows: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

RESULTS AND DISCUSSION

To compare the antimicrobial and antioxidant activities of two commercially available powder-type propolis samples (PKP and BP), we dissolved the propolis samples in distilled water at 37°C. However, they did not completely dissolve. Therefore, we assessed the antibacterial and antioxidant activities of the two propolis samples by filtering them using a 0.45 μm Millipore filter to exclude potential contamination from specific sources. We found that the antibacterial activity was significant-



	MIC_{50} (μg)
BP	950.17 ± 25.06
PKP	625.43 ± 13.37

	MIC_{50} (μg)
BP	1540.23 ± 38.04
PKP	670.19 ± 20.24

Fig. 1. Antimicrobial activity of propolis samples. (A) Plate assay of the antibacterial activity of PKP and BP propolis samples with and without filtering. (B) Antimicrobial activity of PKP and BP propolis samples against bacteria ($n=3$). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

ly higher in the propolis samples that were not filtered compared to the filtered ones (Fig. 1A). This result indicates that non-filtered propolis samples were much more effective than filtered propolis samples. In both propolis samples, regardless of filtering, the antibacterial activity was higher in the PKP samples compared to the BP samples (Fig. 1B), and both samples exhibited stronger antibacterial activity against *B. thuringiensis* than *E. coli* (Fig. 1B). Thus, our results confirmed that propolis acts as an antimicrobial agent, exhibiting stronger activity against Gram-positive bacteria than Gram-negative bacteria (Przybyłek and Karpiński, 2019).

We assessed the effect of propolis samples on cell viability against H_2O_2-mediated toxicity in NIH 3T3 cells. Interestingly, the cell viability assays revealed a concentration-dependent cell protection effect of propolis samples, with the PKP sample showing higher viability compared to BP (Fig. 2A). Additionally, our results showed that propolis samples decreased the levels of iron and ROS in NIH 3T3 cells exposed to H_2O_2 (Fig. 2B and 2C), indicating that propolis exhibits an antiapoptotic effect by reducing the levels of iron and ROS, which are mediators of cell damage (Park *et al*., 2021). Thus, we confirmed the antioxidant activity of propolis

