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원산지별 프로폴리스 추출물의 화장품 소재로서의 생리활성 비교연구

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Comparative Study of the Biological Activity of Propolis Extracts with Various Countries of Origin as Cosmetic Materials

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요 약: 프로폴리스는 식물에서 채취한 수지에 꿀벌의 분비물이 합쳐져 만들어진 아교성 물질로 세균이나 바이러스로부터 자신을 보호하는 기능을 한다. 본 연구에서는 한국, 중국, 브라질 유래 프로폴리스 추출물의 항산화, 항균, 미백, 항염 활성을 비교하고, 이들 추출물의 화장품소재로서의 응용가능성을 살펴보았다. 플라보노이드, 폴리페놀함량분석과 자유라디칼 소거능 시험을 통해 항산화 활성을 확인한 결과 한국, 중국, 브라질 프로폴리스 추출물 모두 유의한 항산화 효능을 보였다. 피부에 상재하는 미생물에 대한 항균효능을 MIC 시험법을 통해 측정한 결과 *C. acnes* 균에서 한국 프로폴리스 추출물이 다른 추출물에 비해 우수한 항균력을 보였다(KPE: 62.5 ㎍/mL, CPE: 250 ㎍/mL, BPE: 500 ㎍/mL). 또한, 한국 프로폴리스 추출물은 멜라닌 세포의 멜라닌 생성을 억제하였고, 마우스대식세포에서 리포폴리사카라이드로 유도된 염증인자인 산화질소와 PGE_2 생성을 나머지 두 추출물보다 우수하게 억제하였다. 이들 결과를 종합하면 프로폴리스 추출물은 항산화, 항균, 항염 효능소재로 응용될 수 있으며, 특히 항균, 항염, 미백에서 우수한 효능을 보인 한국프로폴리스추출물의 응용가능성이 우수함을 확인할 수 있었다.

Abstract: Propolis is a sticky resinous substance that is formed by the combination of honeybee secretions and resin of plants, which serves to protect from bacteria and viruses. This study aims to evaluate the efficacy of propolis extract from Korea (KPE), China (CPE), and Brazil (BPE) through antioxidant, antibacterial, whitening, and anti-inflammatory tests, and to examine their potential as cosmetic materials. KPE, CPE, and BPE showed significant antioxidant activities on flavonoid/polyphenol content and free radical scavenging activity. The antibacterial effect of propolis on skin flora was determined by measuring the minimal inhibitory concentration (MIC). KPE showed better antibacterial efficacy than CPE and BPE in *C.* acnes (KPE, CPE, and BPE: (62.5, 250, and 500) ㎍/mL, respectively). Furthermore, KPE inhibited the melanin synthesis in human epidermal melanocytes and production of nitric oxide and PGE_2 induced by lipopolysaccharide (LPS) in mouse macrophages, which showed better than did CPE or BPE. Taken together, the propolis extracts can be applied to antioxidant, antibacterial, and anti-inflammatory ingredient for cosmetics, while KPE showed superior potential in antibacterial, anti-inflammatory, and whitening efficacies.

Keywords: *Propolis, Antioxidant, Antibacterial, Anti-inflammation, Melanin synthesis*

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1. Introduction

Propolis, referred to as bee glue, is collected by honeybee from the resin of plant, flower, and leaves of trees. It is mixture of beeswax and honeybee secretions, and is used as a physical sealant for gaps in the hive[1]. Propolis was used in traditional medicine with the expectation of wound healing and anti-infection. It has been reported to offer benefits of immunomodulation, anti-inflammation, anti-oxidation, chemoprevention, anti-microbial properties, and so forth[2]. Previous studies have investigated the efficacy of propolis extract and propolis-derived ingredients on skin. Photo-protective effects by suppressing DNA damage, protein oxidation, and metalloprotenases (MMPs) production and anti-inflammatory effect on keratinocytes have been reported[3,4]. In addition, hair growth stimulating effect through the proliferation of keratinocytes and anti-melanogenic effects through suppressing the transactivation activity of microphthalmia-associated transcription factor (MITF) have been observed[5,6].

