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Characterization of antibacterial and anti-inflammatory activities of wheat germ extract using supercritical extraction and High Performance Liquid Chromatography(HPLC)

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ABSTRACT: This study examined the content of 2,6-dimethoxy-p-benzoquinone (DMBQ), an antibacterial active substance, using supercritical extraction and chloroform solvent extraction to extract DMBQ ingredients in wheat germ extracts. The final DMBQ contents in the extract based on the extraction method for WG-1 and WG-2 were 14.5 and 13.6 mg/g, respectively. By measuring the antioxidant activities of these extracts using the oxygen radical absorbance capacity method, all extracts showed similar peroxyl radical scavenging activities. it is estimated that the antioxidant activity was influenced by water-soluble phytochemicals and the DMBQ content in wheat germ extract. Furthermore, the DMBQ contents were similar when antibacterial activity against Propionibacterium acnes (P. acnes) of wheat germ extract was analyzed according to the extraction method; however, the supercritical extract showed higher activity than that of the chloroform solvent extract. Moreover, the anti-inflammatory effect increased in a concentration-dependent manner when the anti-inflammatory effect was examined, thus confirming that the wheat germ supercritical extract had a higher anti-inflammatory effect.

KEYWORDS: Wheat germ extract;Propionibacterium acnes; Anti-inflammatory agents; Antibacterial activity; 2,6-dimethoxy-p-benzoquinone;supercritical extraction

I. INTRODUCTION

[1].The number of skin diseases caused by environmental pollution, stress, and electromagnetic waves has recently increased domestically and internationally. Due to the effect of the COVID-19 pandemic, most of the population wears masks for longer, causing inflammatory acne, a skin disease. Moreover, skin problems, such as acne, are developing due to the lack of breathability regardless of age, causing the emergence of a serious problem.

[2,3].Well-known bacterial species, including Staphylococcus epidermidis, Staphylococcus aureus, and Streptococcus pyogenes, are normally present on human skin and cause inflammation. Moreover, Propionibacterium acnes is known as a bacterium that causes acne. [4].Hormonal changes, bacterial infection, and hereditary factors have been reported to cause acne; however, the exact cause and treatment of acne are not clearly known to date.

[5].Antibacterial products currently used to treat skin diseases, such as acne, are mostly synthetic substances and are highly likely to cause adverse reactions, such as skin allergy and inflammation. Therefore, the development of natural substances with antibacterial effects against bacteria that cause these inflammatory skin diseases is being actively researched .

[6,7].Wheat (Triticum vulgare) is a crucial food ingredient and among the top three crops in the world together with rice and barley. In 2021, the annual wheat consumption per capita of South Korea was 34 kg, which is the second largest after rice. The global annual wheat production is 689,940,000 t, making it one of the cereal crops with the largest cultivation area and production. In South Korea, 26,000 t of wheat were annually

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produced in an area of 10,000 ha as of 2015. [8,9].Wheat has been reported to have various physiologically active effects, including arteriosclerosisprevention, anti-inflammatory, andanticancer and allergyinhibitory effects.

[10,11].Wheat germ is a by-product of the milling process for producing flour. It is used for feed or is discarded because the oil ingredient of wheat germ causes rancidity and the bitter taste characteristic of wheat germ, affecting the flavor. However, wheat germ accounts for 2–3% of wheat grain and is known to have high value as a food additive because it contains abundant nutrients, such as alpha-tocopherol (vitamin E), vitamin B complex, protein, dietary fiber, and minerals. [12, 13].Furthermore, wheat germ contains methoxy-substituted benzoquinones suchas 2,6-dimethoxy-p-benzoquinone (DMBQ) and several anticancer effects have been reported.[14,15,16].Moreover, studies on wheat germ extracts containing DMBQ have reported immunity-boosting effects, anti-inflammatory effects, and antibacterial and antioxidant activities. [17,18,19].Wheat germ extracts have whitening, anti-wrinkle, anti-aging, and skin-soothing effects as a cosmetic raw material through various studies. However, there has been no study on the antibacterial activity of P. acnes, aknown causative agent of acne.

[20].The general extraction method of natural products consists of extracting toxic solvents, such as n-hexane, ether, chloroform, and ethyl acetate.Recently, however, an eco-friendly supercritical fluid extraction method that does not use a solvent has been actively studied.

