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Antioxidant and Antibacterial Effects of Mixed Extracts of *Phyllanthus emblica, Geranium* (*Pelargonium graveolens*) and *Commiphora myrrha*: Possibility of Natural Materials for Acne Treatment

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Various skin diseases are occurring due to external factors such as urbanization and increase in environmental pollution and wearing masks due to COVID-19. Accordingly, various functional cosmetics are being released, but as some side effects are reported, research on functional cosmetics materials using natural plants is necessary. Therefore, in this study, the antioxidant, antibacterial and anti-inflammatory effects of *Phyllanthus emblica*, *Geranium* (*Pelargonium graveolens*), and *Commiphora myrrha* mixed extracts (PGC) that pharmacological efficacy has been verified were analyzed and their potential as functional cosmetics raw materials was examined. Four extracts (PGC-1~4) were prepared according to the extraction method. ABTS and DPPH radical scavenging activity experiments were conducted for the antioxidant efficacy of the extracts. In addition, paper disc experiments and LPS inflammation-inducing cytokine experiments were conducted to examine the antibacterial and anti-inflammatory effects. In addition, a cell viability test was performed to confirm cytotoxicity. As results of the study, all extracts showed antioxidant, antibacterial, and anti-inflammatory effects without cytotoxicity, and in particular, PGC-4, a fermentation and ultrasonic extract, showed the best efficacy. This means that the extraction yield of useful components varies depending on the extraction method.

Keywords: ***Phyllanthus emblica, Geranium* (*Pelargonium graveolens*), *Commiphora myrrha,* antioxidant, antibacterial effect**

Introduction

The skin is an organ that is always in contact with the external environment and plays an important role in protecting the human body from the outside. However, various skin diseases are occurring due to external factors such as urbanization and increases in environmental pollution and indirect effects such as psychological and emotional stress. Skin diseases are not easily cured because many people tend not to regard skin diseases as diseases despite that skin diseases require treatment based on an accurate judgment by an expert since there are differences in the site of onset, shape, and color from person to person [1]. Antioxidants, which are mainly present in natural plants as substances for improving skin diseases, prevent and delay skin diseases by reducing the reactivity of active oxygen in the body [2, 3].

As microorganisms that cause skin diseases, *Staphylococcus aureus* and *Propionibacterium acnes* have been reported [4]. *S. aureus* is a skin flora, and there are reported cases indicating that atopy is induced by a colony of *S. aureus* in more than 95% of the skins of patients with atopic dermatitis [5]. *P. acnes* is known as the bacteria that mainly cause acne, and is distributed

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in hair follicles and sebaceous glands. The lipolytic enzyme of *P. acnes* decomposes sebum and produces free fatty acids in the hair follicles thereby causing inflammation in the cells around the pores [6]. In particular, acne is a chronic disease that is experienced by 85% of adolescents and appears throughout the skin. Androgen secretion is the main cause of adolescent acne as it is known that acne is caused by the proliferation of skin flora in cases where skin pores have been clogged because hormones and external influences prevented sebum from being discharged out of the follicular ducts [7].

As such, abnormal proliferation of skin flora not only can cause skin diseases but also can cause damage to cells or barrier function around pores [8]. Currently, reported ingredients of acne treatment products include triclosan [9], benzoyl peroxide [10], azelaic acid [11], retinoid [12], tetracycline [13], erythromycin [14], roxithromycin [15], and clindamycin [16], but tolerance problems due to excessive use of acne treatments are being raised. Therefore, the development of materials having improvement effects on the occurrence of excessive inflammation of the skin without causing side effects is acutely necessary.

Meanwhile, the fruit of *Phyllanthus emblica* is a fruit that prevents various diseases and aging, has effects for beauty and health, is known as a rejuvenating fruit. *P. emblica* is a small deciduous tree, which grows naturally and is cultivated throughout India. In addition, *P. emblica* contains various antioxidants such as vitamin C, phenolic compounds, tannins, phyllembelic acid, phyllembelin, rutin, curcuminoids, and emblicol [16], and antioxidant activity [17] has been reported in relation to the foregoing.

Geranium (*Pelargonium graveolens*) is a perennial plant belonging to the family *Geranaceae* and is native to China and Egypt. Among the constituents of *Geranium*, geraniol, linalool, and citronellol are known to have very strong oxidative actions because they have strong binding force since they have one or more hydroxyl groups (-OH) in their molecular structures as with phenolic compounds [18]. Antioxidant and anti-inflammatory actions [19], and antibacterial effects on *P. acnes, S. aureus*, etc. were reported in the leaf extract of *P. graveolens* [20]. *Commiphora myrrha*, which is a traditional Chinese herbal medicine, has been reported to have various activities, including anti-inflammatory activity [21], cytotoxicity [22], and antibacterial effects [23].

Whereas many applied studies on phytotherapy, which uses plants as drugs without separating certain components, have been conducted in the past, recently, studies using scientific extraction methods to extract certain useful components from plants have been actively in progress. Therefore, verifying the efficacy appearing when the mixed extracts of *P. emblica, P. graveolens*, and *C. myrrha*, of which the pharmacological effects such as antioxidant and antibacterial effects required for skin disease remedial agents have been individually verified act complexly seems to be very meaningful for the development of materials of eco-friendly skin disease remedial agents.

