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Sensory evaluation of a body lotion formulated with hot spring water from Deokgu, Korea

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덕구온천수로 제조된 바디로션의 관능평가

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Abstract The purpose of this study was to evaluate hot spring water (HSW) from Deokgu as a cosmetic ingredient in the preparation of a body lotion. The HSW was tested for its suitability as an aqueous-phase main component. Microbiological and chemical stability tests of the HSW were carried out. Microorganisms including E. coli were not detected or detected below the detection limits, and no harmful heavy metals were found. The cytotoxicity of the HSW was also considered, and its pH determined over a period of three months. Further, sensory characteristics were assessed for consumer acceptance by performing sensory tests on body lotions formulated using either Deokgu HSW or distilled water. Skin moisturization, irritation and tension reinforcement were found to be enhanced when using the HSW lotion rather than that formulated with distilled water. Taken together, the results of this study show that the use of HSW in cosmetic formulations contributes to the efficacy of these products.

요 약 이 연구의 목적은 바디로션 제조 시 화장품 첨가제로서의 덕구온천수를 평가하기 위함이다. 주요 수용성 구성성분으로서의 온천수의 적합성을 시험하였다. 온천수의 미생물 및 화학적 안전성 시험이 수행되었다. 대장균을 비롯한 미생물은 검출되지 않았거나 검출한도 이하로 검출되었으며, 유해한 중금속은 전혀 검출되지 않았다. 온천수의 세포독성이 평가되었으며, 온천수의 pH 변화를 3달 동안 측정하였다. 또한 덕구온천수 및 증류수로 제조된 바디로션에 대해서 관능시험을 수행함으로써 소비자의 적합성을 관능특성 분석을 통하여 평가하였다. 증류수로 제조된 바디로션보다 온천수를 사용한 바디로션을 피부에 적용하였을 때, 피부보습, 피부트러블 및 피부 장벽강화가 증진되었다. 종합해 보았을 때, 이 연구의 결과는 화장품 제조에 있어서 온천수를 사용하는 것은 화장품의 효능을 증진시킬 수 있음을 보여 준다.

Keywords : Hot Spring Water, Cosmetics, Cytotoxicity, Sensory Evaluation, Transepidermal Water Loss

1. Introduction

The classification of hot springs varies depending on the researcher or country, but is generally divided according to temperature, dissolution type, liquid ion concentration, solubility of minerals or development status [1]. They are also classified as hypotonic, isotonic or hypertonic and divided into cold (<20 °C),

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hypohot (20-30 °C), hot (30-40 °C) or hyperhot (>40 °C) springs depending on the water temperature [2,3].

Balneotherapy is a therapeutic method that uses hot water baths to treat various types of disease; this traditional therapy that has been practiced in many countries around the world [4,5]. Although spa therapy started in Europe and America in the 1800s, many studies have been conducted recently because of its treatment effect on skin diseases such as atopic dermatitis and psoriasis [6]. The commonly known effects of hot spring water (HSW) are its blood circulation-enhancing effects due to the capillary blood being expanded by the warm bath, the chemical action of the HSW and the analgesic effect created by the heat [7,8]. The hot temperature of the water relaxes nerve tension, stimulating the parasympathetic nervous system which in turn stimulates the secretion of acetylcholine to induce relaxation of the mind and body. It relieves muscle tension and spasm of the muscles [9]. and aids buoyancy, weight control and skin care. It is known that the effects of hot spring therapy are a combination of the effects of both mineral water and baths [10,11]. Despite its long history and tradition, however, the mechanism by which it improves various kinds of skin disease has yet to be clarified [12,13].

Deokgu hot spring is the representative one of Korea and the only natural one in Korea. The water that it generates-around 2,000 tons per day-is known to be effective not only as drinking water but also for the digestive organs, because of its low content of minerals. It is mainly composed of sodium bicarbonate, contains many components-such as fluorine, potassium, calcium, iron and carbonic acid-and is weakly alkaline. Furthermore, Deokgu HSW is known to be effective for skin diseases, neuralgia and skin beauty, as well as diabetes and chronic dyspepsia. However, the manufacture of biotechnology products using HSW has not been actively developed.