The chemical composition of propolis is qualitatively and quantitatively variable, because propolis is derived from animals, but contains bioactives that derive from the plant-based diet of bees. More than 300 different phytonutrient components, such as flavonoids, phenolic acids and esters, diterpenes, sesquiterpenes, lignans, and aromatic aldehydes, have been identified in propolis[7]. Flavonoid and polyphenol of propolis have been demonstrated to exert various biological properties that include anti-microbial, anti-inflammatory, and antioxidant activity. The flavonoid constituents of propolis prevent bacterial activity and growth through the increase of bacterial membrane permeability and inhibition of nucleic synthesis, formation of biofilm, and metabolism of bacteria[8]. Chrysin is a flavonoid found in propolis, which shows anti-microbial, anti-photoageing, and anti-melanogenic activities[9]. Caffeic acid phenethyl ester, phenolic compound of propolis, inhibits TNFα-induced NF-κB activation and the expression of inflammatory cytokine in keratinocytes, and decreases melanin synthesis in B16-F10 melanoma cells[6,10].

In this study, we evaluate the biological properties of propolis from Korea, China, and Brazil by measuring the anti-oxidant, anti-bacterial, anti-melanogenic, and anti-inflammatory activities.

2. Experimental

2.1. Materials

Kojic acid, sodium hydroxide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), aluminium chloride ($AlCl_3$), gallic acid, sodium carbonate (Na_2CO_3), and potassium acetate (CH_3CO_2K) were purchased from Sigma-Aldrich (USA). Medium 254 and human melanocyte growth supplement (HMGS) were obtained from Thermo Fisher Scientific (USA). 3-[4,5-Dimethyl-2-thiazolyl]-2,5 diphenyl-2H-tetrazoliumbromide (MTT) was obtained from Duchefa (Haarlem, Netherlands).

2.2. Preparation of Extracts from Propolis

The Korean propolis was obtained from Hallasan sikpoom (Jeju, Korea). Brazilian propolis and Chinese propolis were obtained from Wax Green Inc. (Brazil) and Hunan Nutramax Inc. (China), respectively. Each propolis samples were split and extracted in 95% ethanol at 80 °C for 120 min. This extraction was maintained at under − 4 °C for 12 h and filtered through 5 μm filter. The extract was evaporated and powderized by freeze dryer.

2.3. Total Polyphenol and Flavonoid Contents Assay

The total phenolic contents were determined using modified Fallin-Ciocaleu method with the previous report[11]. Fifty microliters of propolis extract with methanol was mixed with 450 μL of distilled water and 50 μL of Folin-Ciocalteu's phenol reagent. After standing at room temperature for 5 min, 350 μL of 7% Na_2CO_3 solution was mixed with the extract mixture. Then, the mixture was allowed to stand at room temperature for 90 min. The absorbance of mixture was measured at 750 nm using a spectrophotometer (Epoch™ microplate spectrophotometer, BioTek, Winooski, USA). The calibration curve was drawn using the gallic acid standard, and total phenolic content was expressed as μg of gallic acid equivalents per mg of dry weight (μg GAE/mg).

The total flavonoid contents were measured by the colorimetric method of aluminum chloride with some modification[12]. One hundred microliters of propolis extract with methanol and 300 μL of 95% ethanol in a 1.5 mL Eppendorf tube were mixed with 20 μL of the 10% $AlCl_3$

solution and 20 μL of 1 M CH_3COOK. The mixture was followed by the addition of 560 μL distilled water and allowed to stand at room temperature for 30 min. The absorbance of mixture was measured at 415 nm. The total flavonoid content was expressed in terms of the quercetin equivalent (μg QE/mg).

2.4. DPPH Radical Scavenging Activity

Propolis extracts were dissolved at different concentrations in methanol. Then diluted solution was mixed with 0.2 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol. The mixture was kept at RT for 30 min under dark condition and absorbance was measured at 517 nm by spectrophotometer (Epoch, BioTek, USA). Ascorbic acid was used as the standard to compare the result. DPPH radical scavenging activity was calculated using the following equation:

$$\text{Percent scavenging (\% inhibition)} = ((Ac - As)/Ac) \times 100,$$

where, Ac is the absorption of control, and As is the absorption of tested extract.