Therefore, in this study, the antioxidant and antibacterial effects of the mixed extracts (PGC) of *P. emblica, Geranium (P. graveolens)*, and *C. myrrha*, whose pharmacological effects have been verified, will be analyzed and their stability as raw materials of eco-friendly skin disease remedial agents will be reviewed.

Materials and Methods

Experimental sample preparation

P. emblica fruits, *P. graveolens* leaves and *C. myrrha* fruits were washed and dried at 60℃ for 48 h. After grinding to a size of 40 mesh or less, they were mixed at a constant weight (100 g: 100 g: 100 g) to prepare a PGC mixture. This PGC mixture was prepared from PGC-1 to 4 samples according to the extraction conditions, and the details are as follows (Table 1).

PGC-1. Water was added to the PGC mixture in an amount of 10 times weight and irradiated for 2 h at 50℃ with a frequency of 50 kHz using a sonicator. Then, the extract extracted under reflux was repeated 3 times at 60℃ for 6 h, filtered through a 0.45 μm membrane filter (Amicon, USA), and then ethanol was removed with a

Table 1. Sample preparation conditions.

	PGC-1	PGC-2	PGC-3	PGC-4
Extraction method	Ultrasonic wave	Ultrasonic wave ethanol	Fermentation ultrasonic wave	Fermentation ultrasonic wave ethanol

vacuum concentrator to prepare PGC-1.

PGC-2. Ethanol was added to the PGC mixture in an amount of 10 times weight, and the mixture was irradiated at 50℃ with a frequency of 50 kHz using a sonicator for 2 h. Then, extraction was performed by refluxing 3 times at 60℃ for 6 h and the obtained extract was filtered with a 0.45 μm membrane filter (Amicon). Then, ethanol was removed using a vacuum concentrator to prepare PGC-2.

PGC-3. Water was added to the PGC mixture in an amount of 10 times weight, and *L. acidophilus* (KCCM 32820), *L. plantarum* (KCCM 11322) and *L. bulgaricus* (KCCM 40266) were inoculated at a concentration of 10^6 CFU/g, respectively, and fermented at 30℃ for 3 days. After taking the supernatant by centrifugation, it was irradiated for 2 h at 50℃ with a frequency of 50 kHz using a sonicator. Thereafter, 10 times the weight of water was added to the obtained ultrasonic extract, and reflux extraction was performed at 60℃ for 6 h, repeated three times. The extract was filtered through a 0.45 μm membrane filter (Amicon, USA), and then ethanol was removed using a vacuum concentrator to prepare PGC-3.

PGC-4. Water was added to the PGC mixture in an amount of 10 times weight, and *L. acidophilus* (KCCM 32820), *L. plantarum* (KCCM 11322) and *L. bulgaricus* (KCCM 40266) were inoculated at a concentration of 10^6 CFU/g, respectively, and fermented at 30℃ for 3 days. After taking the supernatant by centrifugation, it was irradiated for 2 h at 50℃ at 50 kHz frequency using a sonicator. Thereafter, 70% ethanol of 10 times the weight of the obtained ultrasonic extract was added, and reflux extraction was performed at 60℃ for 6 h, repeated three times. The extract was filtered through a 0.45 μm membrane filter (Amicon, USA), and then ethanol was removed using a vacuum concentrator to prepare PGC-4.

Antioxidant activity test

ABTS radical scavenging activity. In order to measure the ABTS radical scavenging activity, each of the samples PGC-1 to 4 is diluted in water to a concentration of 100, 250, 500, and 1000 μg/ml. 7 mM ABTS (Sigma-Aldrich, USA) and 2.45 mM potassium persulfate are mixed and reacted at room temperature for 12 h in the dark to form ABTS cations. Then, ethanol was added so that the absorbance value at 734 nm was 0.70 ± 0.02. 100 μl of the sample (PGC-1 to 4) and 100 μl of the prepared ABTS solution are added to a 96-well plate, reacted at room temperature for 7 min, and measured at 734 nm. Compared with the blank test solution, the ABTS clearance rate was calculated as a percentage (%) as follows.

ABTS radical scavenging ability (%) =
[Control – (Sample – Blank)]/Control × 100

(Control: Absorbance of ABTS reagent, Sample: Absorbance of Sample + ABTS reagent, Blank: Absorbance of Sample + Blank)

DPPH radical scavenging activity. To measure DPPH radical scavenging activity, put 100 μl of the sample prepared in the ABTS experiment (PGC-1 to 4) and 100 μl of 0.2 mM DPPH into a 96-well plate. After 30 min, absorbance at 517 nm using a microplate reader was measured. Compared with the blank test solution, the DPPH radical scavenging rate was calculated as a percentage (%) as follows.