The purpose of this study was to evaluate the safety, stability and cytotoxicity of Deokgu HSW and its applicability as a cosmetic ingredient. A body lotion containing the HSW was also prepared and a sensory evaluation (skin moisturization, skin irritation and skin tension reinforcement) conducted to analyse potential consumers' preferences.

2. Materials and Methods

2.1 Analysis of heavy metals and microorganisms

Heavy metals in Deokgu HSW were analysed using an Inductively Coupled Plasma Mass Spectrometer (Varina ICP-MS). Standard materials were purchased from Agilent (CA, USA). The concentration of heavy metals was determined according to the method established by Cobina et al [14]. One liter of HSW was collected, to which 0.5 mL of concentrated nitric acid (HNO_3) was added, and the resulting solution transported to the laboratory at a maintained temperature of 4 °C. In the laboratory, samples were filtered through Whatman 0.45 μm membrane filter paper, and 100 mL of the filtered samples was mixed with 5 mL HNO_3. Argon gas was used for the heavy metal analysis, the operating conditions being as follows: nebulizer gas flow rate, 0.9 L/min; auxiliary gas flow, 0.3 L/min; plasma gas flow, 15 L/min; reaction gas flow (helium), 4 mL/min; ICP RF power, 1600 W. Samples were prepared by diluting 1.0 mL of the water samples to 10.0 mL with 0.3% ultrapure nitric acid, and analysed using ICP/MS. Each sample was analysed three times, and the results expressed as mean ± SE. The standard solution was diluted with 0.4-20 ng/mL and the concentration calculated by scoring the calibration curve.

The presence of bacteria, *E. coli* and fungi in HSW was determined using a dry, rehydratable film media (Petrifilm AC for total bacteria count, Petrifilm EC for *E. coli* count and Petrifilm YM for total fungi count) [15]. HSW samples were serially diluted, and 1 mL of the appropriate dilution was plated onto triplicate

Petrifilm plates. Inoculated Petrifilm plates were incubated at 35 ± 1 °C for 24−48 h for total bacteria and *E. coli* counts, and at 25 ± 1 °C for 72−120 h for total fungi count. Colonies were counted on the Petrifilm plates, the average number of colonies being calculated by multiplying by the dilution factor.

2.2 Cytotoxicity of Deokgu HSW

The cytotoxicity of HSW was evaluated using the Cell Counting Kit-8 (CCK-8) according to the manufacturer's instructions. One hundred μL of B16-F10 melanocytes (ATCC LOT. 60508145) cell suspension (5000 cells/well) was dispensed into a 96-well plate and pre-incubated at 37°C for 24 h in a 5% CO_2 incubator to fix the cells to well plates. Dulbecco's Modified Eagle Medium (DMEM) medium (100 μL) was dispensed into each well and 10 μL of HSW was added to the test group. Ten μL of distilled water and 10 μL of 100% ethanol were used as the negative control and positive controls, respectively. Each test well was incubated at 37 °C for 24 h. Ten μL of CCK-8 solution was added to each well and incubated for 1 h, and absorbance measured at 450 nm. The blank was measured with the sample in the absence of cells. The survival rate (%) = $(A_s-A_b)/(A_c-A_b) \times 100$, where A_s is the absorbance of the HSW sample, A_b is the absorbance of a blank sample and A_c is the absorbance of the sample treated with distilled water.

2.3 Body lotion preparation

Deokgu HSW was also used as a solvent in the production of a body lotion. A body lotion formulated with purified water was used for comparison purposes. All additives used in the body lotion fell within the permitted list of ingredients for cosmetics of the Korean Food and Drug Administration. The additives were classified into oil phase and water phase, the constituents of which are shown in Table 1. The oil phase and water phase were stirred on a hot plate (85 °C or more), and the body lotion was prepared by adding the well-mixed water phase to the oil phase.