[21,22].While the solvent extraction method has the advantages of low equipment installation cost and large-capacity extraction, it also has several disadvantages, including the destruction of active ingredients during high-temperature extraction and toxicity of organic solvents remaining in the final extract. [23,24,25].In contrast, the supercritical extraction method, which is known as a green chemistry method, has a disadvantage (i.e., the actual industrialization is difficult due to high equipment installation cost and small quantity that can be extracted simultaneously). However, it has a considerably small possibility of destruction and degeneration of active ingredients due to heat because the critical point (31.1 ° C and 73.8 atm) is low, and it is also non-toxic. Particularly, it has excellent selectivity, such as fractionation and separation, because the pressure and temperature of a supercritical fluid can be manipulated. As a result, high-purity DMBQ in wheat germ can be extracted while water-soluble phytochemicals, such as carotenoids, flavonoids, isoflavones, glucosinolates, sulforaphone, anthoxanthin, and anthocyanins, can also be extracted without loss.

Therefore, this study investigates the difference in the DMBQ content of wheat germ extract by extracting wheat germs with various functionalities using supercritical extraction and solvent extraction methods. In addition, the antibacterial, anti-inflammatory, and antioxidant activities of wheat germ solvent extract and supercritical extract are compared and analyzed.

II. MATERIALS AND METHODS

2.1 Materials

Propionibacterium acne KCTC 3314, the strain used for the antibacterial activity test, and the human keratinocyte HaCaT cell used for cytotoxicity and inflammation alleviation experiments were provided by the Korea Institute of Oriental Medicine. Wheat germs used in the experiment for extraction were provided by Daehan Flour Co., Ltd.

2.2 Fabrication of wheat germ extracts

2.2.1Fabrication of chloroform extract

The prepared wheat germs were crushed, and 2.5 L of secondary distilled water was added to 100 g of crushed wheat germs and stirred for 1 h. Subsequently, 1 L of chloroform solution was added to this solution and the mixture was re-stirred for 30 min to recover the solvent layer through centrifugation. The chloroform layer was removed after centrifugation, and the chloroform was removed from the remaining filtrate layer again in the same manner three times. Chloroform layers were collected, washed with distilled water, and concentrated at 40 °C using a vacuum evaporator (EYELA, Tokyo, Japan). Finally, the concentrated material was redissolved in chloroform and filtered using a 0.45-μm PTFE filter (Hyundai Micro Seoul, Korea).

2.2.2Fabrication of wheat germsupercritic-al extract

After drying the wheat germ powder, the temperature of the supercritical fluid extractor (supercritical fluid extraction (SFE) System, Thar Technologies, Inc., USA) was set to 50–80 ℃. Then, the temperature of the separator was maintained at 30–60 ℃, and the temperature of the cooler was maintained from − 1 to − 5 ℃. Subsequently, 100 g of wheat germ powder as the raw material was injected into the supercritical fluid extractor, and the pressure of the extractor was adjusted to 60–300 bar. After adjusting the CO_2 flow rate to 10–80%, ethyl alcohol as an auxiliary solvent was injected at a flow rate of 0.5–2.0 mL/min. Raw materials were

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extracted at 30 min intervals for 300 min when the pressure reached 200–300 bar. After extraction, the sample was mixed with ethyl alcohol and filtered using a 0.45-μm PTFE filter.

2.3HPLC Analysis and yield of DMBQ in wheat germ extracts

HPLC analysis was performed using the RP-Amide C16 (250 mm × 4.6 mm) Column (Supelco, Bellefonte, PA, USA) to analyze the content of DMBQ in the chloroform solvent wheat germ extract and supercritical wheat germ extract. The analytical method was established using the standard DMBQ (Sigma-Aldrich Co., St. Louis, Mo., USA) to quantify the DMBQ content in the wheat germ extract by HPLC. It was measured at 275 nm, which is the maximum absorption wavelength of DMBQ in the case of a UV detector. Furthermore, the DMBQ content, which is a standard material for extract standardization for each extraction process, was measured using HPLC under the conditions listed in Table 1. The final yield of the final extract obtained from 100 g wheat germ was calculated as

$$Extract\ yield\ (\%) = \frac{Weight\ after\ extraction}{Weight\ before\ extraction} \times 100$$