DPPH radical scavenging ability (%) =
[Control – (Sample – Blank)]/Control × 100

(Control: Absorbance of DPPH reagent, Sample: Absorbance of Sample + DPPH reagent, Blank: Absorbance of Sample + Blank)

Antibacterial and anti-inflammatory activity test

Paper disc experiment. Paper disc experiment was conducted to test the antibacterial activity of acne-causing bacteria. The acne-causing bacterium was *P. acnes* (KCTC 3314), which was purchased from the Center for Biological Resources (KCTC) and cultured at 37℃ in Reinforced Clostridial Broth medium. The cultured *P. acnes* was smeared with a sterile cotton swab by 100 μl in a solid medium (agar 20 g/l) adjusted to 1×10^6 CFU/ml. Sample PGC-1~4 was diluted with water to a concentration of 500 μg/ml, and 20 μl of each was slowly absorbed on a paper disc (diameter 6 mm, Roshi Kaisha., Japan), dried, and the solvent was vola-

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tilized. The paper disc on which the sample (PGC-1~4) was absorbed was adhered to and cultured on the plate medium on which the strain was smeared. Then, the clear zone (mm) created around the disc was measured to compare the antibacterial activity.

LPS inflammation-inducing cytokine analysis. In order to confirm the decrease in cytokines, the control and samples (PGC-1~4) in which the production of inflammatory cytokines (TNF-α) in RAW 246.7 cells were induced through LPS treatment were compared. Cytokines are signaling substances secreted when macrophages are activated by foreign antigens. In order to evaluate the production of inflammation-related cytokines (TNF-α) in the cell culture medium, RAW 264.7 cells were aliquoted in a 96-well plate at a concentration of 1×10^4 cells/well, and then cultured for 12 h at 37℃ in a 5% carbon dioxide incubator. Thus, the cells were completely attached. Thereafter, samples (PGC-1 to 4) at concentrations of 0, 100, 250, and 500 μg/ml and 1 μg/ml of LPS, an inflammation-inducing substance, were added and re-cultured for 24 h. Thereafter, TNF-α was quantified using a Mouse TNF-α Quantikine ELISA kit (R&D systems, USA).

Cell viability experiments

For MTS analysis, RAW 264.7 cells were cultured in 100 IU/ml penicillin, 100 IU/ml streptomycin, and 10% fetal bovine serum DMEM (Dulbecco's modified Eagle's medium) at 37℃ and 5% carbon dioxide incubator. The cultured RAW 264.7 cells were aliquoted at 1×10^4 cells/ml, incubated for 24 h, and a sample (PGC-1~4) diluted to 100–1,000 μg/ml was added and incubated for another 24 h. Then, 20 μl of MTS reagent was added, and after incubation for 2 h, absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated by the following formula.

Cell viability (%) = [(Exp. – Blank)/Control] × 100

(Exp: absorbance of the extract containing cells, Blank: absorbance of the extract without cells, Control: absorbance of distilled water containing cells)

Statistics and data processing

All experiments in this study were used for analysis based on the results of three or more independent runs under the same conditions, and all experimental results were expressed as mean ± standard deviation. After calculating the mean and standard deviation of the experimental results, statistical significance was verified by t-test.

Results and Discussion

Results of antioxidant activity test

Radicals, one of reactive oxygen species (ROS), cause aging phenomena such as wrinkles and pigmentation when they have been accumulated in the body. ABTS and DPPH used in this experiment are used as substrates for measurement of antioxidant activity, and are known as indicators of antioxidant activity of phenolic substances such as phenol and flavonoids [24].

The measurement of antioxidant capacity using ABTS radicals is a method that uses the principle that ABTS radicals generated by reaction with potassium persulfate lose the blue-green color, which is their unique color, when they are in contact with antioxidants, and there are differences in radical scavenging activity among antioxidants [25]. It is known that the content of phenolic substances is proportionally and positively correlated with ABTS radical scavenging activity [26]. In the result of analysis on ABTS radicals, the activity of all samples showed a tendency to increase in proportion to the concentration, and ascorbic acid, a positive control, was found to have the highest activity of 99.5% at the concen-

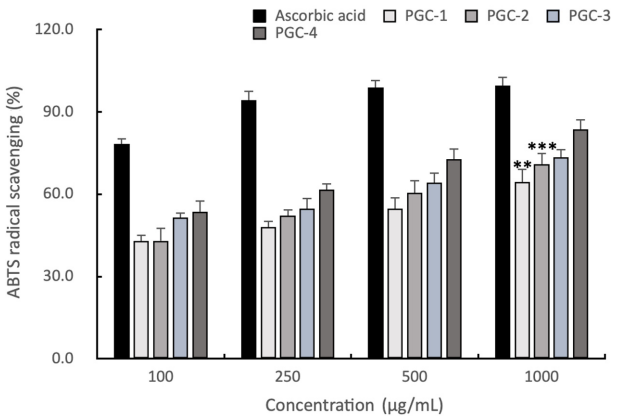

Fig. 1. ABTS radical scavenging activity of PGC mixture. ABTS radical scavenging activity was measured by 100, 250, 500, 1000 ug/ml concentration of PGC mixture. Ascorbic acid was used as positive control. Values are presented mean ± SD. *, $p < 0.05$; **, $p < 0.01$