Table 1. Body lotion composition containing Deokgu HSW

Compound	Contents (%)
Deoggu HSW	60.05
Aloe Barbadensis Leaf Water	10
Olive Oil	8
Avocado Oil	7
Cetearyl Olivate	4
Glycerin	3
Hexanediol	2
Shea butter	2
Cetearyl Alcohol	1
Betaine	1
Grapefruit Seed Extract	0.5
Perfume	0.5
Carbomer	0.5
Allantoin	0.5
Tocopherol	0.2
loe Barbadensis Flower Extract	0.05
Portulaca Oleracea Extract	0.05
Centella Asiatica Extract	0.05
Houttuynia Cordata Extract	0.05
Laminaria Japonica Extract	0.05
Propolis Extract	0.05
Oligo-chitosan	0.01
Collagen	0.0075
Sericin	0.0025

2.4 Sensory evaluation of the body lotion

In this study, two body lotions-one formulated with Deokgu HSW, the other with purified water-were carefully prepared (see Table 1 for the composition of the HSW body lotion). Sensory evaluation was then conducted on untrained female volunteers between the ages of 18 and 55 years to determine potential consumers' preferences-women being the main users of body lotions [16,17].

2.4.1 Skin moisturization test

The effects on the skin moisturization of the resulting body lotion formulated with Deokgu HSW were compared with that of a body lotion formulated with purified water. The skin moisturizing effect was tested on 21 subjects. All the subjects were female, their average age being 44.5 years. Subjects were

selected for the study according to selection and exclusion criteria. The skin condition of the subjects was checked, and then a device evaluation was performed. The test site was the upper arm area of the subject. Subjects were kept clean and dry in order to make the measurement conditions the same, and the skin was kept stable in a location in which a constant temperature (22 ± 2 °C) and humidity (40-60%) could be maintained for at least 30 min.

The test site was measured using a corneometer (CM825, Courage and Khazaka Electronic Co., Germany). After a minimum of five tests, the mean values of three measurements-that is, excluding the maximum and minimum values-were recorded. Measurements were taken before (0), and then two and four weeks after, use. Improvement in skin moisturization (%) with the test product was calculated as follows: i.e. change in skin moisturization = skin moisturization after use - skin moisturization before use; and skin moisturization improvement (%) = (skin moisture change/skin moisturization before use) × 100.

2.4.2 Skin irritation test

The body lotion formulated with Deokgu HSW was used by 21 subjects for two and four weeks, and its effect evaluated using a questionnaire. We investigated whether adverse reactions such as erythema, oedema, scaling, itching, stinging, burning, tightness and prickling occurred with use of the test product. When any adverse skin reaction occurred, a grade was given according to its severity (0: none, 1: mild, 2: severe, 3: very severe).

2.4.3 Skin Tension Reinforcement Test

The effect of Deokgu HSW on transdermal water loss was also investigated. Prior to using the body lotion, subjects' transdermal water loss (TEWL) was measured using a Tewameter. A chamber containing 1 % sodium lauryl sulfate was attached to the test site for 24 h. After 24 h it was removed and the condition of the test site observed. Where it could be confirmed that the stratum corneum had been removed properly, the amount of TEWL at the test site was measured. Where removal of the stratum corneum had been inadequate, a new chamber was attached and the amount of TEWL measured after re-examination of the test site.

2.5 Statistical analysis

All the experiments were repeated three times. Statistical analysis was performed using SPSS 18.0 software. Independent t-tests were performed for comparisons between the two groups. All statistical results were considered to be statistically significant at 5 % ($p < 0.05$), the most frequently used biometric statistical analysis.

3. Results

3.1 The safety, stability and cytotoxicity of Deokgu HSW

The concentrations of heavy metals, total bacteria, total fungi and *E. coli* in Deokgu HSW were analysed (Table 2). Heavy metals such as Pb, As, Hg, Sb and Cd were not detected. Total bacteria and fungi were visible at <10 cells/mL, which is not significantly higher than the detection limit, and *E. coli* was not detected at all. The lack of heavy metals and an extremely low microbial content suggest that Deokgu HSW is suitable for use in cosmetics and as drinking water.