Table 1.Conditions for DMBQ content analysis of wheat germ extract using HPLC

Mobile phase	A: 0.025 M Potassium dihydrogen phosphate, B: ACN
Column	RP-Amide C16 (SUPELCO 25 cm × 4 mm, 5 μm)
Detector	Agilent DAD 1260, 275 nm
Flow	0.7 mL/min
Column temperature	40 ℃

2.4 Measurement of antioxidant activity of wheat germ extract using ORAC method

The peroxyl radical scavenging activity was measured using a modified version of the method proposed by Mariana et al. After adding 50 μL of hydroxyflavanone solution (Sigma-Aldrich Co. St. Louis, MO, USA) in 80 nM fluorescein solution melted in 100 μL of 75 mM phosphate buffer, 50 μL of 20 mM 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) solution (Sigma-Aldrich Co. St. Louis, MO, USA) was added. Then, fluorescence was measured at 37 ℃ using the GENios fluorescence detector (TECAN Trading AG, Switzerland). The fluorescence was measured at an interval of 2 min. The result was calculated as the difference in the area between the fluorescence of the sample and the blank. Trolox was used as the standard, and all results were converted to Trolox values.

2.5 In vitro efficacy evaluation of wheat germ extract

2.5.1 Evaluation of antibacterial activity for acne bacteria

P. acnes KCTC 3314 was used to confirm the growth inhibitory effect on Propionibacterium acnes (P. acnes), an acne-causing bacterium. Reinforced clostridial medium (BD Difco, USA) was pre-cultured for 48 h in an RC liquid medium to test under anaerobic conditions, and 1% of the sample solution diluted with DMSO was added to the RC medium diluted to a concentration of 1.5 × 10^8 CFU/mL. The inhibition rate (%) of P. acnes was confirmed by culturing at 37° C under anaerobic conditions for 48 h. The bacterial inhibition rate was determined by measuring the absorbance at 600 nm to determine the number of proliferated P. acnes bacteria. The inhibition rate of the growth concentration inhibited by the sample treatment compared to the growth concentration of the control group was calculated as

$$Inhibition\ rate\ (\%) = (1 - \frac{O.D.of\ Sample}{O.D.of\ Control} \times 100$$

2.5.2 Evaluation of anti-inflammation effect in skin keratinocytes

To verify the anti-inflammatory effect on human keratinocyte HaCaT cells,they were cultured in a DMEM medium, to which 10% Heat inactivated fetal bovine serum100 units/mL of Penicillin, and 100 μg/mL of Streptomycin were added at 37 °C in 5% CO_2 environment.

Cell counting kit-8 (CCK-8) assay was performed to verify cytotoxicity. HaCaT cells were treated with each concentration in a 96-well plate and cultured for 24 h. After adding CCK-8 at a concentration of 10% and reacting at 37 °C for 2 h, absorbance was measured at 450 nm using a microplate spectrophotometer. Then, the relative cell viability (%) was calculated through comparison with the control group using the following equation:

$$Cell\ survival\ rate\ (\%) = \left(\frac{O.D.of\ the\ sample - added\ group}{O.D.of\ the\ control}\right) \times 100$$

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Cultured human keratinocytes were inoculated into a 6-well plate to analyze inflammatory chemokine. Then, they were treated with 10 ng/mL of TNF-α and 10 ng/mL of IFN-γ and simultaneously Samples were co-cultured with 25, 50, and 100 μg/mL concentrations. After 24 h, the secretion of chemokine separated to the medium was measured with an ELISA kit. Moreover, 100% activity was calculated by the difference in cytokine secretion between the group treated with TNF-α and IFN-γ and the untreated group.

Furthermore, the anti-inflammatory activity in the skin keratinocytes was evaluated for the extracted samples based on the extraction method. The anti-inflammatory activity of the samples was evaluated for the chloroform extract, wheat germ supercritical extract, and standard DMBQ.

III. RESULTS AND DISCUSSION

The analysis results for wheat germ extracts in this study are presented in this section. The result values were represented as WG-1 and WG-2 for the wheat germ chloroform extract and the wheat germ supercritical extract, respectively.

3.1 HPLC analysis and yield of DMBQ for wheat germ extracts

As a result of HPLC analysis, the peak of DMBQ was detected at 15 min, as shown in Fig. 1. Furthermore, as a result of the verification of the UV spectrum of the DMBQ peak at 15 min, the maximum absorbance was measured in the range of 275–300 nm.