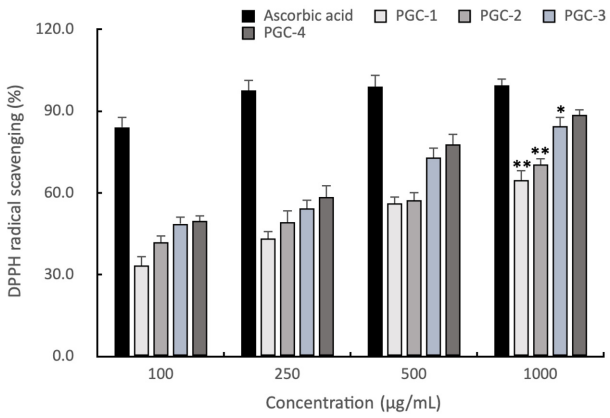

Fig. 2. DPPH radical scavenging activty of PGC mixture. ABTS radical scavenging activity was measured by 100, 250, 500, 1000 ug/ml concentration of PGC mixture. Ascorbic acid was used as positive control. Values are presented mean ± SD. *, $p < 0.05$; **, $p < 0.01$

tration of 1000 μg/ml. PGC-4 showed the highest activity at 83.5% at 1000 μg/ml, which is similar to the activity of a positive control, followed by PGC-3, PGC-2, and PGC-1 at 73.4%, 70.9%, and 64.5%, respectively at 1000 μg/ml (Fig. 1). In particular, at 1000 μg/ml, the ABTS radical scavenging activity of PGC-4 was significantly higher than that of PGC-3 ($p < .05$), PGC-2 ($p < .05$), and PGC-1 ($p < .01$). As a result, it was found that the antioxidant effect of PGC-4 was excellent.

In addition, with regard to the measurement of DPPH radical scavenging activity, since DPPH radicals are reduced by antioxidant active substances and the inherent indigo blue color of DPPH becomes pale so that antioxidant activity can be observed visually, DPPH radical scavenging activity is used as a measure to evaluate the antioxidant and antiaging activities of phenolic substances such as phenols and flavonoids against fat [27].

The result of DPPH radical analysis showed the same trend as the above-mentioned result of ABTS analysis. PGC-4, PGC-3, PGC-2, and PGC-1 showed DPPH radical scavenging activity of 88.7%, 84.5%, 70.9%, and 64.5% at 1000 μg/ml, respectively (Fig. 2). In this case, ascorbic acid, which was a control, was analyzed to have DPPH radical scavenging activity of 99.4% at 1000 μg/ml. In particular, at 1000 μg/ml, the DPPH radical scavenging activity of PGC-4 was not statistically different from that of PGC-3, but there was significantly different from that of PGC-2 ($p < .001$) and PGC-1 ($p < .01$). Therefore, it was found that the antioxidant effect of PGC-4 was excellent.

Free radicals that cause oxidative stress in the human body are generated by environmental pollution or drinking. In addition, reactive oxygen species and nitric oxide generated by free radical reactions *in vivo* cause protein inactivation, tissue damage, genetic mutation, and the like, and are reported as a major cause of aging and diseases such degenerative diseases. Therefore, it is important to search for natural plants that have fewer side effects and have antioxidant components. As a result of examination of the antioxidant efficacy of PGC-1~4, it was found that the antioxidant components of the samples increased concentration-dependently. In particular, the antioxidant effect of PGC-4 extracted through fermentation and ultrasonication was the best. The results of ABTS and DPPH radical analysis also showed that PGC-4 had a significantly higher radical scavenging ability than PGC-1~3, although it was lower than that of positive control. Given these results, it seems that polyphenols present in natural plants were effectively extracted to enhance antioxidant activity, and it is judged that the polyphenols can effectively protect cell membranes and intracellular substances from oxidative stress.

Results of antibacterial and anti-inflammatory activity tests

Various bacteria exist on the skin, and some of them combine with immune antibodies secreted from the sweat glands to act for skin immunity, but sebum, sweat, and various chemicals discharged from the body are decomposed into inflammation causing substances by the skin flora to cause skin troubles [28]. Representative flora that causes skin inflammation includes *P. acnes*. *P. acnes* mainly resides around and inside the hair follicles and secretes lipase, one of the enzymes that break down most of the human body's lipids, to break down neutral fat or triglycerides, the main components of sebum, into free fatty acids thereby causing inflammation and expanding the inflammation to cause acne [29]. In order to develop inhibitors of antibiotic-resistant bacteria, recently, studies to extract natural antibacterial substances from plant extracts have been actively in progress.

As shown in Table 2, as a result of analysis of the anti-

Table 2. Paper disc experiment result.

Strains	Size of clear zone (diameter, mm)			
	PGC-1	PGC-2	PGC-3	PGC-4
Propionibacterium acnes	5.8 ± 0.6	5.4 ± 0.9	11.8 ± 1.3	14.6 ± 1.6

bacterial activity against *P. acnes*, which is an acne-causing bacterium, whereas the clear zone of PGC-2 showed the lowest activity at 5.4 ± 0.9 mm, the clear zone of PGC-4 showed activity at 14.6 ± 1.6 mm, which is close to 3 times the activity of the clear zone of PGC-2. The clear zone of PGC-3 showed activity of 11.8 ± 1.3 mm, which was higher than the activity of the clear zone of PGC-1 or PGC-2, but lower than that of clear zone of PGC-4. According to previous studies, *Geranium* (*P. graveolens*) and *C. myrrha* contain various physiologically active compounds such as phenolic compounds [31, 32]. In particular, PGC-4 showed a statistically significant difference compared to PGC-1 ($p < 0.05$) and PGC-2 ($p < 0.01$). In particular, it is thought that the antibacterial effect appeared thanks to geraniol, linalool, and citronellol, which are constituents of *Geranium* (*P. graveolens*).