Table 2. Evaluation of heavy metal and total microbes in the body lotion containing Deokgu HSW

Test items	Detection limits	Deokgu HSW
Pb (μg/g)	< 20	N/D
As (μg/g)	< 10	N/D
Hg (μg/g)	< 1	N/D
Sb (μg/g)	< 10	N/D
Cd (μg/g)	< 5	N/D
Total bacteria (cells/mL)	< 1,000	< 10
Total fungi (cells/mL)	< 1,000	< 10
E. coli (CPU/g(mL))	N/D	N/D

Changes in the pH at 4°C and 25°C were measured to evaluate the stability of Deokgu HSW (Fig. 1). The pH value was found to be stable over three months-8.58 ± 0.14 and 8.49 ± 0.13, at 4°C and 25°C, respectively. There was no statistically significant difference in pH during the test period ($p < 0.05$).

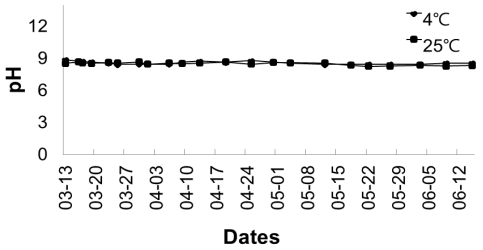

Fig. 1. The pH changes of Deokgu HSW for 3 months

The cytotoxicity of Deokgu HSW was evaluated using melanocytes cells and the Cell Counting Kit-8 (Fig. 2). The survival rates of the cells treated with HSW and purified water were 98.37 % and 99.27 %, respectively. On the other hand, cell viability was found to be 8.7 % when 100 % ethanol was used as a positive control.

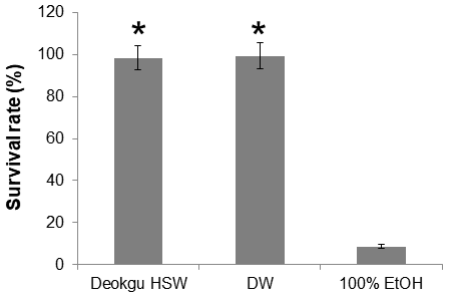

Fig. 2. Cytotoxicity test of Deokgu HSW with 100% ethanol as the positive control. Data are expressed as means ± SE and obtained from triplicate experiments. * denotes significant difference compared with the controls ($p < 0.05$).

3.2 Sensory evaluation of the body lotions

Changes in skin moisturization following application of the body lotion were then measured and improvement in moisturization analysed. After two weeks of treatment with the HSW or purified water body lotion, skin moisture was measured as 77.58 AU and 76.92 AU, respectively; this was not a statistically significant difference. After four weeks of treatment, skin moisture values with the HSW and distilled water body lotions were 78.49 AU and 76.95 AU, respectively (Fig. 3A); thus, improvement in skin moisturization at two and four weeks was 1.55 % and 2.71 %, respectively (Fig. 3B). These results show that the body lotion containing Deokgu HSW made a statistically significant improvement to skin moisturization compared with the body lotion containing distilled water ($p < 0.05$).

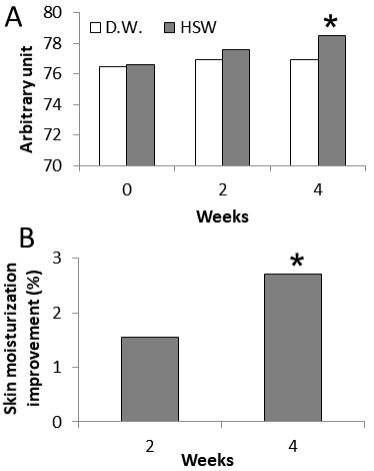

Fig. 3. Effect of the body lotion formulated with Deokgu HSW on skin moisturization (A) and improvement over time (B). Data are expressed as the average of three of five test results (i.e. excluding maximum and minimum scores). * denotes significant difference compared with the controls ($p < 0.05$).

The skin irritation test consisted of a visual inspection and a questionnaire on the degree of irritation to the skin after using the body lotion. This was carried out on the 21 subjects who had used the HSW body lotion for four weeks. The results showed that erythema, oedema, scaling, itching, stinging, burning, tightness and prickling did not appear in any of the subjects (Table 3).