2.5. Microbial Strains and Growth Condition

The strains of (*Cutibacterium acnes* (*C. acnes*) (ATCC 6919), *Staphylococcus aureus* (*S. aureus*) (ATCC 12600), and *Staphylococcus epidermidis* (*S. epidermidis*) (ATCC 14990) used in this study, were obtained from American Type Culture Collection (Manassas, USA). *C. acnes* was anaerobically cultured in Reinforced Clostridial Medium (RCM, Difco, USA) at 37 °C for 48 h. Two *Staphylococcus* were aerobically cultured in Brain Heart Infusion (BHI, Difco, USA) at 37 °C for 24 h.

2.6. Susceptibility Test of Microbial Strains

The antimicrobial activity was determined by the broth microdilution method in 96 well microplate. C. *acnes* was adjusted to 10^7 colony-forming units (CFU)/mL in RCM and two *Staphylococcus* were adjusted to 10^6 CFU/mL in BHI. Propolis extracts were dissolved in DMSO, and two-fold serial dilutions of each extract were prepared in media at concentrations from (15.6 to 1,000) μg/mL. Each well was inoculated with 10 μL of bacterial suspension. The final concentration of *C. acnes* and *Staphylococcus* in wells were (10^6 CFU/mL and 10^5 CFU/mL, respectively. These microplates were incubated at each culture condition for 24 h. The MIC was defined as the lowest concentration of propolis that inhibits the microorganism growth. Ampicillin was employed as positive control.

2.7. Cell Culture

Human epidermal melanocytes neonatal, moderately pigmented (HEMn-MP) were obtained from Thermo fisher (USA) and maintained in medium 254, containing human melanocyte growth supplement (HMGS, Thermo fisher, USA) at 37 °C, under 5% CO_2. Passage (6 and 7) cells were used in all experiments. The RAW 264.7 cell (Korean Cell Line Bank, Korea), mouse macrophage cell line was maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA), containing 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (Invitrogen, USA), at 37 °C, under 5% CO_2.

2.8. Measurement of Cell Viability

Cell viability assay was determined by using the MTT assay. After treatment, MTT reagent (1 mg/mL) was added to each well and incubated for an additional 3 h. After that, the supernatant was removed, and dimethylsulphoxide (DMSO) was added to dissolve the formazan crystal produced from MTT. Absorbance was measured at a wavelength of 570 nm by a spectrophotometer (Epoch™ microplate spectrophotometer, BioTek, Winooski, USA).

2.9. Measurement of Melanin Synthesis

The determination of melanin contents was performed in the manner described below. Human epidermal melanocytes, neonatal cells (HEMn) were seeded into 6 well plates at 1.0 x 10^5 per well. The HEMn were treated with propolis extracts or 50 μg/mL of kojic acid as a positive control. After 5 days, the cells were washed with phosphate buffered saline (PBS) and scraped with a cell scraper. The supernatant after centrifugation was discarded, and the cell pellet was lysed with 1 N NaOH for 1 h at 60 °C. Melanin content was

Table 1. Total Phenolic and Flavonoid Contents of Propolis Extracts

	Total phenolic content (μg GAE/mg propolis extracts)	Total flavonoid content (μg QE/mg propolis extracts)
KPE	131.3 ± 1.9	53.3 ± 2.7
CPE	159.0 ± 1.5	97.1 ± 4.0
BPE	176.2 ± 2.1	36.5 ± 1.6

Table. 2. Minimum Inhibitory Concentration (MIC) of Propolis Extract against *C. acnes, S. aureus* and *S. epidermidis*

Materials	MIC (μg/mL)		
	C. acnes	*S. aureus*	*S. epidermidis*
Ampicillin (antibiotic)	0.097	0.2	1.56
KPE	62.5	1,000	1.000
CPE	500	1.000	1.000
BPE	250	1.000	1.000

estimated by the absorbance at 450 nm and normalized by absorbance of non-treated sample.