Common symptoms appearing on acne-prone skin include hyperkeratosis in pores, increases in sebum secretion, clogging of pores, and the occurrence of inflammation due to increases in *P. acnes*, which is the cause of acne. The types of acne can be divided into non-inflammatory acne, which includes whiteheads (closed comedones) and blackheads (open comedones), and inflammatory acne, which includes papules, pustules, nodules, and cysts. Acne begins as non-inflammatory acnes and progresses into inflammatory acne in most cases [33]. As for acne remedial agents, retinoids, benzoyl peroxide, azeleic acid, and *etc.* are used to remove dead skin cells and sebum, and antibiotics such as clindamycin and erythromycin are used to treat acne skin inflammation [34]. The above remedial agents are widely used in the treatment of acne skin because they have strong antibacterial effects that inhibit the proliferation of *P. acnes*, although there are differences in degrees [35]. However, these antibiotics cause side effects such as skin moisture loss, skin irritation, redness, and itching, and cause a problem of tolerance when they have been used for a long time [36]. In order to overcome the side effects and tolerance of acne remedial agents, various studies are being conducted to discover active ingredients against acne from natural plant sources. As a result of the analysis of antibacterial activity against *P. acnes*, which is the causative agent of acne, in this study, PGC-4 was found to have the highest antibacterial effect, and the antibacterial effect of PGC-3 was found to be higher than that of PGC-1 and PGC-2 although it was lower than that of PGC-4. The natural physiologically active ingredients mainly contained in plants can be divided into four types: alkaloids, terpenes, flavonoids, and phenols [37]. In particular, *Geranium* (*P. graveolens*) and *C. myrrha*, which are natural plants, contain various physiologically active compounds such as phenolic compounds. Physiologically active substances such as geraniol, linalool, and citronellol, which are constituents of *Geranium* (*P. graveolens*), produce compounds with complex and diverse structures while they coexist with living things around them [38], and these compounds appear to have showed antibacterial effects that inhibit microbial growth.

Inflammatory responses are a normal defense mechanism of the body against external stimuli, and continuous inflammatory responses cause tissue damage consequently becoming a cause of various diseases such as arthritis, diabetes, arteriosclerosis, and cancer. When inflammatory responses occur, inflammatory cells such as macrophages secrete inflammatory mediators such as tumor necrosis factor-α (TNF-α). Lipopolysaccharide (LPS), which is known to be an endotoxin, is present in the outer membrane of Gram-negative bacteria to induce the activation of nuclear factor-κB (NF-κB), an intracellular transcription factor, in macrophages or mononuclear cells thereby inducing gene expression of inflammatory cytokines and producing inflammation mediators. Therefore, substances that inhibit the expression of these enzymes are highly likely to be developed as anti-inflammatory agents [32].

Anti-inflammatory agents developed thus far have problems in terms of human safety as they cause gastritis, nephritis, and heart disease [33]. Consequently, the use of them is partially restricted. Therefore, searching for safer anti-inflammatory substances from natural substances is very important now. Hence, in this study, a control group, which is Raw cell 246.7 cells treated with LPS, and PGC-1~4 was incubated for 24 h and the amounts of TNF-α production were examined thereafter.

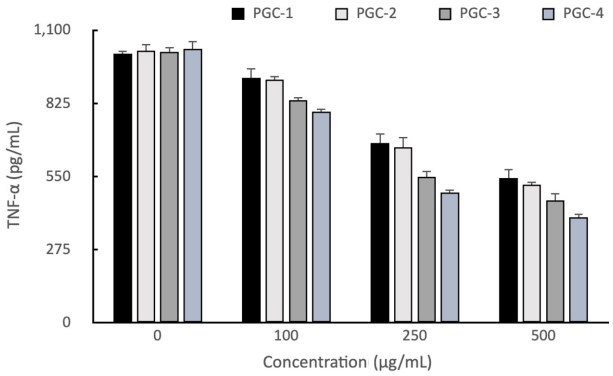

Fig. 3. Inhibitory effects of PGC mixture on TNF-α production in LPS-stimulated RAW 264.7 cells. Cells (1×10^4 cells/well) were treated by LPS (1 ug/ml) for 24h in the presence of PGC mixture (0, 100, 250, 500 ug/ml). Supernatants were collected, and the TNF-α concentration in the supernatants was determined by ELISA.

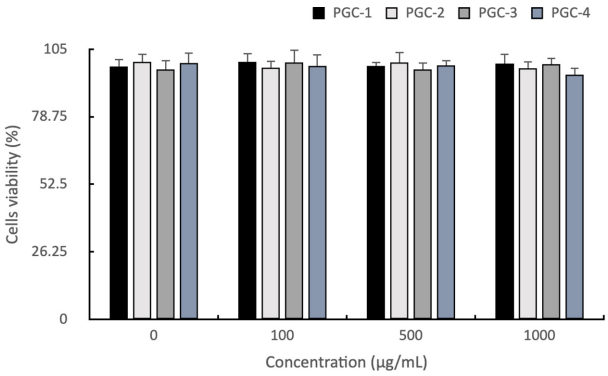

Fig. 4. Effect of PGC mixture on the cell viability by MTS assay. The cultured RAW 264.7 cells were aliquoted at 1×10^4 cells/ml and incubated for 24 h. Cells were treated with PGC mixture diluted to 100-1,000 μg/ml and incubated for 24 h.