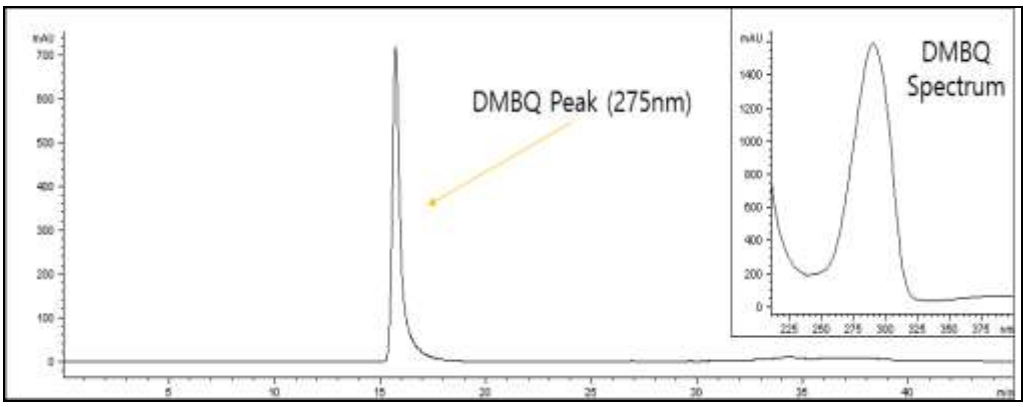

Fig. 1.DMBQ analysis conditions and HPLC profile (UV wavelength of 275 nm).

Furthermore, to investigate the reliability and detection limit of the established analytical method, BQ, is the standard material, was prepared at concentrations of 0.01, 0.025, 0.05, 0.1, and 0.5 mg/mL, and the detection limit and analysis reliability for each concentration were examined. As a result, the DMBQ concentration up to 0.01 mg/mL could be detected, as shown in Fig. 2, and a calibration curve with an R^2 value of 1.00 was obtained.

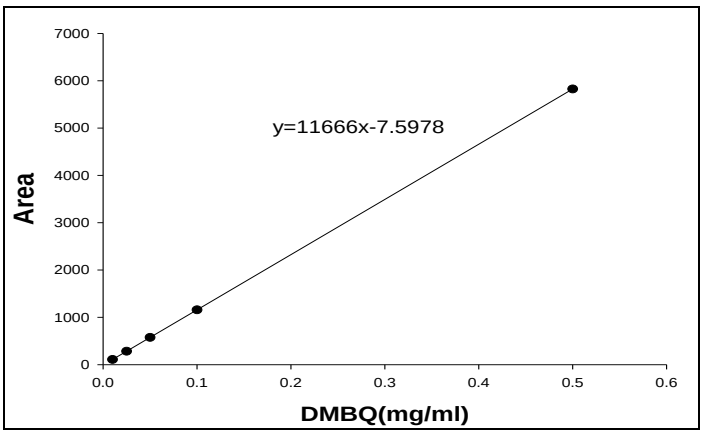

Fig. 2. Calibration curve of the standard DMBQ.

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The results of HPLC are shown in Fig. 3 to analyze the DMBQ content of two types of extracted samples, WG-1 and WG-2, using the obtained HPLC analysis method.