2.10. Measurement of Nitric Oxide Production

The concentration of NO in the culture medium was determined as nitrite, a major indicator of NO production based on the Griess reaction. The RAW 264.7 cells were seeded at 2×10^5 cells per well in 24 well culture plates. The cells were pretreated with the propolis extracts at increasing concentrations (1, 5, 10) μg/mL or 500 nM of 1400 w (Sigma-Aldrich, USA) as a positive control for 2 h and then stimulated with LPS (100 ng/mL) for 24 h. 50 µL of Griess reagent (Promega, USA) was mixed with equal volume of cell culture supernatant for 30 min at room temperature. The absorbance was measured by a spectrometer at a wavelength of 540 nm[13].

2.11. Measurement of PGE_2 Production

The PGE_2 concentrations were quantified by using a commercially available ELISA kit (R&D systems, USA) according to the manufacturer's instructions. The RAW 264.7 cells were seeded at 2×10^5 cells per well in 24 well culture plates. The cells were treated with (1, 5 and 10) μg/mL of propolis extracts or positive control 0.1 nM celecoxib (Sigma-Aldrich, USA) and induced by LPS (100 ng/mL) for 24 h. The cell culture supernatants were collected and assayed for PGE_2. The PGE_2 concentrations were determined using a standard curve. All samples and standards were measured in duplicate.

2.12. Statistical Analysis

Statistical significance of data was determined by a Student's t-test. All results were expressed as the means ± standard deviation (N = 3). $^{*}p < 0.05$ and $^{**}p < 0.01$ were considered to be significant.

3. Results and Discussion

3.1. Total Polyphenol, Total Flavonoid Content and Antioxidant Activity of Propolis

The propolis ethanolic extracts from Korea, China, and Brazil were compared in terms of their total phenolic and flavonoid contents. The total phenolic contents of (131.3, 159.0, and 176.2) μg GAE/mg were observed in KPE, CPE, and BPE, respectively (Table 1). The total flavonoid contents were determined as (53.3, 97.1, and 36.5) in KPE, CPE, and BPE, respectively.

To compare the free radical scavenging activity of propolis extracts, we performed the DPPH radical scavenging assay. KPE, CPE, and BPE increased the free radical scavenging activity in a concentration-dependent manner. CPE showed stronger activity, compared to KPE and BPE. The result

shows a correlation with the total phenolic and flavonoid contents of propolis extract. This can be attributed to the redox property of the constituents[14].

3.2. Antimicrobial Activity of Propolis

Propolis has been used for anti-infection, and its antimicrobial activities have been confirmed. The antibacterial effects of propolis on *C. acnes*, *S. aureus*, and *S. epidermidis* were determined by measuring the MIC. *C.* acnes is a gram positive, anaerobic and lipophilic bacterium that plays an important role in inflammatory acne[15]. *S. aureus* not only causes skin infections, but also aggravates the symptom of