As a result, the TNF-α concentration of the control group treated only with LPS was found to be the highest (Fig. 3). The TNF-α concentration decreased concentration-dependently in all samples, and the lowest TNF-α concentration was shown at a concentration of 500 μg/ml. In particular, PGC-4 was shown to inhibit TNF-α production more compared to any other samples, and the amount of TNF-α produced was shown to be 396.4 ± 21.2 μg/ml at 500 μg/ml, showing a TNF-α inhibitory ability of 61.5% compared to the control group.

TNF-α, an inflammatory cytokine, is produced by macrophages during inflammatory processes [39], and has been reported as a multifunctional cytokine that can exert inflammatory activity and cytotoxicity to a wide range of lymphoid, non-lymphoid, and tumor cells [39]. When inflammatory mediators have been produced excessively, inflammatory responses actively occur, excessive immune responses are caused, and various diseases are aggravated. Therefore, if inflammatory cytokines can be suppressed, not only inflammation but also various diseases can be suppressed [40]. Therefore, to identify the anti-inflammatory efficacy of the samples in this study, the effects of the samples on pro-inflammatory cytokines (TNF-α) were investigated. When RAW 264.7 macrophages treated with LPS alone were compared with the control group, it was found that TNF-α production increased significantly, TNF-α concentrations decreased concentration-dependently in all samples (PGC-1 to 4), and the lowest TNF-α concentration was shown at a concentration of 500 μg/ml. From these results, it was found that compared to other samples, PGC-4 was involved more effectively in the regulation of TNF-α among pro-inflammatory cytokines and this was assumed to be a difference due to the extraction method.

Result of MTS experiment

Recently, among plant extracts, physiologically active components such as antioxidant effects against free radicals and anti-inflammatory effects against inflammation such as dermatitis have been identified. Accordingly, it is widely applied as a treatment for skin diseases. However, the most important thing is to ensure safety in the use of these extracts, and therefore, verification of cytotoxicity is required. The cytotoxicity of PGC-1 to 4 was tested in RAW 264.7 cells and based on the result, it was identified that the cell viability was at least 95% at all concentrations (Fig. 4). Therefore, it was identified that PGC-1 to 4, which are all samples, have no cytotoxicity.

As described above, the antioxidant, antibacterial, and anti-inflammatory effects of the mixed extract of *P. emblica*, *Geranium* (*P. graveolens*) and *C. myrrha* were confirmed. The results as such are judged attributable to the complex actions of various active ingredients involved in the antioxidant activity of *P. emblica* [16], the antibacterial activity of *Geranium*, *P. graveolens* [20], and the anti-inflammatory activity of *C. myrrha* [21]. In particular, PGC-4 extracted using fermentation and ultrasonic extraction methods showed excellent antioxidant, anti-inflammatory, and antibacterial effects,

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indicating that fermentation and ultrasonic extraction methods effectively extract active ingredients involved in antioxidant, anti-inflammatory, and antibacterial effects.

Ultrasound-assisted extraction has been used to improve the efficiency of extraction of functional compounds from various plant materials, and this method has the advantage of simplifying the extraction process by increasing the extraction speed [41]. It is known that ultrasonic waves generate cavitation bubbles and that as the bubbles are collapsed, plant cell walls are destroyed to increase the discharge of organic compounds inside plant cells. In addition, the ultrasonic energy impact effect induces pressure rise so that high extraction efficiency can be obtained [42]. In this study, the antioxidant, anti-inflammatory, and antibacterial effects of PGC-4 and PGC-3 applied with ultrasonic extraction were shown to be high. This is judged to be because the cavitation formed during ultrasonic irradiation created high pressure to effectively destroy the plant cell walls so that the travel range of the extracts was shortened, and diffusion occurred easily. Kim *et al*. (2009) [43] stated that the extraction yield could be increased because ultrasonic energy destroyed plant tissues so that those components that could be hardly eluted with conventional extraction methods could be extracted. In addition, in a study of the antioxidation of *Oenothera odorata* extract, Kim and Lee (2016) [44] identified that active ingredients such as polyphenols and flavonoids were shown to be higher in the ultrasonic extraction method than in room temperature stirring extraction.

Fermentation is a process to generates energy by decomposing sugar without oxygen [45]. According to a study conducted by Park *et al*. (2012) [46], the total polyphenol content of extract of *Gastrodia elata Blume* that underwent yeast (*S. cerevisiae*, KCCM 50583) fermentation was found to be at least three times that of the extract that was not fermented. The reason why the efficacy of PGC-4 was shown to be excellent in this study seems to be the fact that enzymes such as lipase, protease, and amylase increased phenol components thanks to various yeasts involved in the fermentation process, so that the antioxidant effect and melanin production inhibitory effects were improved.