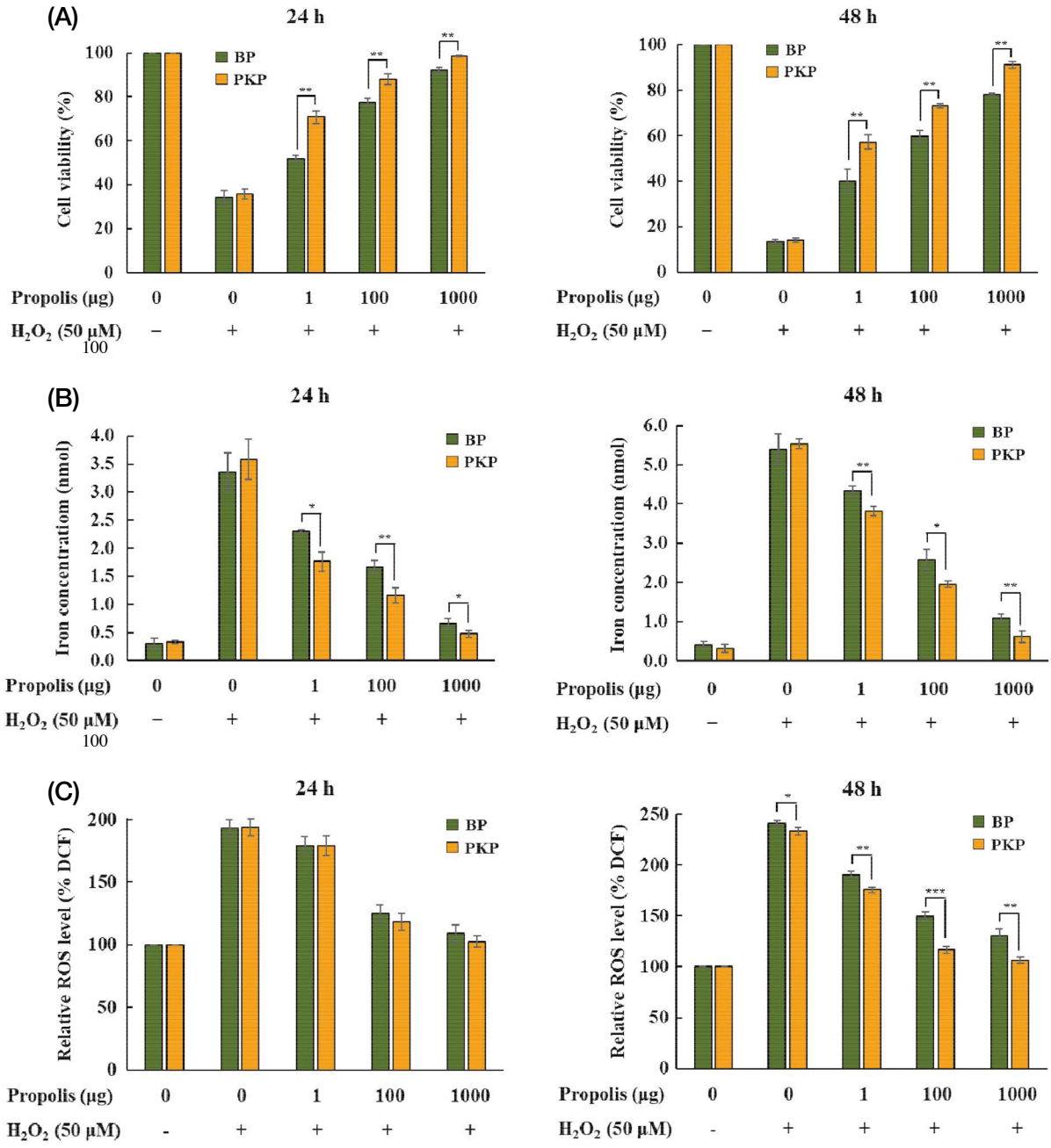


Fig. 2. Antioxidant activity of propolis samples. NIH 3T3 cells were treated with PKP and BP propolis samples and H_2O_2. The percentage of cell viability (A), iron concentration (B), and relative ROS level (C) were determined at 24 and 48 h ($n=3$). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

in H_2O_2-exposed NIH 3T3 cells, underscoring its role as an antioxidant agent.

The findings of this study have confirmed that propolis acts as both an antimicrobial and antioxidant agent. Despite numerous attempts to increase its solubility in water, propolis, also known as bee glue, does not completely dissolve due to its resinous nature (Marcucci, 1995). Therefore, we were interested in investigating whether filtering propolis partially dissolved would affect its biological properties. The chemical composition and biological activity of propolis vary depending on the extraction methods and procedures (Taddeo *et al*., 2016; Šuran *et al*., 2021), types of propolis (Machado *et al*., 2016), and collection regions (Siheri *et al*., 2016;

Wang *et al*., 2016). Previous studies have demonstrated significant variations in the total phenolic contents and biological properties of ethanol-extracted propolis from 20 different regions in Korea (Wang *et al*., 2016). Similarly, the chemical and antimicrobial profiling of propolis collected from different regions within Libya has resulted in its classification into several groups (Siheri *et al*., 2016). Collectively, the biological and pharmacological activities of propolis depend on its chemical composition, which varies according to geographical locations and even within the same countries (Przybyłek and Karpiński, 2019).

In conclusion, our data demonstrate that propolis samples, when subjected to filtering, act as antimicrobial agents and antioxidants, effectively protecting against oxidative stress. These findings highlight the crucial role of the two propolis samples with filtering, ensuring their reliability in terms of antimicrobial and antioxidant effects.

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CONTRIBUTION OF AUTHORS

BYK, HJY, and KSL performed experiments and analyses. KSL and BRJ designed the study and drafted the manuscript. All authors helped write the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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