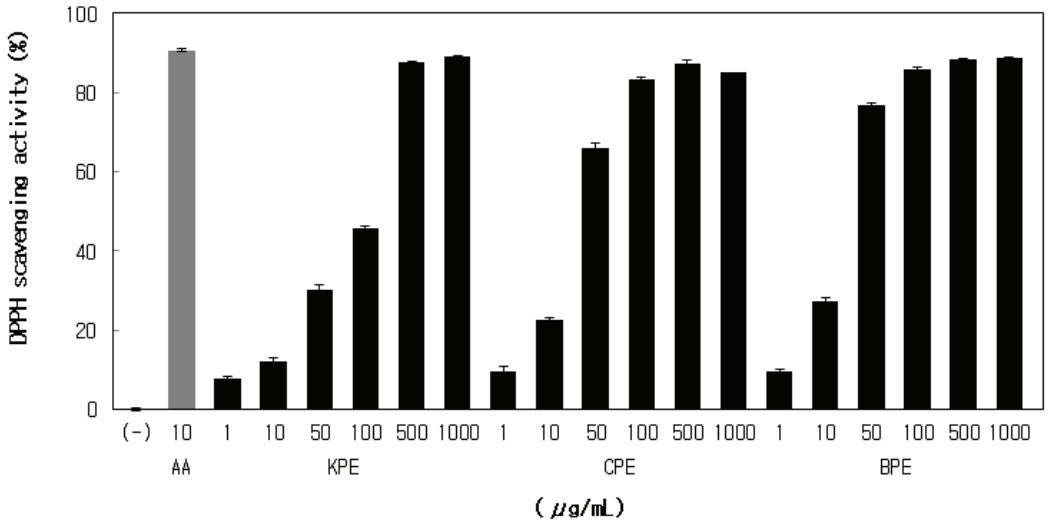

Figure 1. Free radical scavenging activity of propolis extract. Ascorbic acid (AA) was used as a positive control. The value were expressed as mean ± SD.

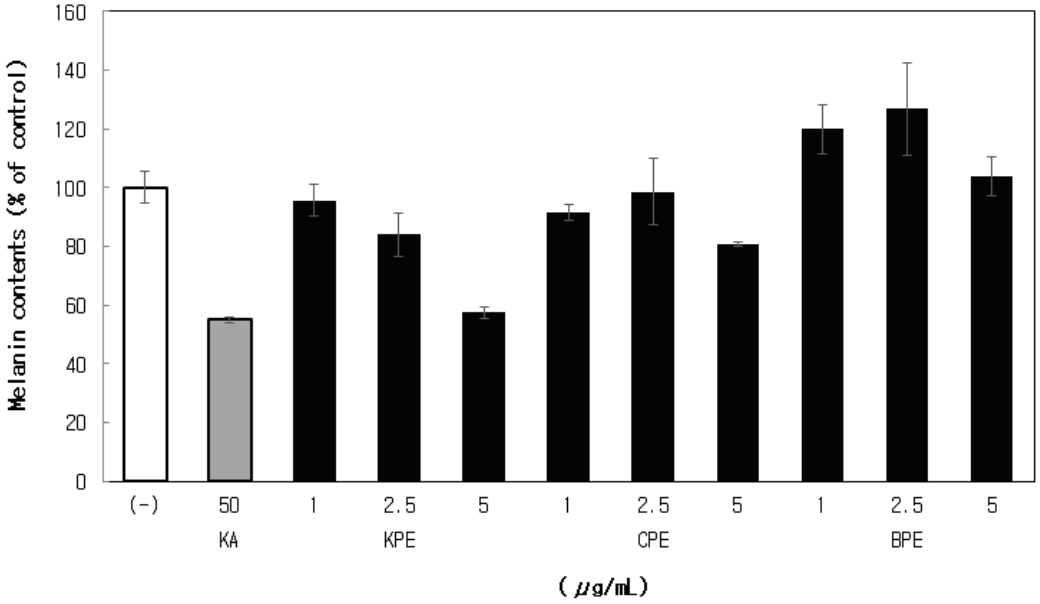

Figure 2. Anti-melanogenic effect of propolis extracts. Melanin content in human epidermal melanocytes incubated with the indicated concentration of propolis extracts (1, 2.5, 5 μg/mL). Kojic acid (KA) was used as a positive control. The value were expressed as mean ± SD.