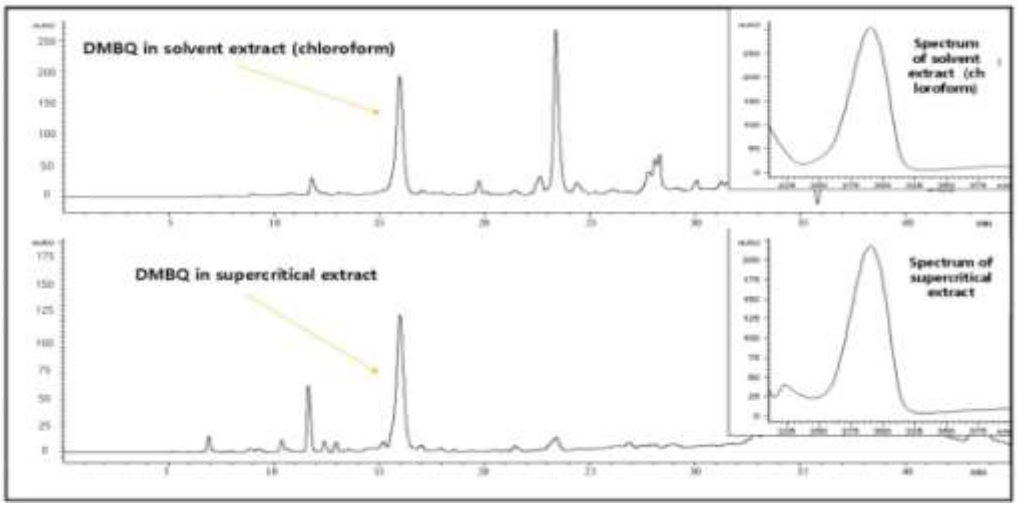

Fig 3.DMBQ content analysis graph of thewheat germ extract by HPLC.

The results of WG-1 (15.9 min) and WG-2 (15.8 min) were analyzed for the DMBQ content at each retention time (RT) value, as shown in Table 2. As a result, the final DMBQ contents in the extract based on the extraction method for WG-1 and WG-2 were 14.5 and 13.6 mg/g, respectively. Moreover, final extraction yield of the final extract obtained from 100 g wheat germ based on the extraction method was 5.50% for WG-1 and 5.40% for WG-2, indicating similar levels.

Table 2. Results of the comparison of the DMBQ content and extraction yield based on the extraction method

	Wheat germ powder (g)	Final extract weight (g)	Final extraction yield of DMBQ (%)	DMBQ content (mg/g-final extract)
WG-1	100	5.50	5.50	14.5
WG-2	100	5.40	5.40	13.6

3.2 Analysis of antioxidant activity of wheat germ extract by oxygen radical absorbance capacity (ORAC) assay

Fig. 4 shows the $ORAC_{ROO\cdot}$ assay result of the wheat germ extract. Also, the result of the analysis of the wheat germ extract by diluting it 40,000 times from the existing concentration is shown in Table 3. The peroxyl radical scavenging activity was 47,612 ± 7,680 μM TE/g-fresh weight (F.W) for WG-1 and 47,308 ± 6,438 μM TE/g-F.W for WG-2, indicating similar values.

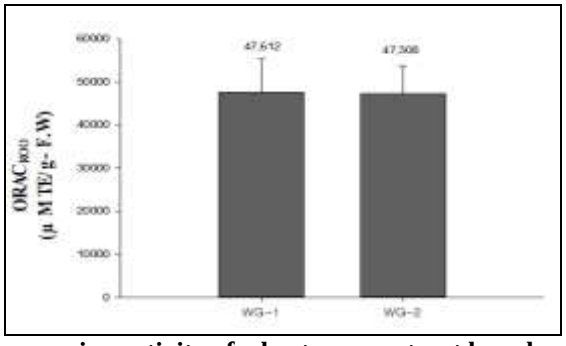

Fig. 4. Peroxyl radical scavenging activity of wheat germ extract based on the extraction method.

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Table 3. ORAC value result of wheat germ extract

ORAC value	WG-1	WG-2
μM TE/g-F.W	47,612 ±7,680	47,308 ± 6,438

3.3 Evaluation of antibacterial activity for Propionibacterium acnes (P. acnes)

The wheat germ chloroform extract, wheat germ supercritical extract, and index ingredient DMBQ were diluted by 5000, 2500, 1250, and 625 μg/mL concentrations to measure inhibition rates. As a result, the antibacterial activity was higher in WG-2 than that in WG-1, as shown in Table 4. This suggests that the antibacterial activity of wheat germ extract influenced the DMBQ content and the antibacterial activity of water-soluble phytochemicals in the supercritical extract.

As shown in Table 5, the standard DMBQ shows an inhibition rate of approximately 86% even at a concentration of 62.5 μg/mL.