Conclusion

As a result of the study, all extracts (PGC-1 to 4) showed antioxidant, antibacterial, and anti-inflammatory effects without cytotoxicity, confirming their potential as natural raw materials for the development of acne treatments. In particular, as the fermented and ultrasonic extract PGC-4 showed the most excellent efficacy, if the fermentation and ultrasonic extraction method were applied to the mixed extraction of *Phyllanthus emblica*, *Geranium* (*P. graveolens)*, and *Commiphora myrrha*, as a natural material, it is judged that the practicality will increase.

Conflicts of Interest

The authors have no financial conflicts of interest to declare.

References

1. Bae CH, Cho WY, Kim HJ, Ha OK. 2020. An experimental comparison of cnn-based deep learning algorithms for recognition of beauty-related skin disease. *J. Korea Soc. Comput. Info.* **25**: 25-34.
2. Pande G, Akoh CC. 2010. Organic acids, antioxidant capacity, phenolic content and lipid characterisation of Georgia-grown underutilized fruit crops. *Food Chem*. **120**: 1067-1075.
3. Dasaroju S, Gottumukkala KM. 2014. Current trends in the research of *Emblica officinalis* (Amla): A pharmacological perspective. *Int. J. Pharm. Sci. Rev. Res.* **24**: 150-159.
4. Kim SC, Ahn KJ, Hann SK, Kim JW, Sung KJ, Kye YC, *et al.* 2003. Clinico-epidemiologic study on the abuse, misuse, and adverse effects of topical dermatologic drugs. *Korean J. Dermatol.* **41**: 1129-1135.
5. Zaenglein AL. 2018. Acne vulgaris. *New Eng. J. Med.* **379**: 1343-1352.
6. Webster GF. 1995. Acne vulgaris: state of the science. *Arch. Dermatol.* **135**: 1101-1102.
7. Brown SK, Shalita AR. 1998. Acne vulgaris. *Lancet* **351**: 1871-1876.
8. Weon JB, Ahn JH, Ma CJ. 2011. Antibacterial activity of some medicinal plants against *Propionibacterium acnes*. *Korean J. Pharmacog.* **42**: 98-101.
9. Lee NH, Choi EH, Ahn SK, Lee SH. 1998. A clinical study on the effect of a facial cleanser consisting of 1% triclosan and 0.5% Ku Shen on acne vulgaris. *Korean J. Dermatol.* **36**: 871-876.
10. Leem MH, Jung EH. 2005. Changes of pH, sebum, moisture excreation level of skin surface after benzoyl peroxide lotion treatment on acne skin. *Korean J. Aesthetics Cosmetol.* **3**: 37-48.
11. Bojar RA, Holland KT, Cunliffe WJ. 1991. The in-vitro anti-microbial effects of azelaic acid upon *Propionibacterium acnes* strain P37. *J. Antimicrob. Chemother.* **28**: 843-853.