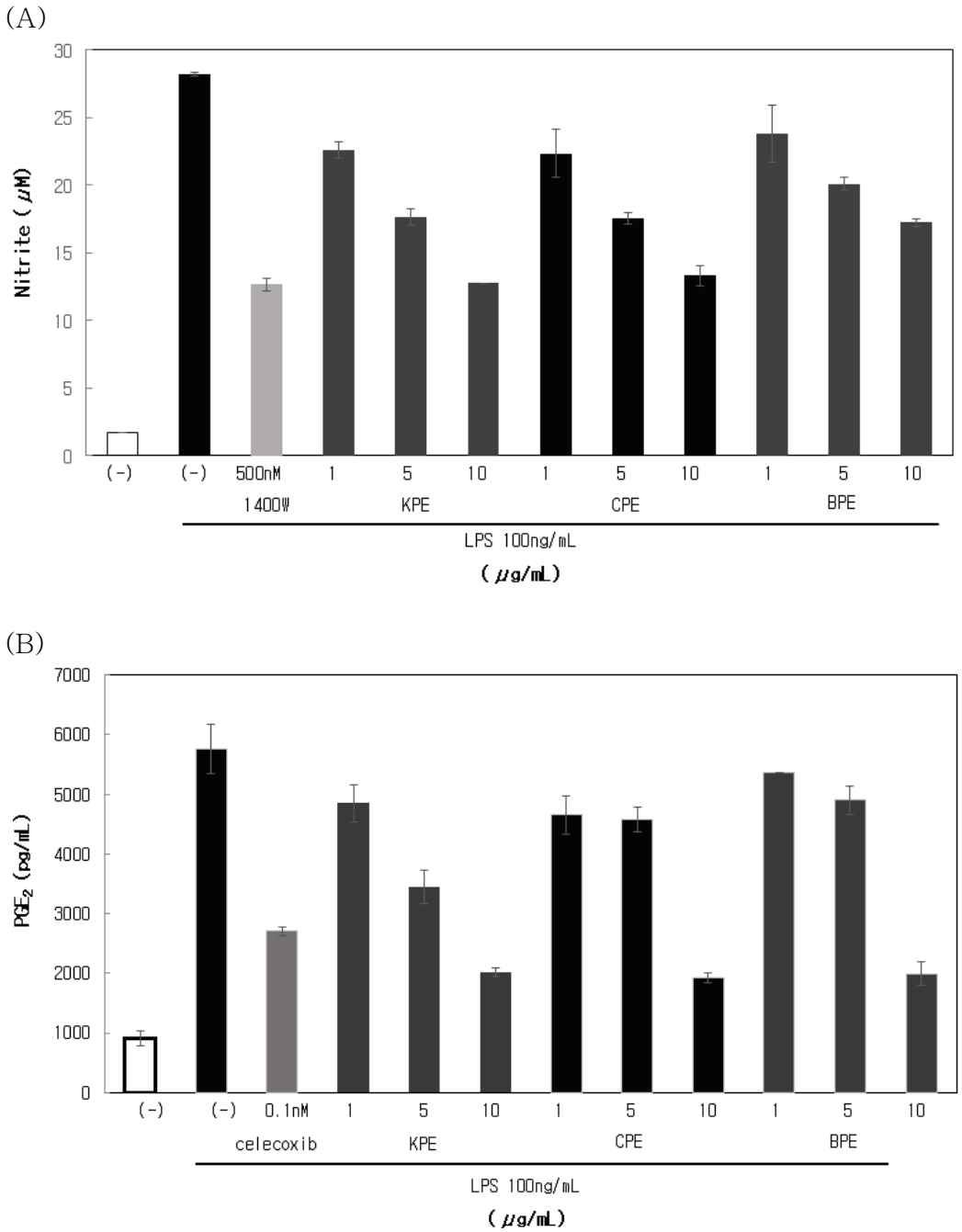

Figure 3. Anti-inflammatory effect of propolis extracts. Effects of propolis extracts on (A) NO production, and (B) PGE_2 expression, in LPS-induced RAW 264.7 cells. The cells were pretreated with the indicated concentrations of propolis extracts for 2 h, and then further incubated with LPS (100 ng/mL) for 24 h. Celecoxib was used as a positive control. The amount of NO produced was then determined by Griess assay. PGE_2 release was determined using ELISA. The values are expressed as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. LPS-treated.

atopic dermatitis by virulence factors, such as biofilm, superantigens, and α-toxin[16]. *S. epidermidis*, commensal microorganism of skin, is considered to be an important opportunistic pathogen[17].