Table 4. Inhibition activity (%) for wheat germ extracts

Conc. (μg/mL)	Inhibition activity (%) for wheat	
	WG-1	WG-2
5,000	32.7 ± 1.9	101 ± 2.7
2,500	9.90 ± 2.3	63.1 ± 2.1
1,250	6.30 ± 3.4	11.9 ± 3.7
625	1.50 ± 2.0	9.80 ± 1.4

Table 5. Inhibition activity (%) of DMBQ

Conc. (μg/mL)	Inhibition activity (%) of 2,6-Dimethyl-4-benzoquinone
500	108 ± 3.5
250	95.6 ± 6.3
125	96.0 ± 3.4
62.5	86.1 ± 1.8

3.4 Evaluation of anti-inflammatory efficacy in skin keratinocytes

Cell viability and chemokine inhibitory activity were evaluated at 25, 50, and 100 μg/mL using HaCaT cells regarding WG-1 and WG-2 samples used to evaluate the anti-inflammatory efficacy of wheat germ extract and DMBQ as a standard material. The survival rate was measured treating HaCaT cells by extract concentration to calculate the concentration that does not show cytotoxicity. As a result, cytotoxicity was not confirmed even at the highest concentration of 100 μg/mL, as shown in Fig. 6. Therefore, the anti-inflammatory efficacy test was carried out based on these concentrations, and the results are shown in Fig. 7. Based on the measurement, the chemokine inhibitory activity increasedin a concentration-dependent manner, and the anti-inflammatory effect was higher in WG-2 than that in WG-1. It is estimated that both the DMBQ content and water-soluble phytochemicals in the supercritical extract influenced the anti-inflammatory effect as in the results of the antibacterial activity of wheat germ extract.

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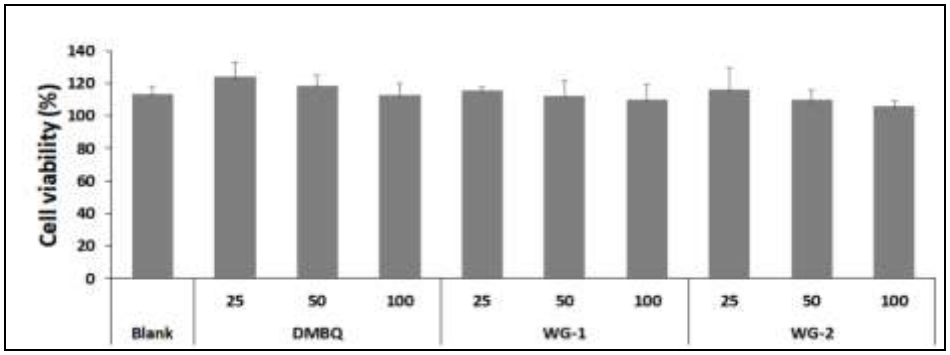

Fig. 6. Cell survival rate of the HaCaT cell based on the concentration of wheat germ extract.

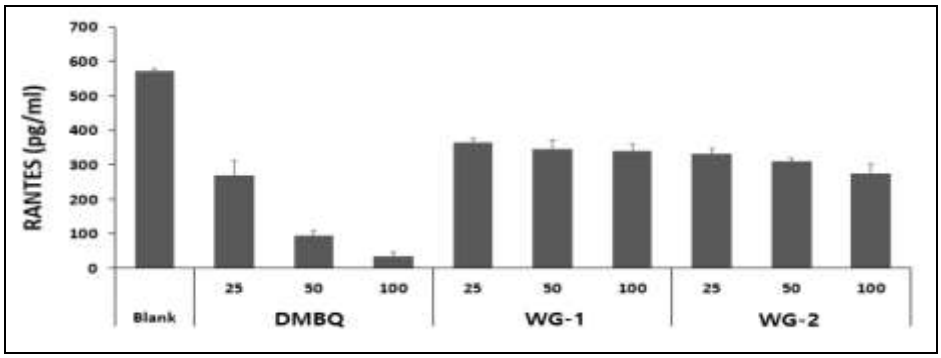

Fig. 7. Chemokine inhibitory activity of HaCaT cells based on the concentration of wheat germ extract.