The effect of the Deokgu HSW body lotion on improving skin barrier function was assessed by measuring transdermal water loss. Improvements in transepidermal water loss (TEWL) after one week of treatment were recorded as 90.72 ± 3.99 % and 91.72 ± 4.69 % for the purified water lotion and HSW lotion, respectively; after two weeks, the respective figures were 94.92 ± 3.08 % and 96.79 ± 2.14 % (Fig. 4). When TEWL improvements in the two lotion test groups were compared, a statistically significant improvement effect of skin barrier function was found for the HSW lotion following two weeks of treatment ($p < 0.05$). These results suggest that Deokgu HSW helps to improve skin barrier function.

Table 3. Skin irritation test of body lotion formulated with Deokgu HSWs.

Skin troubles	Weeks	
	2	4
Erythema	0	0
Edema	0	0
Scaling	0	0
Itching	0	0
Stinging	0	0
Burning	0	0
Tightness	0	0
Prickling	0	0
0, none; 1, mild; 2, severe; 3, very severe		

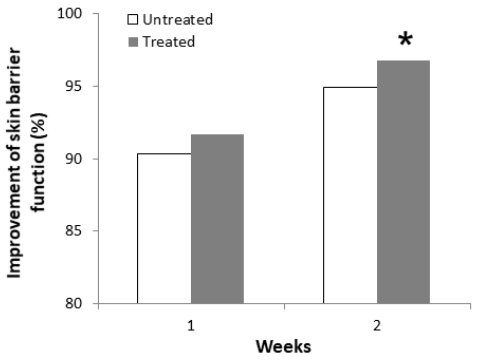

Fig. 4. Effect of body lotion formulated with Deokgu HSW on skin tension reinforcement. Data are expressed as the average of three of five results (i.e. excluding maximum and minimum scores); * denotes significant difference compared with the controls ($p < 0.05$).

4. Discussion

As the biotechnology industry develops, there is growing interest in quality of life in an ageing society in which life expectancy has risen. As a consequence of the recent well-being epidemic, many people are looking for effective products; and in order to achieve successful ageing, most attention is focused on cosmetics associated with skin protection. As a result, food and cosmetics that contain natural ingredients have been steadily gaining popularity, and so the need for research to meet these consumer needs is increasing. The purpose of this study was to investigate the applicability of Deokgu HSW as a cosmetic additive, in order to expand the range of applications of HSW.

We conducted a safety of Deokgu HSW and sensory evaluation on body lotions formulated using Deokgu HSW in order to investigate its applicability as a cosmetic additive. In the formulation of body lotions, water is used as an excipient and solvent for transporting and applying active additives, but HSW may also act as an active additive because of the minerals it contains [18]. Deokgu HSW is high in Na^+ (42.8 mg/L) and Ca^{2+} (3.53 mg/L). Its contents of HCO^{3-} (80.9 mg/L) and SiO_2 (50.7 mg/L) are very high, while those of F^- (12.4 mg/L), Cl^- (6.93 mg/L) and SO_4^{2-} (6.76 mg/L) are high relative to that of other ions (unpublished data). It is assumed that the Na^+ in the HSW has the effect of maintaining moisture balance, and that the Ca^{2+} has the effect of increasing skin strength, both of which are thought to have a beneficial effect on various skin ailments. Both F^- and SiO_2 are known to be effective for skin disease treatment and skin regeneration [3,6].

The stability and safety evaluation of cosmetic raw materials and ingredients must be checked before manufacturing cosmetics [19-21]. In particular, the sensory evaluation of the manufactured cosmetics is considered to be an essential process for verifying the efficacy of the product as well as for analyzing the

consumer's preference [22,23]. Deokgu HSW is suitable as a hot spring bath or drinking water because its microbial and heavy metal contents are very low (Table 2). We observed no pH change in the water over a three-month period, confirming its stability (Fig. 1). A body lotion formulated with Deokgu HSW was tested and its beneficial effects on skin moisturization (Fig. 3), skin irritation (Table 3) and transdermal water loss (Fig. 4) were verified when sensory evaluation was performed. In conclusion, the use of Deokgu HSW in cosmetic formulations appears to be beneficial not only in terms of marketing but also in terms of efficacy.

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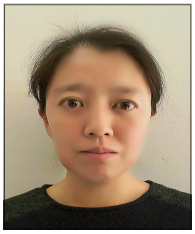

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