The results show that three types of propolis have significant antimicrobial activity against *C. acnes, S. aureus,* and *S. epidermidis*. Among all three, KPE showed the strongest activity compared to CPE and BPE against *C. acnes* (Table 2). KPE exhibited MIC values of 62.5 μg/mL against *C. acnes*. CPE and BPE exhibited MIC values of (500 and 250) μg/mL, respectively. These propolis showed the same MIC value of 1,000 μg/mL against *S. aureus* and *S. epidermidis.*

3.3. Inhibitory Effect of Propolis on Melanin Synthesis

To confirm the inhibitory effect of propolis extract on melanin production, we performed a melanin content assay in human epidermal melanocytes. Figure 2 shows the results. The melanin content of KPE-treated cells decreased in a concentration-dependent manner. At a concentration of 5 μg/mL, the inhibitory effect was similar to the effect of kojic acid that is well known to inhibit and prevent the formation of tyrosine. CPE and BPE did not show any significant change in melanin contents, compared to the untreated group. Therefore, it has been confirmed that while KPE has a significant inhibitory effect on melanin synthesis, CPE and BPE do not.

3.4. Anti-inflammatory Activity of Propolis

NO is known to play a central role in the physiology and the pathophysiology of many human organ systems. The induction of macrophage NO production begins in response to an inflammatory stimulus, such as certain cytokines or microbial products. Abnormal release of NO can lead to the amplification of inflammation and tissue damage[18]. Prostaglandin (PGs) is an eicosanoid lipid mediator that plays a key role in the production of the inflammatory response. There are many contributors to the inflammatory process, and PGE_2 is one of the more significant ones. Biosynthesis is significantly upregulated in inflamed tissue, and they are important mediators of the immune system. PGE_2 is also involved in the process leading to the symptoms of inflammation, such as redness, swelling, and pain[19].

In order to compare the anti-inflammatory effects of propolis extracts, we investigated the inhibitory activity on LPS-induced NO and PGE_2 production. To assess the effects on LPS-induced NO production in RAW 264.7 cells, we measured the level of nitrite using the Griess reaction. Figure 3A shows that KPE, CPE, and BPE significantly inhibited the LPS-induced nitrite production in a concentration-dependent manner. The inhibitory effect of KPE and CPE was up to 55% at 10 μg/mL, and BPE was also up to 39% at 10 μg/mL. Similar to this result, PGE_2 production was also reduced by propolis extracts of (1, 5, and 10) μg/mL (Figure 3B). The PGE_2 inhibition effect of KP was up to 40% at 5 μg/mL, while BPE and CPE showed weak inhibitory effect of about (14 and 20)% at 5 μg/mL. Korean propolis was found to have the most potent effect on PGE_2 accumulation inhibition.

These results suggest that propolis extract inhibits LPS-induced inflammation by suppressing the production of PGE_2 and NO in macrophages. Among the three propolis, Korean propolis is the most effective.

4. Conclusion

In this study, we evaluated the efficacy of propolis extract from Korea, China, and Brazil through antioxidant, anti-bacterial, anti-melanogenic, and anti-inflammatory tests. As a result, KPE, CPE, and BPE showed antioxidant, anti-bacterial, and anti-inflammatory properties, which indicate that they can be applied as antioxidant, antibacterial, and anti-inflammatory ingredients for cosmetics. Furthermore, compared to CPE and BPE, KPE showed superior potential in antibacterial, anti-inflammatory, and whitening efficacies. The difference of biological activity of propolis is related to variance in chemical composition, biochemical analysis need to be performed to verify active compound of extract in further study.