IV. CONCLUSION

We verified the DMBQ content through supercritical extraction and chloroform solvent extraction to extract the DMBQ ingredient inside the wheat germ extract. As a result, the final DMBQ contents in the extract based on the extraction method for the wheat germ chloroform solvent extract and the supercritical extract were14.5 and 13.6 mg/g, respectively.which were at similar levels. As a result of $ORAC_{ROO\text{-}}$ assay for the verification of the antioxidant activity, all extracts showed similar peroxyl radical scavenging activities. The antibacterial activity of the wheat germ extract was higher in WG-2 than that in WG-1. This suggests that regarding the antibacterial activity of the wheat germ extract, both the DMBQ content and water-soluble phytochemicals in the supercritical extract influenced the antibacterial activity. As a result of the verification of the anti-inflammatory effect of wheat germ extract, cytotoxicity was not found at the highest concentration of 100 μg/mL. Based on the result of measuring the chemokine inhibitory activity, it was confirmed that the inhibitory activity was increased in a concentration-dependent manner.Moreover, the anti-inflammatory effect was higher in WG-2 than that in WG-1. This suggests that water-soluble phytochemicals in the supercritical extract and the DMBQ content influenced its anti-inflammatory effect.

Based on the results of this study, the DMBQ content of a level similar to the conventional solvent extraction method, in which toxic solvent ingredients may remain, was extracted by the supercritical extraction method. Furthermore, the supercritical extraction method could stably extract phytochemicals without loss and DMBQ. Therefore, the supercritical extraction method is an efficient extraction method that can further increase antioxidant, antibacterial, and anti-inflammatory characteristics that can create high added value for various uses from wheat germ, which is a by-product of wheat processing. Therefore, its application scope as a functional cosmetic natural material is expected to further expand.

V. ACKNOWLEDGEMENTS

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualization, W.K.; methodology, W.K.; validation, Y.K, W.K. and J.X.; formal analysis, J.X.; investigation, J.X.; resources, W.K.; data curation, J.X.; origimal draft, J.X.; preparation, J.X. and Y. K.; editing, J.X.; visualization, Y.K.; supervision, W.K.; project administration, W.K. All authors have read and agreed to the published version of the manuscript.

REFERENCES

[1]. Kwon, H-R; Kim, J-H; Shin, S-Y.,(2021). "A Study on the Direction of Construction of Problematic Skin Data for Customized Beauty Self-Care," Journal of Business Convergence, 6(4), 95-100.

[2]. Park, J-H; Kwon, H-J., (2017). "Antimicrobial activity against Propionibacterium acnes bacteria of Smilacis glabrae Rhizoma extracts," Journal of the Korea Convergence Society, 8(12), 425-430.

[3]. Ramli, R; Malik, A-S; Hani, A-F-M; Jamil, A., (2011). "Acne analysis, grading and computational assessment methods: an overview," Skin Research and Technology, 18(1), 1-14.

[4]. Hacivelioglu, S; Gungor, A-N-C; Gencer, M; Uysal, A; Hizli,D; Koc, E; Cosar, E., (2013). "Acne severity and the Global Acne Grading System in polycystic ovary syndrome," International Journal of Gynecology and Obstetrics, 123(1), 33-36.

[5]. Kim, Y-D; Kim,Y-M; Mo, E-M., (2017). "Antibacterial, Antioxidant, and Antiaging Effects of the Ethanol Extract of Dolnamul (Sedum sarmentosum) and the Production of the Oil in Water Cream," J. Soc. Cosmet. Sci. Korea , 43(3), 211-221.

[6]. Baek, J-H; Ahn, S-M; Choi, K-M; Jung, M-K. Shin, M-K; Koh, J-S., (2016). "Analysis of comedone, sebum and porphyrin on the face and body for comedogenicity assay," Skin Research and Technology, 22(2), 164-169.

[7]. Kang, C-S; Cheong, Y-K; Kim, B-K., (2016)."Current Situation and Prospect of Korean Wheat Industry," Food Industry and Nutritio, 21(2), 20-24.

[8]. Choe, M; Kim, H-S., (2022). "Effects of Korean Whean on LDL Oxidation and Atherosclerosis in Cholesterol-Fed Rabbits," J. Korean Soc. Food Sci. Nutr, 31(1), 104-108.

[9]. Lee, J-K; Lee, J-H; Choi, Y-H; Choi, Y-S., (2019). "G. H. Ryu, Changes in 2,6-dimethoxy-1,4-benzoquinone and Water Extractable Arabinoxylan Content of Wheat Germ Extract by Enzyme Treatmen," Food Eng. Prog, 23(1), 22-29.

[10]. Choi, B-S; Kang, K-O., (2009). "Studies on the Analysis of Physiological and Antimicrobial Activity of Wheat Germ," Journal of the East Asian Society of Dietary Life, 19(4), 585-592.