12. Aires JR, Pechere JC, Van Delden C, Köhler T. 2002. Amino acid residues essential for function of the MexF efflux pump protein of *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **46**: 2169-2173.
13. Leyden JJ, Kaidbey K, Gans EH. 1996. The antimicrobial effects in vivo of minocycline, doxycycline and tetracycline in humans. *J. Dermatol. Treatment* **7**: 223-225.
14. Lim YH, Kim IH, Seo JJ. 2007. In vitro activity of kaempferol isolated from the impatiens balsamina alone and in combination with erythromycin or clindamycin against *Propionibacterium acnes*. *J. Microbiol.* **45**: 473-477.
15. Kobayashi M, Kabashima K, Nakamura M, Tokura Y. 2009. Effects of oral antibiotic roxithromycin on quality of life in acne patients. *J. Dermatol*. **36**: 383-391.
16. Dasaroju S, Gottumukkala KM. 2014. Current trends in the research of *Emblica officinalis* (CP): A pharmacological perspective. *Int. J. Pharm. Sci. Rev. Res*. **24**: 150-159.
17. Tsuji N, Moriwaki S, Suzuki Y, Takema Y, Imokawa G. 2001. The role of elastases secreted by fibroblasts in wrinkle formation: implication through selective inhibition of elastase activity. *Photochem. Photobiol*. **74**: 283-290.
18. Gates MA, Tworoger SS, Hecht JL, De vivo I, Rosner B, Hankinson SE. 2007. A prospective study of dietary flavonoid intake and incidence of epithelial ovarian cancer. *Int. J. Cancer* **121**: 2225-2232.
19. Akdemir ZS, Tatli II, Saracoglu I, Ismailoglu U, Calis I, Inci SE. 2001. Polyphenolic compound from Geranium pratense and their free radical scavenging activities. *Phytochemistry* **56**: 189-193.
20. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. 2006. In vitro antibacterial activity of some plant essential oils. *BMC Complement. Altern. Med*. **6**: 39.
21. Su S, Wang T, Duan JA, Zhou W, Hua YQ, Tang YP, *et al*. 2011. Anti-inflammatory and analgesic activity of different extracts of *Commiphora myrrha*. *J. Ethnopharmacol.* **134**: 251-258.
22. Sotoudeh R, Gholamnezhad Z, Aghaei A. 2019. The anti-diabetic and antioxidant effects of a combination of *Commiphora mukul, Commiphora myrrha* and *Terminalia chebula* in diabetic rats. *Avicenna J. Phytomed.* **9**: 454-464.
23. Madia VN, De Angelis M, De Vita D, Messore A, De Leo A, Ialongo D, *et al.* 2021. Investigation of *Commiphora myrrha* (Nees) Engl. oil and its main components for antiviral activity. *Pharmaceuticals* **14**: 243-254.
24. Que F, Mao L, Pan X. 2006. Antioxidant activities of five Chinese rice wines and the involvement of phenolic compounds. *Food Res. Int.* **39**: 581-587.
25. Arnao MB. 2000. Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case. *Trends Food Sci. Technol.* **11**: 419-421.
26. Jeong JA, Kwon SH, Lee CH. 2007. Screening for anti-oxidative activities of extracts from aerial and underground parts of some edible and medicinal ferns. *Korean J. Plant Resour.* **20**: 185-192.
27. Aoshima H, Tsunoue H, Koda H, Kiso Y. 2004. Aging of whiskey increases 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity. *J. Agric. Food Chem.* **52**: 5240-5244.
28. Kim MK. 2021. Investigation of antimicrobial activity of rutaceae fruit ethanol extracts against microorganisms-induced skin inflammation. *J. Korea Soc. Comput. Information* **26**: 237-245.
29. Wang SJ, Lee HJ, Cho JY, Park KH, Moon JH. 2012. Isolation and identification of antioxidants from makgeolli. *Korean J. Food Sci. Technol*. **44**: 14-20.
30. Guzik TJ, Korbut R, Adamek-Guzik T. 2003. Nitric oxide and superoxide in inflammation and immune regulation. *J. Physiol. Pharmacol*. **43**: 469-487.
31. Graca VC, Ferreira IC, Santos PF. 2016. Phytochemical composition and biological activities of *Geranium robertianum* L.: A review. *Ind. Crops Prod*. **87**: 363-378.
32. Hamed AM, Awad AA, Abdel-Mobdy AE, Alzahrani A, Salamatullah AM. 2021. Buffalo yogurt fortified with Eucalyptus (*Eucalyptus camaldulensis*) and Myrrh (*Commiphora Myrrha*) essential oils: New insights into the functional properties and extended shelf life. *Molecules* **26**: 6853.
33. Fu LW, Vender RB. 2011. Newer approaches in topical combination therapy for acne. *Skin Ther. Lett*. **16**: 3-6.
34. Koreck A, Pivarcsi A, Dobozy A, Kemeny L. 2003. The role of innate immunity in the pathogenesis of acne. *Dermatology* **206**: 96-105.
35. Witold M, Aleksander K. 2003. Preliminary assessment of alginic acid as a factor buffering triethanol amine interacting with artificial skin sebum. *Eur. J. Pharm. Biopharm*. **55**: 237-240.
36. Kim N, Lim YH, Park SW, Nam ES. 2009. Antimicrobial activities of the anti-acne compounds from natural sources. *Microbiol. Biotechnol. Lett*. **37**: 80-84.
37. Lee JY, Son HJ. 2018. Trends in the efficacy and safety of ingredients in acne skin treatments. *Asian J. Beauty Cosmetol.* **16**: 449-463.
38. Chan GY, Kim HJ. 2011. Antimicrobial effects of extracts of Taraxacum officinale H. on acnes strains. *Int. J. Integrated CAM* **7**: 3-16.
39. Laksmitawati DR, Prasanti AP, Larasinta N, Syauta GA, Hilda R, Ramadaniati HU, *et al.* 2016. Anti-inflammatory potential of gandarusa (*Gendarussa vulgaris* Nees) and soursoup (*Annona muricata* L) extracts in LPS stimulated-macrophage cell (RAW264. 7). *J. Nat. Remedies* **16**: 73-81.
40. Kim SH, Kang SA. 2019. Anti-inflammatory effects of beopje processed curly dock (*Rumex crispus* L.) in LPS- induced murine RAW 264.7 cell lines. *Korean J. Food Nutr.* **32**: 408-416.
41. Jang GY, Kim HY, Lee SH, Kang YR, Hwang IG, Woo KS, *et al.* 2012. Effects of heat treatment and extraction method on antioxidant activity of several medicinal plants. *J. Korean Soc. Food Sci. Nutr.* **41**: 914-920.
42. Chemat F, Khan MK. 2011. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrason. Sonochem.* **18**: 813-835.
43. Kim HY, Choi DH, Kang DG, Lee HS. 2009. Effects of *Oenothera odorata* on vasorelaxation in thoracic and carotid artery. *FASEB Exp. Biol. J*. **23**: 939-18.
44. Kim JH, Lee SH. 2016. Antioxidative and antimicrobial activities

of *Oenothera biennis* extracted by different methods. *Korean J. Food Preserv.* **23**: 233-238.
45. Klein A, He X, Roche M, Mallett A, Duska L, Supko JG, *et al.* 2006. Prolonged stabilization of platinum-resistant ovarian cancer in a single patient consuming a fermented soy therapy. *Gynecol. Oncol*. **100**: 205-209.
46. Park MR, Yoo C, Chang YN, Ahn BY. 2012. Change of total polyphenol content of fermented *Gastrodia elata* blume and radical scavenging. *Korean J. Plant Resour.* **25**: 379-386.