Reference

1. L. Cornara, M. Biagi, J. Xiao, and B. Burlando, Therapeutic properties of bioactive compounds from dierent honeybee products, *Front Pharmacol*, **8**, 412 (2017).
2. S. De Castro, Propolis: Biological and pharmacological activities. Therapeutic uses of this bee-product, *Annu. Rev. Biomed. Sci*., **3**(4), 49 (2001).
3. A. Karapetsas, G. P. Voulgaridou, M. Konialis, I. Tsochantaridis, S. Kynigopoulos, M. Lambropoulou, M. I. Stavropoulou, K. Stathopoulou, N. Aligiannis, P. Bozidis, A. Goussia, K. Gardikis, M. I. Panayiotidis, and A. Pappa, Propolis extracts inhibit UV-induced photodamage in human experimental *in vitro* skin models, *Antioxidants (Basel)*, **8**(5), 125 (2019).
4. J. K. Choi, Y. H. Jang, S. Lee, S. R. Lee, Y. A. Choi, M. Jin, J. H. Choi, J. H. Park, P. H. Park, H. Choi, T. K. Kwon, D. Khang, and S. H. Kim, Chrysin attenuates atopic dermatitis by suppressing inflammation of keratinocytes, *Food Chem. Toxicol.*, **110**, 142 (2017).
5. S. Miyata, Y. Oda, C. Matsuo, H. Kumura, and K. Kobayashi, Stimulatory Effect of Brazilian propolis on hair growth through proliferation of keratinocytes in mice, *J. Agric. Food Chem.*, **62**(49), 11854 (2014).
6. J. Y. Lee, H. J. Choi, T. W. Chung, C. H. Kim, H. S. Jeong, and K. T. Ha, Caffeic acid phenethyl ester inhibits alpha-melanocyte stimulating hormone-induced melanin synthesis through suppressing transactivation activity of microphthalmia-associated transcription factor, *J. Nat. Prod.*, **76**(8), 1399 (2013).
7. S. I Anjum, A. Ullah, K. A. Khan, M. Attaullah, H. Khan, H. Ali, M. A. Bashir, M. Tahir, M. J. Ansari, H. A. Ghramh, N. Adgaba, and C. K. Dash, Composition and functional properties of propolis (bee glue): A review, Saudi *J Biol Sci*, **26**(7), 1695 (2019).
8. I. Przybyłek and T. M. Karpiński, Antibacterial properties of propolis, *Molecules*, **24**(11), 2047 (2019).
9. L. Zhu, Y. Lu, W. G. Yu, X. Zhao, and Y. H. Lu, Anti-photoageing and anti-melanogenesis activities of chrysin, *Pharm Biol.*, **54**(11), 2692 (2016).
10. K. M. Lim, S. Bae, J. E. Koo, E. S. Kim, O. N. Bae, and J. Y. Lee, Suppression of skin inflammation in keratinocytes and acute/chronic disease models by caffeic acid phenethyl ester, *Arch. Dermatol. Res.*, **307**(3), 219 (2015).
11. H. G. Ji, Y. R. Lee, M. S. Lee, K. H. Hwang, E. H. Kim, J. S. Park, and Y. S. Hong, Metabolic phenotyping of various tea (*Camellia sinensis* L.) cultivars and understanding of their intrinsic metabolism, *Food chem,* **233**, 321 (2017).
12. C. C. Chang, M. H. Yang, H. M. Wen, and J. C. Chern, Estimation of total flavonoid content in propolis by two complementary colorimetric methods, *J Food Drug Anal,* **10**(3), 178 (2002).
13. N. S. Bryan and M. B. Grisham, Methods to detect nitric oxide and its metabolites in biological samples, *Free Radic. Biol. Med.*, **43**(5), 645 (2007).
14. J. F. M. da Silva, M. C. de Souza, S. R. Matta, M. R. de Andrade, and F. V. N. Vidal, Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities, *Food Chem,* **99**(3), 431(2006).
15. B. R. Vowels, S. Yang, and J. J. Leyden, Introduction of pro-inflammatory cytokines by a soluble factor of *Propionibacerium acnes*: implications for chronic inflammatory acne, *Infect. Immun.*, **63**(8), 3158 (1995).
16. L. Blicharz, L. Rudnicka, and Z. Samochocki, *Staphylococcus aureus*: an underestimated factor in the pathogenesis of atopic dermatitis?, *Postepy Dermatol Alergol*, **36**(1), 11 (2019).
17. M. Otto, *Staphylococcus* epidermidis – the "accidental" pathogen, *Nat. Rev. Microbiol.*, **7**(8), 555 (2009).
18. N. McCartney-Francis, J. B. Allen, D. E. Mizel, J. E. Albina, Q. W. Xie, C. F. Nathan, and S. M. Wahl, Suppression of arthritis by an inhibitor of nitric oxide synthase, *J. Exp. Med.*, **178**(2), 749 (1993).
19. E. Ricciotti and G. A. FitzGerald, Prostaglandins and inflammation, *Arterioscler. Thromb. Vasc. Biol.*, **31**(5), 986 (2011).