[11]. Lee, J-K; Lee, J-H; Choi, Y-H; Choi, Y-S., (2019). "Changes in 2,6-dimethoxy-1,4-benzoquinone and Water Extractable Arabinoxylan Content of Wheat Germ Extract by Enzyme Treatment," Food Eng. Prog, 23(1), 22-29.

[12]. Seog, H-M; Seo, M-S; Kim, S-R; Park, Y-K; Lee, Y-T., (2002)."Characteristics of barley polyphenol extract(BPE) separatedfrom pearling by-products," Korean Journal of Food Science and Technology, 34(5), 775-779.

[13]. Muhammad, I; Takamatsu, S; Walker, L-A; Mossa, J-S; Fong, H-H; El-Feraly, F-S.,(2003). "Cytotoxic and antioxidant activities of alkylated benzoquinones from Maesa lanceolata," An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 17(8), 887-891.

[14]. Mahmoud, A-A; Mohdaly, A-A; Elneairy, N-A., (2015). "Wheat Germ: An Overview on Nutritional Value, Antioxidant Potential and Antibacterial Characteristics," Food and Nutrition Sciences, 6(2), 265-277.

[15]. Choi, E-M; Lim,T-S; Lee, H-R; Hwang,J-K., (2002)."Immune Cell Stimulating Activity of Wheat Arabinoxylan," KOREAN J. FOOD SCI. TECHNOL, 34(3), 510-517.

[16]. Kang, B-K; Kim, M-J; Jeong, D-H; Kim, K-B-W-R; Bae, N-Y; Park, J-H; Park, S-H; Ahn, D-H., (2016)."Anti-inflammatory effect of wheat germ oil on lipopolysacchaide-stimulated RAW 264.7 cells and mouse ear edema," Microbiology and Biotechnology Letters, 44(3),236–245.

[17]. Yang, T-J; Oh, H-N; Applicant CJ Cheil Jedang Co., Ltd., (2018). "Skin external agent for skin whitening comprising an extract of fermented wheat germ," Korean Intellectual Property Office, Registration

No. 1019296580000.
[18]. Yang, T-J; Oh, H-N; Applicant CJ Cheil Jedang Co., Ltd., (2017). "Skin external agent for improving skin wrinkle comprising an extract of fermented wheat germ," Korean Intellectual Property Office, Registration No. 1019296570000.
[19]. Yang, T-J; Oh, H-N; Applicant CJ Cheil Jedang Co., Ltd., (2019). "External composition for soothing effect on the skin comprising an extract of fermented wheat germ," Korean Intellectual Property Office, Registration No. 1019551110000.
[20]. Ju, Y-W; Lee, M-Y; Woo, M-J; Byun, S-Y.,(2005). "The Current status of Supercritical Fluid Extraction Technology and Industrial Applicationns," KSBB Journal, 20(5), 329-337.
[21]. Kim, J-S; Lee, J-A., (2021). "A Study on Anti-oxidant and Anti-wrinkle Effect of Supercritical Fluid Extraction of Black Carrot as a FunctionalCosmetic Material," Journal of Convergence for Information Technology, 11(12), 236-243.
[22]. Lim, S-B; Jwa, M-K; Ko, Y-H; Yoo, I-J., (1997). "Supercritical Carbon Dioxide Extraction of Dried Egg Yolk," Journal of the Korean Society of Food Science and Nutrition, 26(5), 860-865.
[23]. Shin, S-L; Lee, C-H., (2011). "Antioxidant Activities of Ostrich Fern by Different Extraction Methods and Solvents," Journal of Life Science, 21(1), 56-61.
[24]. Chae, J; Kim, J; Jo, H; Lee, J-Y., (2021). "Comparison of Antioxidant, Wrinkle Improvement, and Whitening Efficacies of Extracts from Pinuskoraiensis Cone Scale Using Extraction Methods," Journal of Korean Society of Forest Science, 110(3) 431-439.
[25]. Lee, M-S; Lee, K-B; Han, M-K; Park, S-S., (2001). "Effect of Extraction Conditions on Yield and Quality of Extracts in Astragalas manbranaceus Bunge, Angelica gigas Nakai," The Korean Journal of Food And Nutrition, 14(6), 543-547.